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Inheritance and diversity of simple sequence repeat (SSR) microsatellite markers in various families of *Picea abies*

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A large number of sequence-specific SSRs were screened by using electrophoresis on metaphore agarose gels with the bands visualized by ethidium bromide staining. Many SSRs appeared as codominant and many as dominant markers, with presence or absence of bands. A simple Mendelian inheritance pattern for most codominant and dominant SSR loci was found. For many codominant SSR markers, null alleles were detected. The proportion of dominant microsatellites detected in this study (close to 50 %) was much higher than that commonly reported in many other studies. A high proportion of dominant markers together with a high frequency of codominant markers with null alleles may represent two important limitations for the use of microsatellites in different studies. On the other hand, many polymorphic codominant SSR microsatellite markers were found to be highly repeatable, and can be used for population studies, seed certification, quality control of controlled crosses, paternity analysis, pollen contamination, and mapping of QTL in related families.

In this paper, we report on the inheritance pattern and diversity of codominant and dominant SSR microsatellites in seven families of *Picea abies* sharing a common mother.

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Picea abies is one of the most widespread tree species in Scandinavia and northern Europe used for production of pulp wood and timber. Traditional methods for the genetic improvement and conservation of this species have been practised in many countries. For progress in this work in the future, highly polymorphic genetic markers are needed. Examples of the types of markers that are being developed are simple sequence repeat (SSR) microsatellite markers.

SSR markers belong to the family of repetitive non-coding DNA sequences. SSRs are used for analysis of population genetic diversity, either natural or artificial, in gene flow studies (STREIFF et al. 1998, 1999), parentship analysis (LEXER et al. 1997), or construction of linkage maps (BELL and ECKER 1994; AKKAYA et al. 1995; BRONDANI et al. 1998). The presence of microsatellite SSRs in forest tree species has been reported in several studies (CONDITT and HUBBELL 1991; SMITH and DEVEY 1994; DOW et al. 1995; KOSTIA et al. 1995; CHASE et al. 1996; ECHT et al. 1996, 1999; VEN and MCNICOL 1996; WHITE and POWELL 1997; HICKS et al. 1998; RAJORA et al. 2001).

The development of SSR primers for the amplification of microsatellites in *Picea abies*, which involves the screening of enriched or non-enriched genomic libraries for microsatellite sequences, repeat types, PCR product sizes, and in some cases inheritance pattern has been reported by PAGLIA et al. (1998), PFEIFFER et al. (1997) and SCOTTI et al. (1999, 2002).

The main objective of this work was to test the Mendelian inheritance of numerous nuclear SSR markers in *Picea abies*, which could be used for mapping and population genetic studies in the future. We found a high proportion of dominant microsatellites (close to 50 %), which is much higher than that commonly reported, and high frequency of null alleles. Microsatellites are always described as generally codominant markers. Here we report on the result of the inheritance and the level of diversity of numerous polymorphic codominant and dominant SSR markers in different families of *Picea abies*.

MATERIAL AND METHODS

Plant material and DNA extraction

The material for this study originated from crosses between plus trees selected in southern Sweden with a central European origin. Controlled crosses were carried out with clone N2022 used as common female and seven clones N2024, E2006, M2016, N2018, M1001, M2006, M2013 used as different males giving rise to 7 families with identification numbers F 1500, F 1600, F 1700, F 1800, F 1900, F 2000, F 2100. All material was obtained from the Forestry Research Institute of Sweden (SkogForsk). The parental clone originated from the Maglehem seed orchard in Skåne, and the progenies from the Vetlanda progeny trial in Småland, trial number S21F772783. The trial was established in 1979 with 3-yr-old seedlings. Needle samples were collected from the parents and from 29–37 progenies per family in the field tests in southern Sweden, i.e. all living trees in the selected families.

DNA was extracted from needle tissue using a standard CTAB (hexadecylatrimethylammonium bromide) procedure following DOYLE and DOYLE (1990).

Simple sequence repeat (SSR) loci

67 SSR loci with dinucleotide and trinucleotide repeats were tested. Many SSR primers did not amplify or amplified badly despite using different annealing temperatures, but 51 SSRs produced codominant or dominant markers. The list of the dinucleotide and trinucleotide amplified SSRs with their names, repeat sequences, forward and reverse primer sequences, size estimate, number of alleles, and their annealing temperatures have been published by PAGLIA et al. (1998), PFEIFFER et al. (1997) and SCOTTI et al. (2002).

For PCR reactions of all SSRs, we adapted some modifications of procedures in PAGLIA et al. (1998) and PFEIFFER et al. (1997), as described below. Each reaction contained 0.15 μM of F and R end primers, 5–10 ng of *Picea abies* genomic DNA, 2 mM $MgCl_2$, 200 μM of each nucleotide, 1 unit Ampli Taq Gold polymerase, diluted with $1 \times$ PCR Gold Buffer (Perkin Elmer) to a total volume of 13 μl. All PCR reaction consisted of 1 cycle of 10 minutes with incubation at 95°C, 40 cycles of 45 s of denaturation at 94°C, 45 s of annealing at 57°C, and 45 s of extension at 72°C, with a final extension of 10 minutes at 72°C, on a MJ Research model PTC-100 thermocycler (MJ Research, Watertown, Mass.).

Amplification products from different SSR loci were analysed in the 3.5 % metaphore agarose gel (BioProduct).

The SSRs patterns were screened for polymorphic bands. The band positions were assigned with discrete values from 1 to 9, ranging from the smallest (1) to the greatest (9) length of the DNA fragments after electrophoresis. For the sake of simplicity, numbers 1 to 9 are used as genotype codes for different codominant marker alleles. These are derived from the relative mobility of the band on the gel. For dominant SSRs, we used allelic designation 1 for presence of band and 0 for abscence of band (null allele).

The SSR marker loci were tested for segregation according to the Mendelian hypothesis using chi-square (χ^2) goodness-of-fit values. 67 SSRs have been tested for amplification and 51 were found reliable for inheritance study.

We found several illegitimate individuals among progenies, which may be a result of errors in harvesting or through outcrossing, or a mutation event that causes changes in the band migration pattern.

RESULTS

Codominant and dominant SSR markers

Twenty-nine codominant and twenty-two dominant SSRs markers with presence or absence of bands were scored in this study. Parent and progeny genotypes for codominant and dominant SSRs, family number, observed and expected genotype distribution, chi-square (χ^2) and P-value for significance for all markers are shown in Table 1. The overall pattern of inheritance shows that most markers had a Mendelian behaviour. In few cases a significant deviation from Mendelian segregation was observed. This may be explained by the number of tests performed. In our case only 3 out of 133 test show the p-value below 5 %. An overall test of the p-value, using the fact that the sum of $-2 \ln (p)$ has a chi-square distribution under the null hypothesis was not significant ($p = 0.56$).

In Fig. 1A, 1B, 1C, 1D and 1E the inheritance and segregation patterns for SSR "SPAGC1", in parents and progenies of seven families are presented.

The female clone N2022 has genotype (23) and the male clones N2024, E2006, N2018, M1001, M2013, were homozygous (55). Progeny from these crosses all segregate in two categories with genotypes 25 and 35 for families F 1500, F 1600, F 1700, F 1900, and F 2100 (Fig. 1). In family F 1800, the male clone N2018 has genotype (25). The segregation in the progeny gives rise to four categories of genotypes (22), (23), (25) and (35) (Fig. 1B). In family F 2000, the male clone M2006 has genotype (26), and segregation among the progeny produced four categories

Table 1. *Inheritance study of codominant and dominant SSR markers. A, B, and C are different loci. In case of dominant marker 1 is present and 0 absence of band.*

Primer	Family No.	Codominant	Dominant	Parent genotype	Progeny genotype	Expected segregation	Observed segregation	Chi-square	P-value
AC1B8	F 1500	A		57 22	25 27	1:1	17:17	0	1.000
AC1B8	F 1600	A		57 67	56 57 67 77	1:1:1:1	3:7:7:7	1.9	0.593
AC1B8	F 1700	A		57 22	25 27	1:1	17:19	0.1	0.752
AC1B8	F 1800	A		57 57	55 57 77	1:2:1	10:11:14	5.7	0.058
AC1B8	F 1900	A		57 45	45 55 47 57	1:1:1:1	8:8:4:5	1.9	0.593
AC1B8	F 2000	A		57 67	56 57 67 77	1:1:1:1	8:2:7:9	4.4	0.221
AC1B8	F 2100	A		57 57	55 57 77	1:2:1	2:5:5	1.7	0.192
EAC1C8	F 1600		A	00 10	10 00	1:1	15:9	1.5	0.221
EAC1E3	F 1900		A	10 00	10 00	1:1	17:11	1.2	0.273
EAC1E3	F 2000		A	10 00	10 00	1:1	19:17	0.1	0.752
EAC1E3	F 2100		A	10 00	10 00	1:1	6:4	0.4	0.527
EAC1F4	F 1500	A		14 12	11 12 14 24	1:1:1:1	10:10:8:7	0.7	0.873
EAC1F4	F 1600	A		14 33	13 34	1:1	11:8	0.4	0.527
EAC1F4	F 1900	A		14 23	12 13 24 34	1:1:1:1	11:11:4:8	3.8	0.284
EAC1F4	F 2000	A		14 35	13 15 34 45	1:1:1:1	9:12:6:9	2	0.572
EAC1F4	F 2100	A		14 34	13 14 34 44	1:1:1:1	9:6:10:9	1	0.801
EAC1G5	F 1800	A		15 23	12 13 25 35	1:1:1:1	7:13:8:7	2.8	0.094
EAC1G5	F 1900	A		15 34	13 14 35 45	1:1:1:1	8:9:4:7	2	0.572
EAC1G5	F 2100	A		15 34	13 14 35 45	1:1:1:1	9:10:8:8	0.31	0.958
EAC2C8	F 1700	A		13 22	12 23	1:1	22:14	1.6	0.206
EAC2C8	F 2100	A		13 13	11 13 33	1:2:1	4:10:17	4.8	0.091
EAC2H7	F 1900	A		80 36	38 68 30 60	1:1:1:1	8:6:9:10	1.1	0.777
EAC2H8	F 1600		A	00 10	10 00	1:1	14:9	1	0.317
EAC2H8	F 1700		A	00 10	10 00	1:1	22:15	1.3	0.254
EAC2H8	F 1800		A	00 10	10 00	1:1	20:17	0.2	0.655
EAC2H8	F 1900		A	00 10	10 00	1:1	17:16	0.03	0.862
EAC2H8	F 2000		A	00 10	10 00	1:1	13:20	1.4	0.237
EAC6A6	F 1500	A		24 45	24 25 44 45	1:1:1:1	6:5:2:12	9.4	0.024
EAC6A6	F 1700	A		24 13	12 23 14 34	1:1:1:1	3:7:11:13	6.9	0.075
EAC6A6	F 1800	A		24 35	23 25 34 45	1:1:1:1	5:5:4:9	2.5	0.475
EAC6B1	F 1600		A	00 10	10 00	1:1	13:11	0.1	0.752
EAC6B2	F 1600	A		23 33	23 33	1:1	16:8	2.6	0.107
EAC6C02	F 1700	A		33 40	34 30	1:1	22:15	1.3	0.254
EAC6C10	F 1600		A	00 10	10 00	1:1	12:12	0	1.000
EAC6D11	F 1500	A		34 23	23 33 24 34	1:1:1:1	8:13:5:9	3.7	0.296
EAC6D11	F 1700	A		34 57	35 37 45 47	1:1:1:1	8:4:14:10	5.7	0.127
EAC6D11	F 2000	A		34 24	23 34 24 44	1:1:1:1	4:7:11:12	4.8	0.187
EAC6E9	F 1600		A	00 10	10 00	1:1	15:9	1.5	0.221
EAC6F4	F 1500	A		46 14	14 44 16 46	1:1:1:1	6:13:7:7	3.7	0.296
EAC6F4	F 1700	A		46 17	14 47 16 67	1:1:1:1	7:16:9:5	7.4	0.060
EAC6F4	F 1800	A		46 27	24 47 26 67	1:1:1:1	10:7:12:8	2.2	0.532
EAC6F4	F 2000	A		46 24	24 44 26 46	1:1:1:1	9:7:9:10	0.5	0.919
EAC6F5	F 1700	A		40 30	34 40 30 00	1:1:1:1	13:5:11:7	4.4	0.221
EAC6G3	F 1600	A		35 24	23 34 25 45	1:1:1:1	5:7:6:6	0.2	0.978
EAC6G8	F 1500		A	00 10	10 00	1:1	21:14	1.4	0.237
EAC7A7	F 1900		A	00 10	10 00	1:1	15:18	0.2	0.655
EAC7B9	F 1500	A		34 34	33 34 44	1:2:1	4:17:13	3.8	0.150
EAC7B9	F 1700	A		34 34	33 34 44	1:2:1	4:21:8	3.4	0.183
EAC7B9	F 1900	A		34 44	34 44	1:1	21:13	1.8	0.180
EAC7B9	F 2000	A		34 46	34 36 44 46	1:1:1:1	9:6:11:6	1.2	0.753
EAC7C11	F 1700	A		40 30	34 40 30 00	1:1:1:1	7:13:10:7	2.6	0.457
EAC7D10	F 1700		A	10 00	10 00	1:1	20:17	0.2	0.655
EAC7D10	F 1800		A	10 00	10 00	1:1	16:20	0.4	0.527
EAC7D10	F 2000		A	10 10	(11 10 10) 00	3:1	28:7	0.4	0.527
EAC7D10	F 2100		A	10 10	(11 10 10) 00	3:1	23:12	1.6	0.206
EAC7E6	F 1600	A		50 30	35 50 30 00	1:1:1:1	4:8:4:8	2.6	0.457
EAC7F6	F 1600		A	00 10	10 00	1:1	16:8	2.6	0.107
EAC7F8	F 1800		A	10 00	10 00	1:1	16:21	0.6	0.439
EAC7G7	F 1600		A	00 10	10 00	1:1	9:15	1.5	0.221
EAC7H7	F 1500	A		57 55	55 57	1:1	11:23:1	4.2	0.040

Table 1. (*Continued*)

Primer	Family No.	Codominant	Dominant	Parent genotype	Progeny genotype	Expected segregation	Observed segregation	Chi-square	P-value
EAC7H7	F 1600	A		57 07	50 57 (77 70)	1:1:2	5:10:6	3.6	0.165
EAC7H7	F 1700	A		57 00	50 70	1:1	18:16:3	0	1.000
EAC7H7	F 1800	A		57 46	45 56 47 67	1:1:1:1	2:13:9:11	7.8	0.050
EAC7H7	F 1900	A		57 50	(50 55)57 70	2:1:1	18:4:8	1.4	0.237
EAC7H7	F 2000	A		57 55	55 57	1:1	17:14	0.2	0.655
EAC7H7	F 2100	A		57 57	55 57 77	1:2:1	3:7:2	0.5	0.779
EATC1B2	F 1500	A		34 33	33 34	1:1	21:14	1.4	0.237
EATC1B2	F 1600	A		34 33	33 34	1:1	12:7	1.3	0.254
EATC1B2	F 1700	A		34 33	33 34	1:1	21:16	0.6	0.439
EATC1B2	F 1800	A		34 33	33 34	1:1	24:13	3.2	0.074
EATC1B2	F 1900	A		34 33	33 34	1:1	22:13	2.3	0.129
EATC1B2	F 2000	A		34 33	33 34	1:1	28:8	11	0.001
EATC1B2	F 2100	A		34 33	33 34	1:1	7:5	0.3	0.584
EATC2C1	F 1500	A		37 36	33 36 37 67	1:1:1:1	14:10:5:6	5.8	0.122
EATC2C1	F 1600	A		37 46	34 36 47 67	1:1:1:1	9:7:4:3: 1	3.5	0.321
EATC2C1	F 1700	A		37 34	33 34 37 47	1:1:1:1	14:9:5:9	4.4	0.221
EATC2C1	F 1900	A		37 34	33 34 37 47	1:1:1:1	11:9:7:8	0.9	0.825
EATC2C1	F 2000	A		37 34	33 34 37 47	1:1:1:1	8:10:7:9	0.6	0.896
EATC2C1	F 2100	A		37 34	33 34 37 47	1:1:1:1	9:10:9: 5	1.8	0.615
L2AGH1	F 1600	A		68 57	56 67 58 78	1:1:1:1	3:9:7:3	4.9	0.179
L3B3A1	F 1500	A		22 23	22 23	1:1	17:17	0	1.000
L3B3A1	F 1700	A		22 12	12 22	1:1	20:15	0.7	0.403
L3B3A1	F 1800	A		22 12	12 22	1:1	21:15	1	0.317
SPAC1H8	F 1600	A		33 05	35 03	1:1	10:13	0.3	0.584
SPAC1H8	F 1800	A		33 24	23 34	1:1	20:15:3	0.7	0.403
SPAGC1	F 1500	A		23 55	25 35	1:1	14:20	1	0.317
SPAGC1	F 1600	A		23 55	25 35	1:1	14:10	0.7	0.403
SPAGC1	F 1700	A		23 55	25 35	1:1	21:15	1	0.317
SPAGC1	F 1800	A		23 25	22 25 23 35	1:1:1:1	6:9:12:9:1	2	0.572
SPAGC1	F 1900	A		23 55	25 35	1:1	11:22:1	3.6	0.058
SPAGC1	F 2000	A		23 26	22 26 23 36	1:1:1:1	7:8:10:10:1	0.7	0.873
SPAGC1	F 2100	A		23 55	25 35	1:1	15:20	0.7	0.403
SPAGC2	F 1500	A		10 20	12 10 20 00	1:1:1:1	8:11:11:5	2.8	0.423
SPAGC2	F 1700	A		10 40	14 10 40 00	1:1:1:1	10:7:15:5	6.1	0.107
SPAGC2	F 1800	A		10 01	(10 11 10) 00	3:1	26:8	0.9	0.343
SPAGC2	F 2000	A		10 20	12 10 20 00	1:1:1:1	13:12:5:6	5.5	0.139
SPAGD1	F 1500	A		4013	10 30 14 34	1:1:1:1	11:9:8:7	0.9	0.825
SPAGD1	F 1700	A		40 23	20 30 24 34	1:1:1:1	12:6:7:12	3	0.392
SPAGG3	F 1500	A		34 34	33 34 44	1:2:1	12:15:8	1.6	0.449
SPAGG3	F 1600	A		34 35	33 35 34 45	1:1:1:1	5:10:3:6	4.3	0.231
SPAGG3	F 1700	A		34 23	23 33 24 34	1:1:1:1	12:12:7:6	3.3	0.348
SPAGG3	F 1800	A		34 45	34 35 44 45	1:1:1:1	8:13:11:5	3.9	0.272
SPAGG3	F 1900	A		34 44	34 44	1:1	18:15	0.2	0.655
SPAGG3	F 2000	A		34 23	23 33 24 34	1:1:1:1	11:13:7:5	4.4	0.221
SPAGG3	F 2100	A		34 44	34 44	1:1	21:14	1.4	0.237
SPAGH1	F 2000	A		10 10	(11 10 10) 00	3:1	27:8	0.2	0.655
EAC1G5	F 1600		B	10 00	10 00	1:1	11:13	0.1	0.752
EAC1G5	F 1800		B	10 00	10 00	1:1	20:14	0.7	0.403
EAC1G5	F 1900		B	10 00	10 00	1:1	22:13	2.3	0.129
EAC1G5	F 2100		B	10 00	10 00	1:1	18:14	0.5	0.480
EAC2H8	F 1800		B	00 10	10 00	1:1	17:20	0.2	0.655
EAC6B1	F 1600		B	00 10	10 00	1:1	13:11	0.1	0.752
EAC6B2	F 1600		B	10 10	(11 10 01) 00	3:1	14:10	3.5	0.061
EAC6G3	F 1600	B		23 03	20 23 (30 33)	1:1:2	5:6:13	0.2	0.905
EAC6G8	F 1500		B	10 00	10 00	1:1	22:13	2.3	0.129
EAC7C11	F 1700		B	00 10	10 00	1:1	16:21	0.6	0.439
EAC7D10	F 2100		B	10 00	10 00	1:1	19:16	0.2	0.655
EAC7H7	F 1500	B		57 57	55 57 77	1:2:1	7:11:8	0.6	0.741
EAC7H7	F 1600	B		57 50	(50 55) 57 70	2:1:1	9:9:6	2.2	0.333
EAC7H7	F 1700	B		57 50	(50 55) 57 70	2:1:1	18:7:8	0.3	0.861
EAC7H7	F 1800	B		57 46	45 56 47 67	1:1:1:1	14:12:4:6	7.5	0.058

Table 1. (*Continued*)

Primer	Family No.	Codominant	Dominant	Parent genotype	Progeny genotype	Expected segregation	Observed segregation	Chi-square	P-value
EAC7H7	F 1900	B		57 07	50 57 77 70	1:1:2	9:5:16	0.6	0.741
EAC7H7	F 2000	B		57 88	58 78	1:1	16:17	0.03	0.862
EAC7H7	F 2100	B		57 57	55 57 77	1:2:1	2:8:2	1.3	0.254
L2AGH1	F 1900		B	10 10	(10 11 10) 00	3:1	21:10	0.8	0.371
L2AGH1	F 2000		B	10 10	(10 11 10) 00	3:1	26:8	0.03	0.862
L2AGH1	F 2100		B	10 10	(10 11 10) 00	3:1	23:10	0.4	0.527
SPAGC2	F 1500		B	10 00	10 00	1:1	14:21	1.4	0.237
SPAGC2	F 1700		B	10 00	10 00	1:1	20:17	0.2	0.655
SPAGC2	F 1800		B	10 00	10 00	1:1	19:18	0	1.000
SPAGC2	F 2000		B	10 10	(11 10 10) 00	3:1	24:12	1.3	0.254
SPAGH1	F 2000	B		12 13	11 13 12 23	1:1:1:1	6:12:10:7	2.6	0.457
EAC7D10	F 2100		C	00 10	10 00	1:1	19:16	0.2	0.655

of genotypes (22), (23), (26), and (36) (Fig. 1D). In Fig. 1B, an unexpected genotype (55), and in Fig. 1D genotype (44), appeared as illegitimate individuals. The illegitimate individuals are marked by a capital letter (I) in Fig. 1. For locus SPAGC1, the pattern of variation in different crosses was compatible with simple Mendelian inheritance.

For many codominant SSRs we found a null allele segregating in the progenies. Such alleles are not amplified and therefore in heterozygous individuals the presence of alternative alleles is detected on the gel. Codominant microsatellites that appeared with at least one null allele in one or both of the parents were found for loci EAC2H7, EAC6C02, EAC6F5, EAC6G3, EAC7C11, EAC7E6, EAC7H7, SPAC-1H8, SPAGC2, SPAGD1, and SPAGH1 (Fig. 2).

For SSR, EAC7H7 (Table 1), two segregating codominant loci A and B were found with null alleles. For both loci, the female parent carried double bands, representing a heterozygote genotype with two alleles (57), while many male parents were heterozygous with one band and one null allele. Segregation patterns for these two loci are compatible with simple Mendelian inheritance. For locus A in two families F 1500 and F 1700 four illegitimate individuals were observed.

SSR locus SPAGC2 also consisted of two loci, one codominant locus with a null allele and one dominant locus with a null allele (Table 1). Locus A in F 1500 had a heterozygous female with genotype (10) and a heterozygous male with genotype (20). Both female and male carried one null allele each, which segregates in the progenies, giving 4 different genotype classes 12: 10: 20: 00 types. Locus B appeared as a dominant marker with the presence of a band in the heterozygous female (10), and absence of band (null allele) in the homozygous male (00). The pattern of segregation for both codominant locus A and dominant locus B followed Mendelian inheritance.

All dominant markers with the presence of band (1) or absence of band (0) in parents with crosses homozygote × heterozygote or heterozyote × heterozyote segregated in the 1:1 or 1:3 ratios. The frequency of null alleles differed for different codominant SSR loci. There were 22 null alleles for 29 codominant loci in 8 clones and 97 crosses. For dominant SSR, there were either heterozygous genotypes (10) carrying null alleles or homozygous genotypes (00).

DISCUSSION

An inheritance study of microsatellite SSR is a fundamental prerequisite for using these molecular markers in genetic studies. The foremost aim of this investigation was to find a large number of polymorphic and highly repeatable microsatellite markers for mapping QTL in full-sib families and population studies of *Picea abies*.

Except for a few crosses, the pattern of variation for all polymorphic SSRs was in accordance with Mendelian inheritance (Table 1). For some microsatellites such as SpAGC1, SpAGD1, SpAGC2, SpAGG3, and SpAC1H8, Mendelian inheritance has already been reported for single loci by using megagametophytes from a few heterozygote trees (PFEIFFER et al. 1997).

For many markers only one family was studied. It is easy to understand that if two alleles appear in the cross, one being a null allele, there is a dominant/recessive situation. Even when the number of families are too few, the evaluation of marker loci as codominant or dominant may become difficult. This is true if the marker in both parents of one family appear to be heterozygote with one band and one null allele each. In this case it is difficult to asign the marker as

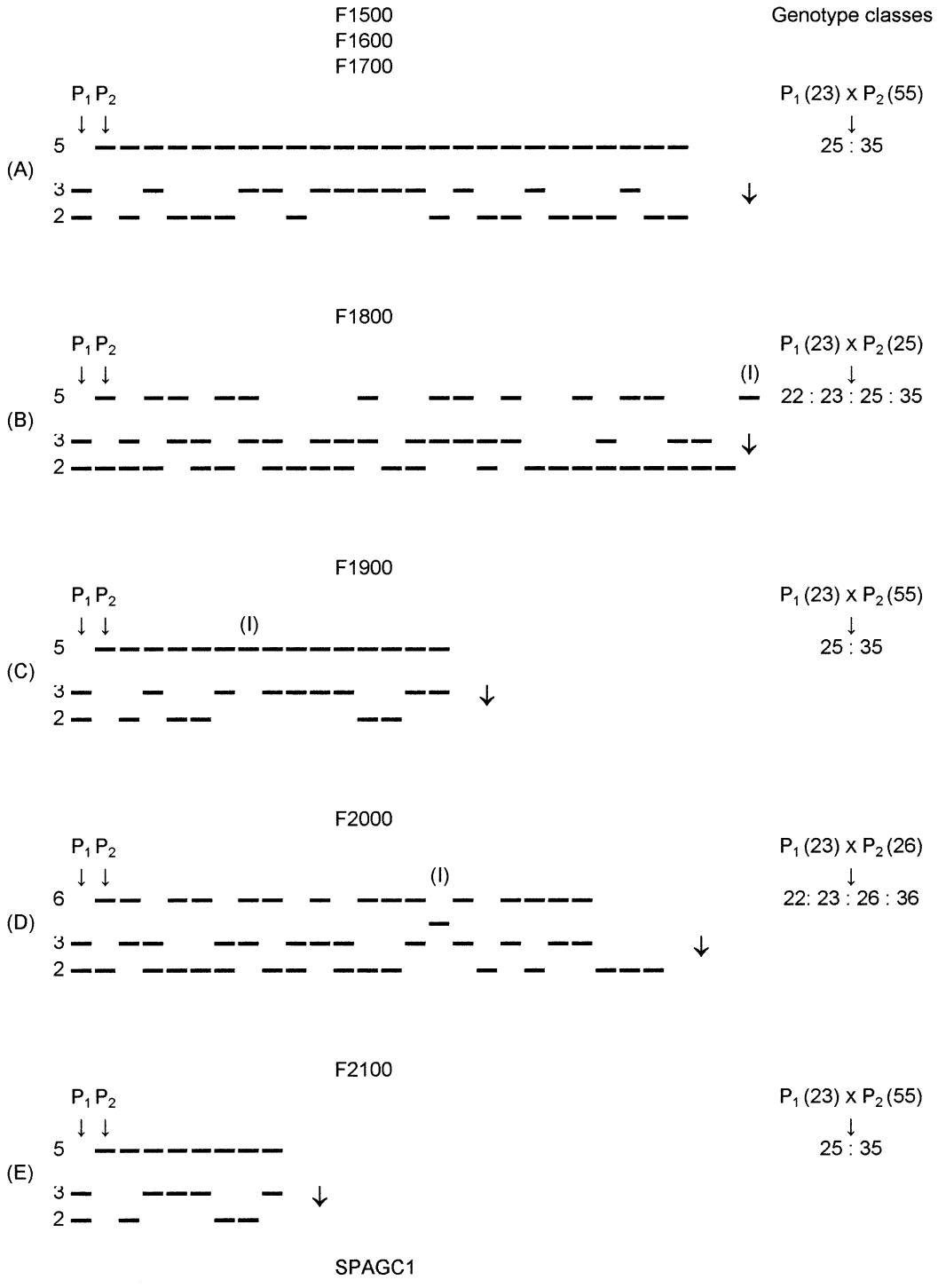

Fig. 1. Inheritance and segregation pattern of alleles are demonstrated for loci SPAGC1 in the parent and progeny of the mapping pedigree. In all crosses, a common female clone N2022 and seven male clones N2024, E2006, N2018, M1001, M2013, M1001 and M2013 with family designations F 1500, F 1600, F 1700, F 1800, F 1900, F 2000, and F 2100 were used. Lanes 1 and 2 are always the two parents P1 as female and P2 as male, followed by several F1 progenies. Arrows indicate the direction of migration on the gel. Genotype of the parents and progenies for each SSRs and families are demonstrated and illegitimate individuals are indicated as (I).

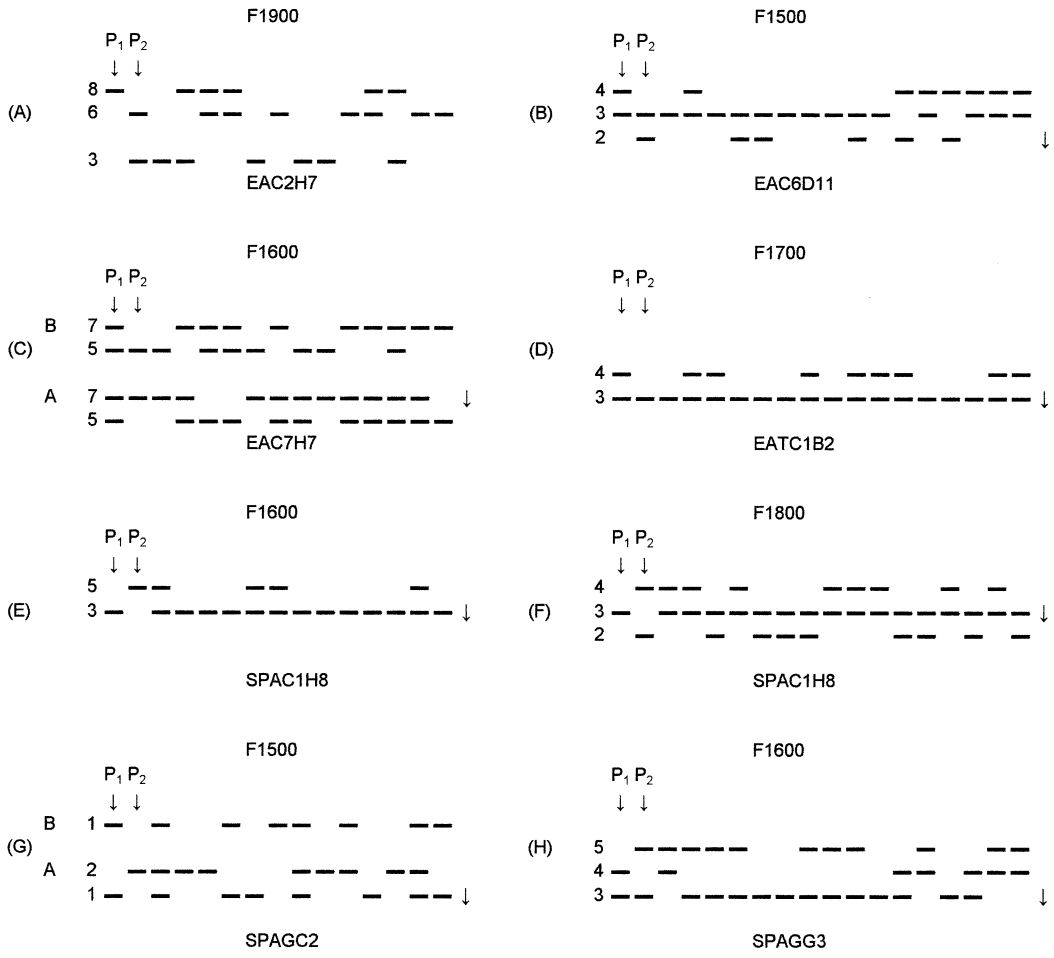

Fig. 2. Inheritance and segregation pattern of alleles are demonstrated for loci EAC2H7, EAC6D11, EAC7H7, EATC1B2, SPAC1H8, SPAGC2, and SPAGG3 in the parent and progeny of the mapping pedigree. In all crosses, a common female clone N2022 and seven male clones N2024, E2006, N2018, M1001, M2013, M1001 and M2013 with family designations F 1500, F 1600, F 1700, F 1800, F 1900, F 2000, and F 2100 were used. Lanes 1 and 2 are always the two parents P1 as female and P2 as male, followed by several F1 progenies. Arrows indicate the direction of migration on the gel. Genotype of the parents and progenies for each SSRs and families are demonstrated and illegitimate individuals are indicated as (I).

one codominant or two dominant loci unless more families are investigated.

A high proportion of dominant microsatellites (close to 50 %) were found in this study. Microsatellites are always described as generally codominant markers. A higher proportion of codominant SSRs with null alleles were found here than commonly reported. Null alleles may result from the mutation in the binding region of the microsatellite primers which may inhibit the amplification reaction and cause a loss of the PCR product.

One explanation for the high accumulation of null alleles found for microsatellites could be due to the fact that microsatellite mostly originate from non-coding regions of the DNA with no functional importance. Mutations in coding genes are more frequently lethal and are more often eliminated from the population.

SSRs consist of multiple tandem copies of mono-, di-, tri-, or tetra nucleotide repeat units, distributed throughout genomes. The frequencies at which changes in repeat number occur at microsatellite loci are much higher than normal mutation rates, ranging from 10^2 to 10^5 per generation (EDWARDS et al. 1992; DI RIENZO et al. 1993). Size differences in a microsatellite of *Pinus sylvestris* were caused by variations in the number of repeats as well as by insertion or deletion and duplication events in the flanking sequences (KOSTIA et al. 1995; KARHU et al. 2000).

In pine species, AC and AG repeats were the most abundant microsatellites found (SMITH and DEVEY 1994; ECHT and MARQUARDT 1997). The presence of

dinucleotide AC and AG repeats in the genome of *Picea abies* was investigated by PFEIFFER et al. (1997). On average, they found one AG repeat microsatellite every 194 kb and one AC repeat microsatellite every 406 kb, corresponding to a total number of 7.4×10^4 AC regions and 1.55×10^4 AG regions in the spruce genome (GOVINDERAJU and CULLIS 1991).

Microsatellites are conserved in many plant species (LAGERCRANTZ et al. 1993; KRESOVICH et al. 1995). As a result of genomic conservation in spruce, primer pairs for many SSR loci in *Picea glauca*, *Picea engelmanni*, *Picea sitchensis*, *Picea mariana*, *Picea rubens* and *Picea abies* successfully amplified specific fragments from genomic DNA and resolved polymorphic microsatellites (RAJORA et al. 2001). Genomic conservation of microsatellites has utility for comparative mapping study among species.

Conclusion

The high proportion of microsatellite codominant loci with null alleles and high frequency of dominant markers with null alleles found may represent two important limitations for use of these markers in studies of population genetics.

For many codominant SSR microsatellites, highly polymorphic patterns with simple mendelian inheritance are found in *Picea abies*. These markers are useful for applications such as multilocus estimation of outcrossing rates in seed orchards and natural stands, inbreeding studies, quality control of controlled crosses, paternity identification, control of artificial pollination, pollen contamination, gene dispersion, marker-trait association and comparative mapping analysis.

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