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ORIGINAL PAPER

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A full saturated linkage map of *Picea abies* including AFLP, SSR, ESTP, 5S rDNA and morphological markers

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Abstract Based on an F_1 progeny of 73 individuals, two parental maps were constructed according to the double pseudo-test cross strategy. The paternal map contained 16 linkage groups for a total genetic length of 1,792 cM. The maternal map covered 1,920 cM, and consisted of 12 linkage groups. These parental maps were then integrated using 66 intercross markers. The resulting consensus map covered 2,035 cM and included 755 markers (661 AFLPs, 74 SSRs, 18 ESTPs, the 5S rDNA and the early cone formation trait) on 12 linkage groups, reflecting the haploid number of chromosomes of *Picea abies*. The average spacing between two adjacent markers was 2.6 cM. The presence of 39 of the SSR and/or ESTP markers from this consensus map on other published maps of different *Picea* and *Pinus* species allowed us to establish partial linkage group homologies across three *P. abies* maps (up to five common markers per linkage group). This first saturated linkage map of *P. abies* could be therefore used as a support for developing comparative genome mapping in conifers.

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Introduction

Recently, molecular marker technologies, especially those based on the polymerase chain reaction (PCR), have been increasingly used in a variety of applications, including population genetics, QTL (quantitative trait loci) detection and marker-assisted-selection. These markers have also been used to construct linkage maps, i.e. genome representations in which loci involved in the control of traits and functions can be integrated and positioned. Genetic mapping can be therefore considered complementary to the ongoing research programmes based on ESTs (expressed sequence tags) and functional genomics. Moreover, using appropriate anchor points, linkage maps of related species can be aligned (comparative mapping) providing relevant information for understanding genomic organisation and evolution.

Norway spruce [*Picea abies* (L.) Karst.] is one of the major conifer species of Europe, where it plays an important role in the forest ecosystems of continental plains and medium-height mountains. It covers a wide natural distribution area, stretching from the western Alps to the Ural and from Scandinavia to Greece. In addition, due to its commercial importance and adaptability, it has been massively used for reforestation and is presently considered naturalised in large areas of Western Europe (Belgium, Germany, central France) and North America (south-eastern Canada and north-eastern USA).

Several incomplete genetic maps of Norway spruce consisting of 17–29 linkage groups have been constructed using RAPD (random amplified polymorphic DNA), AFLP (amplified fragment length polymorphism) and SSR (single sequence repeat, microsatellite) markers (Binelli and Bucci 1994; Bucci et al. 1997; Paglia et al. 1998; Skov and Wellendorf 1998; Troggio et al. 2001). All exceed the haploid chromosome number of the species (n=12) and were established from segregation analysis in the haploid maternal tissue of seeds (megagametophyte). Using this megagametophyte approach allows the avoidance of problems in genotype determination due to marker dominance, but also has disadvan-

tages such as the low quantity of extracted DNA and restriction to maternal effects in QTL detection (Plomion et al. 1997; Chagné et al. 2002). Another partial linkage map, established with AFLP, SSR and ESTP markers, can be consulted on the web site http://www.pierroton.inra.fr/genetics/Picea/.

In the present study, we report the construction of the first saturated linkage map in *Picea abies*. We used the double pseudo-test cross strategy consisting of the construction of two parental linkage maps which were then integrated into a consensus map (Ritter et al. 1990; Grattapaglia and Sederoff 1994). As in most conifer species, the nuclear genome of *P. abies* is very large (37.2 pg/2C, Siljak-Yakovlev et al. 2002) and contains a high proportion of repetitive sequences, hampering the construction of complete genetic maps. For the building of parental maps, we chose therefore a set of AFLP, SSR and ESTP markers expected to ensure good genome coverage and provide anchor points for genome comparisons.

The AFLP method (Vos et al. 1995) yields large numbers of markers well adapted to genome coverage. This type of marker has been shown to be appropriate to saturate genetic maps in species with large genomes such as *Pinus pinaster* (Chagné et al. 2002; Ritter et al. 2002) or *P. taeda* (Remington et al. 1999). However, despite some exceptions, they usually behave as dominant markers and this hinders genotype determination in sporophytic tissues.

Microsatellite markers are considered useful for the construction of high-density maps due to their high polymorphism levels, their co-dominant character, their abundance and wide distribution over the genome. In addition, SSR markers generally display good transferability from one species to another within the same genus (Rajora et al. 2001; Hodgetts et al. 2001; Shepherd et al. 2002) and can be thus used as convenient anchor points in the construction of intraspecific and interspecific consensus maps.

ESTP (expressed sequence tag polymorphism) markers are generated by PCR-amplification with primers designed from cDNA sequences. They usually display less polymorphism than SSRs or AFLPs and their analysis often requires time-consuming experimental approaches such as SSCP (single strand conformation polymorphism) or DGGE (denaturing gradient gel electrophoresis) analysis. However, they have proven to be transferable between species (Perry and Bousquet 1998a; Brown et al. 2001) and, to some extent, between genera (Brown et al. 2001).

Additionally, as a useful complement to assign linkage groups to chromosomes, the nuclear 5S ribosomal DNA which has been shown to be located at a single locus on the long arm of the large metacentric chromosome pair 2 (Lubaretz et al. 1996), was included in this analysis.

Materials and methods

Plant materials

The mapping population consisted of 73 F_1 individuals of an outbred full-sib family. The female parent (TH787F) was an *acrocona* mutant characterised by a dwarf phenotype and early cone formation on main shoots and branches (Fladung et al. 1999). The male parent (Sire5) exhibited the *aurea* character (yellow flushing shoots). The controlled cross was made in 1995 in the experimental field of the Institute for Forest Genetics and Forest Tree Breeding, Grosshansdorf, Germany.

AFLP procedure

Total genomic DNA was isolated from fresh needles using the protocol of Dumolin et al. (1995). Restriction digests and ligation were performed by using the AFLP Core Reagent Kit (Invitrogen, Life Technologies) with 125 ng of DNA. Preamplification was carried out with standard *Eco*RI (E) and *Mse*I (M) adaptors with one, two or three additional nucleotides in a 50 μl reaction volume containing 10 mM Tris-HCl (pH 8.4), 50 mM KCl, 0.2 mM of each dNTP, 0.4 mM of each primer, 2.5 mM $MgCl_2$, 0.75 U *Taq* DNA polymerase (Invitrogen) and 3 μl DNA ligation mixture diluted 10-fold. For selective amplification, 46 primer-enzyme combinations with three to five selective nucleotides were tested in total (Table 1). The reaction mixture (20 μl) was prepared as described above for preamplification except that 5 μl of a 1:5 dilution of the preamplification mixture was used as template with 0.16 mM IRD 800-labelled *Eco*RI primer (MWG) and 0.5 mM standard *Mse*I primer. Thermal cycling conditions for preamplification and selective amplification were as described by Remington et al. (1999). AFLP fragments were resolved on denaturing gels composed of 8% Long Ranger acrylamide (TEBU), 7 M urea and 1× TBE buffer (134 mM Tris, 45 mM boric acid, 2.5 mM EDTA). Electrophoresis was carried out on a Li-Cor automated sequencer (model 4000 L) using 1× TBE running buffer, with run parameters of 1,500 V, 35 mA, 31.5 W, 50°C plate temperature. Polymorphic fragments were scored visually directly on the TIFF image files. Fragment sizes were estimated using the GeneImageIR software v 3.0.

SSR assays

Seventy-eight primer pairs designed for amplification of SSR loci in different *Picea* species, namely *P. abies* (Pfeiffer et al. 1997; Scotti et al. 2000, 2002a, 2002b; Besnard et al. 2003), *P. glauca* (Hodgetts et al. 2001; Rajora et al. 2001; Besnard et al. 2003) and *P. rubens* (Besnard et al. 2003), were analysed in total. Several of these SSR loci had already been mapped in *P. abies* (Paglia et al. 1998; http://www.pierroton.inra.fr/genetics/Picea/). PCR amplifications were carried out in 25 μl containing 10 mM TrisHCl (pH 8.4), 50 mM KCl, 0.2 mM of each dNTP and 0.2 mM of each primer (forward primer labelled with IRD 800), 2.5–5 mM $MgCl_2$, 0.75 U *Taq* DNA polymerase (Invitrogen) and 50 ng of genomic DNA. Thermal cycling was performed in a BioRad icycler as follows: 4 min initial denaturation at 94°C, 35 cycles consisting of a 45 s denaturation at 94°C, a 45 s annealing at temperatures ranging from 48 to 58°C depending on the primer pair, and a 45 s extension at 72°C, before a 10 min final extension at 72°C. Amplification products were electrophoresed under the same conditions used for the AFLP analysis in 6.5% denaturing polyacrylamide gels. Polymorphism was screened visually and markers were scored as either co-dominant (segregation 1:1:1:1) or dominant (segregations 1:1 or 3:1).

ESTP and 5S rDNA analysis

A set of 54 EST primer pairs derived from cDNA sequences of *Pinus taeda* (Harry et al. 1998; Brown et al. 2001; Temesgen et al.

Table 1 The AFLP primer enzyme combinations (PECs) used for the pre-amplification step in italics, the number and size of the polymorphic fragments detected after amplification, and their segregation ratios.

	Primer:enzyme combinations	Total number of polymorphic bands	Size of fragments (bp)	Markers segregating 1:1	Markers segregating 3:1	Distorted markers (P <0.05)
a1	*E.ACA*/*M.CC*GG	31	60–449	25	4	2
a2	*E.ACA*/*M.CC*AG	24	55–329	21	1	2
a3	*E.ACA*/*M.CC*CG	26	68–466	24	1	1
a4	*E.ACA*/*M.CC*GC	25	67–619	23	2	–
a5	*E.ACA*/*M.CC*CA	9	120–269	7	–	2
a6	*E.ACA*/*M.CC*AC	13	61–231	12	–	1
a7	*E.ACA*/*M.CC*TG	37	64–408	28	7	2
a8	*E.ACG*/*M.CC*CA	21	60–410	19	1	1
a9	*E.ACG*/*M.CC*GT	25	68–440	21	4	–
a10	*E.ACG*/*M.CC*GC	28	72–700	24	2	2
a11	*E.ACG*/*M.CC*AT	11	55–378	8	2	1
a12	*E.ACG*/*M.CC*AA	11	101–254	7	1	3
a13	*E.ACG*/*M.CC*AC	37	57–607	30	5	2
a14	*E.ACG*/*M.CC*AG	18	66–500	16	1	1
a15	*E.ACG*/*M.CC*TC	17	60–303	11	6	–
a16	*E.ACG*/*M.CC*TG	17	62–220	13	4	–
a17	*E.ACG*/*M.CC*TA	12	83–252	8	1	3
a18	*E.ACC*/*M.CC*GT	19	69–255	12	5	2
a19	*E.ACC*/*M.CC*TG	6	98–314	6	–	–
a20	*E.ACC*/*M.CC*AG	12	61–169	11	1	–
a21	*E.ACT*/*M.CC*AG	15	62–333	15	–	–
a22	*E.ACT*/*M.CC*CG	34	65–402	29	4	1
a23	*E.ACT*/*M.CC*GG	15	86–289	13	2	–
a24	*E.ACT*/*M.CC*TG	23	76–462	22	–	1
a25	*E.ACT*/*M.CC*GT	14	130–342	14	–	–
a26	*E.ACT*/*M.CC*GC	10	60–231	8	1	1
a27	*E.ACT*/*M.CC*TC	14	62–323	13	1	–
a28	*E.ACT*/*M.CC*TA	17	61–348	15	1	1
a29	*E.TG*GC/*M.CG*TC	13	81–277	9	1	3
a30	*E.TG*GC/*M.CG*GT	10	89–335	7	2	1
a31	*E.TG*GC/*M.CG*AT	14	90–346	12	2	–
a32	*E.TG*GC/*M.CG*CG	8	55–282	7	1	–
a33	*E.TG*GC/*M.CC*GG	12	84–318	9	2	1
a34	*E.TG*GC/*M.CC*AA	4	86–149	2	–	2
a35	*E.TG*AC/*M.CG*AT	12	67–286	12	–	–
a36	*E.TG*AC/*M.CG*TC	7	77–211	5	–	2
a37	*E.TG*AC/*M.CG*GT	6	80–242	5	1	–
a38	*E.AG*CT/*M.AG*CT	23	102–457	18	5	–
a39	*E.AG*CT/*M.AG*CG	18	83–392	16	–	2
a40	*E.AG*CG/*M.A*AACT	12	105–367	11	1	–
a41	*E.AAA*CT/*M.AG*CG	23	101–369	20	3	–
a42	*E.AAA*CT/*M.AG*CT	12	73–278	10	–	2
a43	*E.AAA*CA/*M.AG*CG	22	63–413	19	1	2
a44	*E.AAA*CA/*M.AG*CT	17	72–235	16	1	–
a45	*E.AAACC*/*M.AG*CG	26	58–402	23	1	2
a46	*E.AAACC*/*M.AG*CT	11	87–248	9	1	1
Total		791	55–700	665	79	47

2001), *P. pinaster* (Plomion et al. 1999; Chagné et al. 2003), *P. sylvestris* , *P. banksiana*, *Abies grandis*, *Picea abies* (Schubert et al. 2001) and *P. mariana* (Perry and Bousquet 1998b) were tested. For full details of the primers used see http://www.pierroton.inra.fr/genetics/pinus/primers.html. PCR amplifications were performed as indicated by the authors. Screening for polymorphisms was first carried out directly after electrophoresis of PCR products on agarose or non-denaturing acrylamide gels (8%). In the absence of detectable length polymorphisms, two other approaches were applied depending on the fragment sizes. When the fragment length was superior to 500 bp, PCR products were digested using a set of ten restriction enzymes with a 4-bp recognition site (*Taq*I, *Hpa*II, *Hha*I, *Nde*II, *Rsa*I, *Msp*I, *Alu*I, *Hae*III *Mbo*I and *Hinf*I) prior to electrophoresis (PCR-RFLP). When the fragment length was shorter than 500 bp, a SSCP analysis was performed. PCR fragments were resolved on non-denaturing acrylamide gels (8%) in 0.6× TBE buffer at 15°C for 7 h at constant power (20 W) and then visualised by silver staining according to Bodenes et al. (1996). Amplification of the spacer region of the nuclear 5S rDNA repeat units was carried out according to Trontin et al. (1999) using the P1 and P2 "universal" plant primers.

Linkage analysis

The data set included three different segregation patterns: 1:1 for heterozygous markers in one parent and homozygous or null in the other, 3:1 for dominant markers heterozygous in both parents, 1:1:1:1 for co-dominant multiallelic markers. For each marker, a chi-square test (P<0.05) was used to identify deviations from the expected Mendelian ratios. Linkage analysis was carried out using JoinMap v3.0 software (Van Ooijen and Voorrips 2001) with a minimal LOD of 4.0 and a maximum recombination fraction of 0.3 as the grouping criteria. Recombination rates were converted to

genetic distances in centiMorgans using Kosambi's mapping function (Kosambi 1944). The male and female parental maps were built up based on the 1:1 and 3:1 segregating markers. These maps were then aligned by using intercross markers (segregation ratios 3:1 and 1:1:1:1) and a consensus map integrating all the segregation data was constructed by using the "map integration" function. If the marker order was disturbed when using the integration algorithm, one parental linkage group was fixed as reference prior computing (the "fixed orders" command). Maps were drawn using the Mapchart version 2.0 software (Voorips 2002).

Analysis of marker distribution

Two different methods were used for the calculation of marker distribution on the consensus map. The first consisted of testing whether markers were randomly distributed using a Poisson distribution function $P(x)=e^{-\mu}\mu^x/x!$, where x is the number of markers per 10 cM interval and μ the average marker density in the map. The number of markers in each 10 cM interval was counted and the frequency of each class compared to the expected binomial frequencies by a chi-square test. This method has been previously applied to AFLP markers by Young et al. (1999), Remington et al. (1999), Cervera et al. (2001) and Yin et al. (2003). The second method was to calculate the Pearson correlation coefficient between the number of markers in the linkage groups and the size of the linkage groups as described by Cervera et al. (2001).

Results

AFLP markers

A total of 791 segregating fragments were scored from the 46 primer:enzyme combinations (PEC) analysed (Table 1). The number of polymorphic fragments per PEC ranged from 4 to 37 (17 on average) with sizes between 55 bp to greater than 700 bp. Of these markers, 84% (665) and 9.9% (79) segregated in the 1:1 and 3:1 ratios, respectively. Around 6% (47) showed significant distortion ($P<0.05$) from the expected Mendelian segregation ratios 1:1 (44) and 1:3 (3). The number of maternal markers (359) was significantly higher than the number of paternal markers (306).

Microsatellite markers

Out of the 78 SSR primer pairs tested, 65 (84%) yielded amplification products and 50 of them (including 14 producing a multilocus amplification pattern) generated a total of 80 polymorphic microsatellite markers. Interestingly, the percentage of polymorphisms was higher among the dinucleotide microsatellites (90%) compared to the trinucleotide microsatellites (36%). Of the 80 SSR markers detected, 27 were heterozygous in the female parent, 15 in the male parent, and 33 from both parents displayed segregation ratios 3:1 or 1:1:1:1. Five SSRs exhibited distorted segregation ($P<0.05$).

ESTP and 5S rDNA markers

Among the 54 EST primer pairs selected 41 generated amplification products. All primer pairs derived from *Picea* species amplified; however only seven (44%) were polymorphic. Amplification success was lower with the primer pairs derived from *Pinus* species (68%), but the percentage of polymorphisms reached 48% (12 polymorphic primer pairs). The unique primer pair derived from *Abies* did not amplify. Out of the 19 ESTP markers obtained, 15 were heterozygous in one parent and four in both (segregation ratios 3:1 or 1:1:1:1). Only one (SB 06) showed a distorted segregation ($P<0.01$). For eight markers, polymorphism was based on length variations detected on 2% agarose or 8% non-denaturing polyacrylamide gels and for the other markers, it probably relied on substitutions, which were only revealed after SSCP analysis.

Initially used in Larch species (*Larix decidua, L. kaempferi*), the "universal" plant primers used to amplify 5S rDNA also allowed successful amplification of the nuclear 5S rDNA in our *Picea abies* mapping population. One polymorphic fragment was produced, which was heterozygous in the female parent.

Morphological traits

Among the two morphological traits analysed (early cone formation and *aurea* factor), early cone formation segregated as a dominant marker with a 1:1 ratio, suggesting monogenic inheritance. In contrast, the *aurea* factor could not be mapped.

Parental maps

The female map consisted of 461 markers (389 AFLPs, 60 SSRs, 10 ESTPs, the 5S rDNA and the early cone formation trait) distributed on 12 linkage groups covering 1,920.8 cM with an average distance of 4 cM between two adjacent markers. Linkage groups were composed of 25–62 markers each and their sizes ranged from 127.5 to 209.2 cM (160 cM on average).

The male map included 360 markers (303 AFLPs, 45 SSRs and 12 ESTPs) assigned to 16 linkage groups, which covered 1,792 cM with an average distance of 4.9 cM between two adjacent markers. Linkage group sizes ranged from 94 to 210.9 cM (149.3 cM on average) and the number of markers per linkage group varied between 18 and 51. Both parental maps are shown on the website http://www.neiker.net/UHDfor/.

Consensus map

Homologous linkage groups were identified in the parental maps based on 66 intercross AFLP, SSR or ESTP markers (three to ten intercross markers per paired linkage

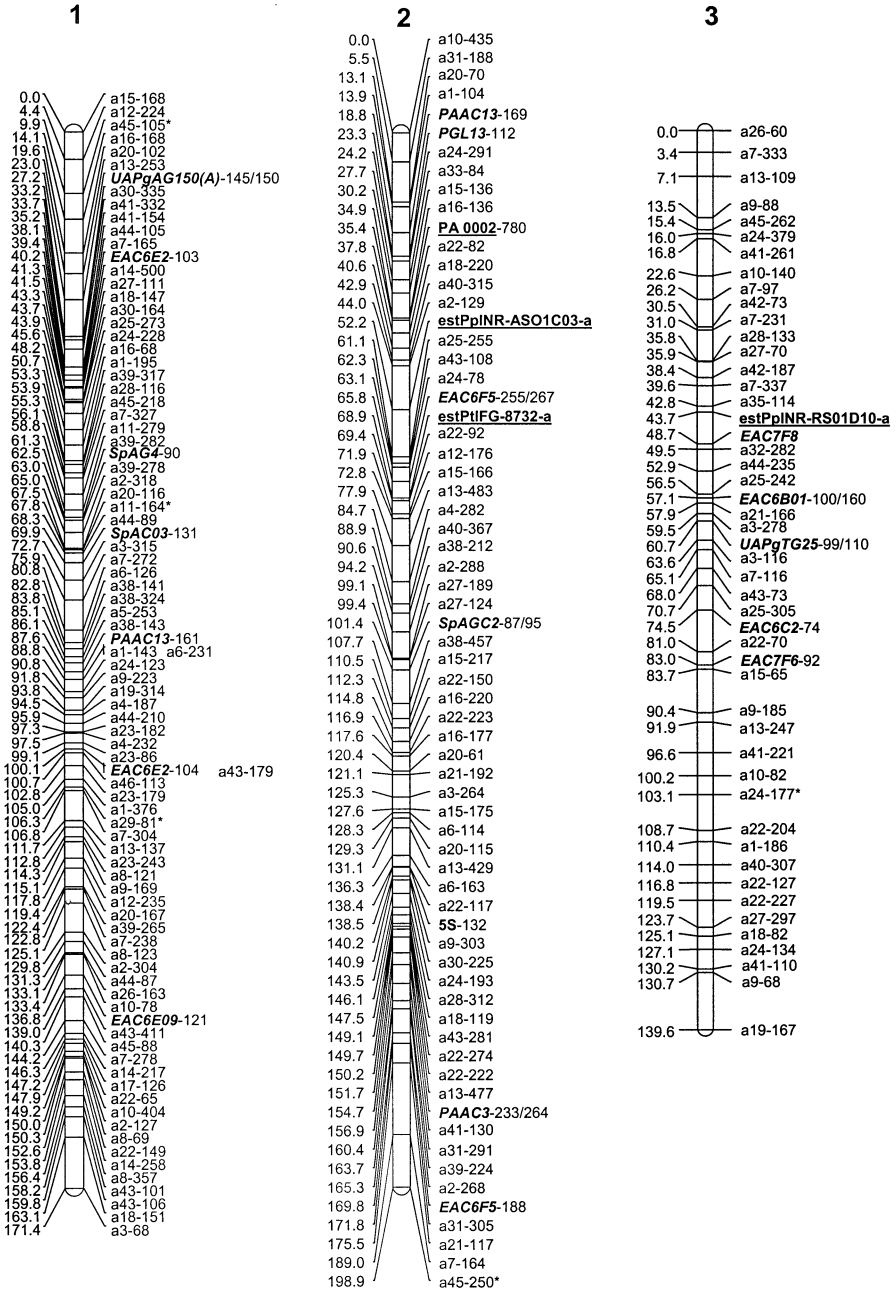


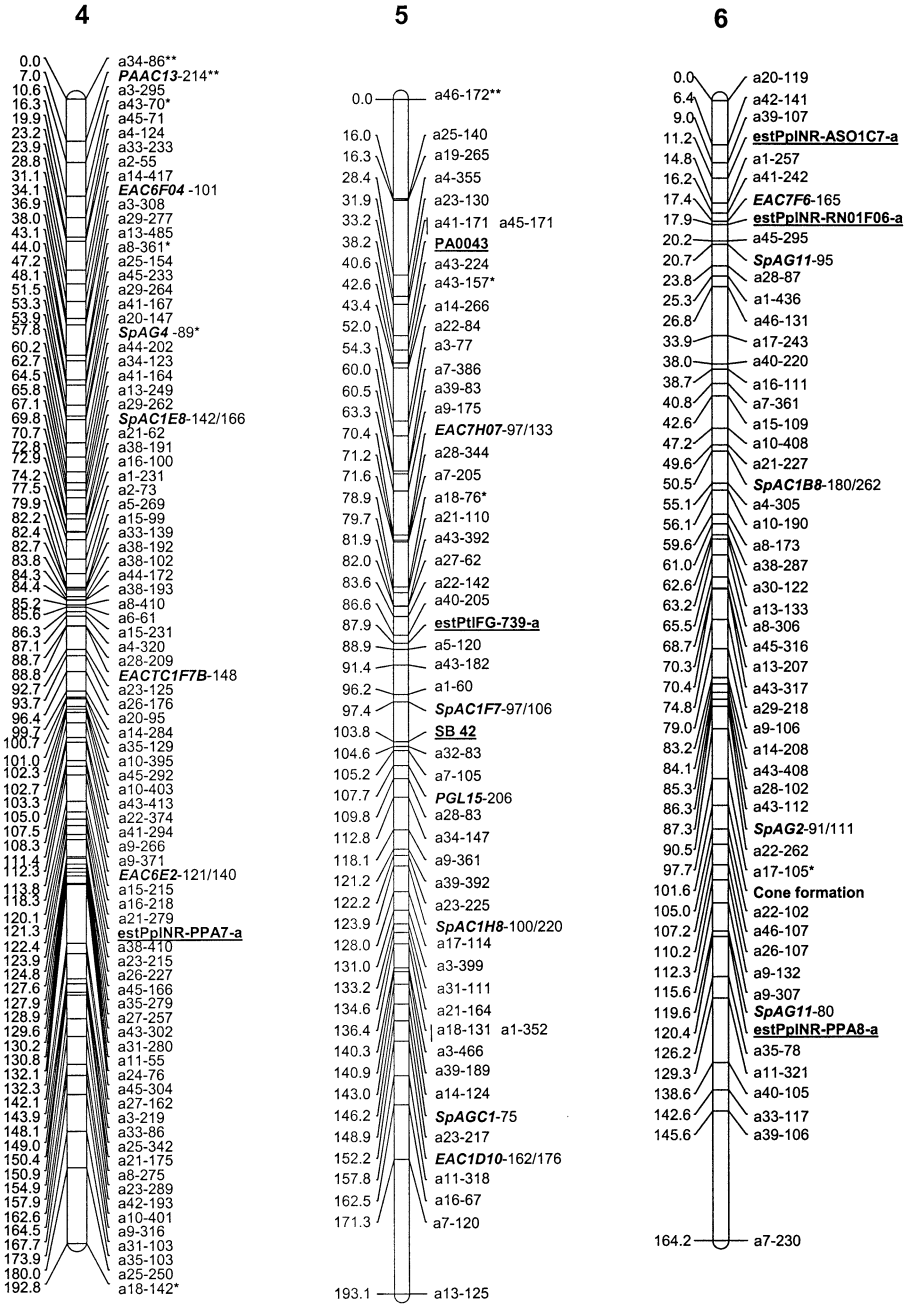
Fig. 1 Consensus linkage map of Norway spruce. Names of framework markers are indicated on the *right* of the linkage groups with the fragment size indicated in bp. Genetic distances, in cM, are indicated on the *left*. AFLP markers are named as in Table 1. SSR markers are designated by the primer pairs in *italics*. ESTP markers *(underlined)* were denoted according to the locus nomenclature of the Treegenes database (http://dendrome.ucdavis.edu/Tree_Page.htm) with the experiment reference omitted for simplification. For ESTPs developed by Schubert et al. (2001) and Perry and Bousquet (1998b) only the clone names were indicated (*PA* and *SB*, respectively) due to incomplete information. Markers showing distorted segregation are labeled with one or two *asterisks* depending on the level of significance ($P<0.05$ or $P<0.01$, respectively) of the χ^2 test

groups). The integrated corresponding data set allowed the construction of a consensus map composed of 755 markers (661 AFLPs, 74 SSRs, 18 ESTPs, the 5S rDNA and the early cone formation) assigned to 12 linkage groups (Fig. 1). The marker orders of the parental maps were not significantly disturbed, except on linkage group 1. The total length of this map was 2,035.2 cM with an average spacing of 2.6 cM between adjacent markers. The size of the linkage groups ranged from 143.1 to 198.9 cM (169.6 cM on average) and the number of markers per linkage group varied between 49 and 89. Thirty-five of the mapped markers showed a distorted segregation. The number of linkage groups of this consensus map corresponded to the haploid chromosome number of the species.

Distribution of markers

Figure 2 shows a comparison between the expected binomial frequencies calculated for an average marker density across the entire map of $\mu=3.25$ and the observed frequency data for each class of marker number per 10 cM interval. No significant deviation was observed ($\chi^2=8.94$; $df=9$; $P<0.05$) indicating that the AFLP markers were uniformly distributed. The significant Pearson's correlation ($r=0.53$ at the 1% level) observed between the

Fig. 1 (continued)

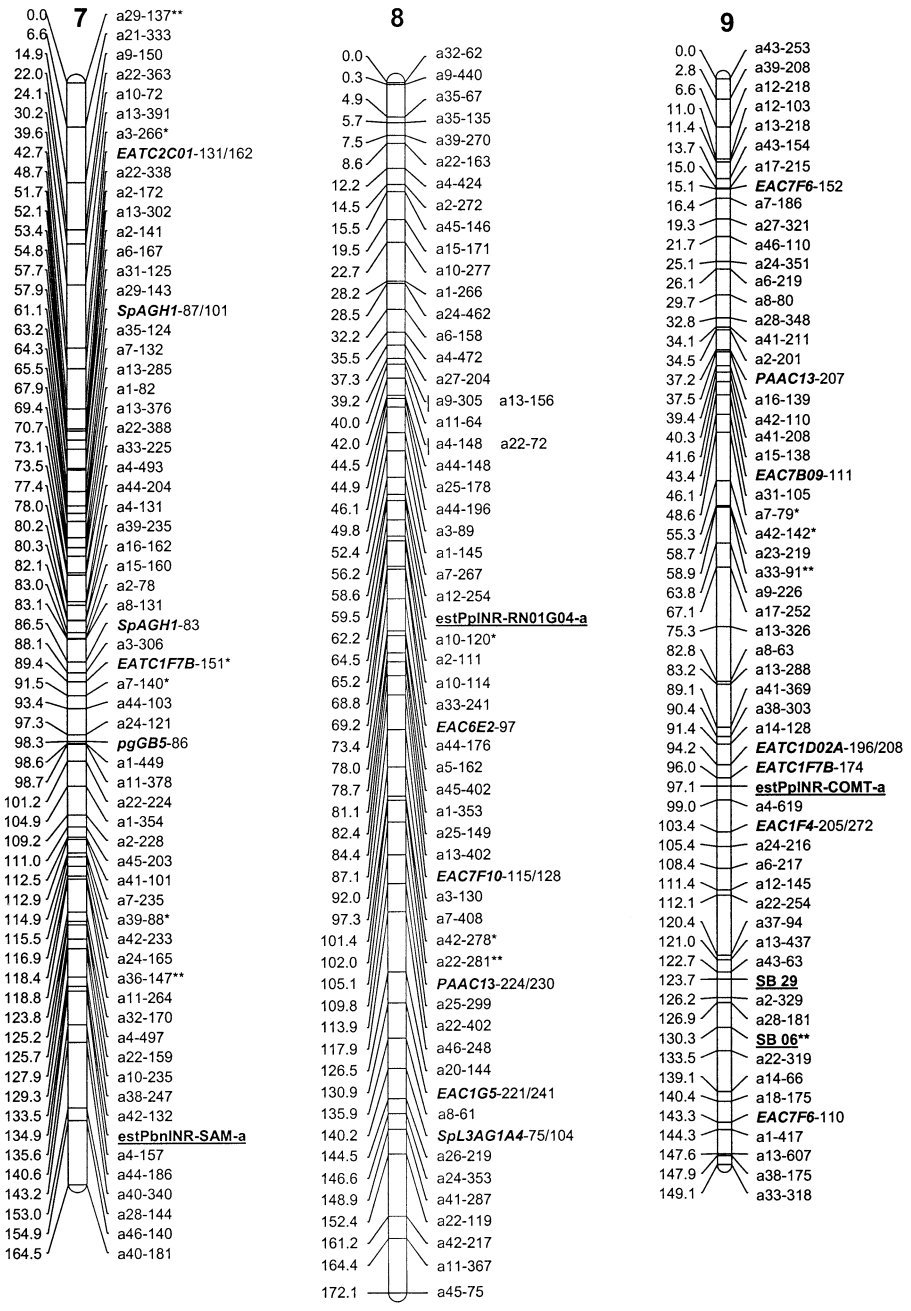


number of markers and the size of the linkage groups confirmed this indication. It was therefore concluded that the consensus map did not contain clusters of AFLP markers. For SSRs and, a fortiori, for ESTPs, application of this statistical analysis was not appropriate owing to insufficient number of markers per 10 cM interval (maximum 2). However a visual examination of the consensus map revealed an apparent random distribution of SSRs with at least five SSR markers present in each linkage group. In contrast, the distribution of ESTP markers seemed not to be uniform, ranging from one marker in linkage groups 3, 4, 7 and 8 to three in linkage groups 2, 5, 6 and 9. The 35 mapped markers which showed distorted segregations were mainly located at the end of the linkage groups (see Lg 1, 2, 4, 5, 7 and 11) and occasionally grouped into clusters (Lg 4 and 9).

Comparison with other published maps for conifers

Intraspecific comparison was made with the two other published maps of *Picea abies* including SSR and ESTP markers, i.e. the maps of Paglia et al. (1998) and of the website http://www.pierroton.inra.fr/genetics/Picea/. Table 2 shows that common SSR/ESTP markers were found between these maps and 11 out of the 12 linkage groups of the consensus map (1–5 common markers per linkage group). In general, a good conservation of the marker

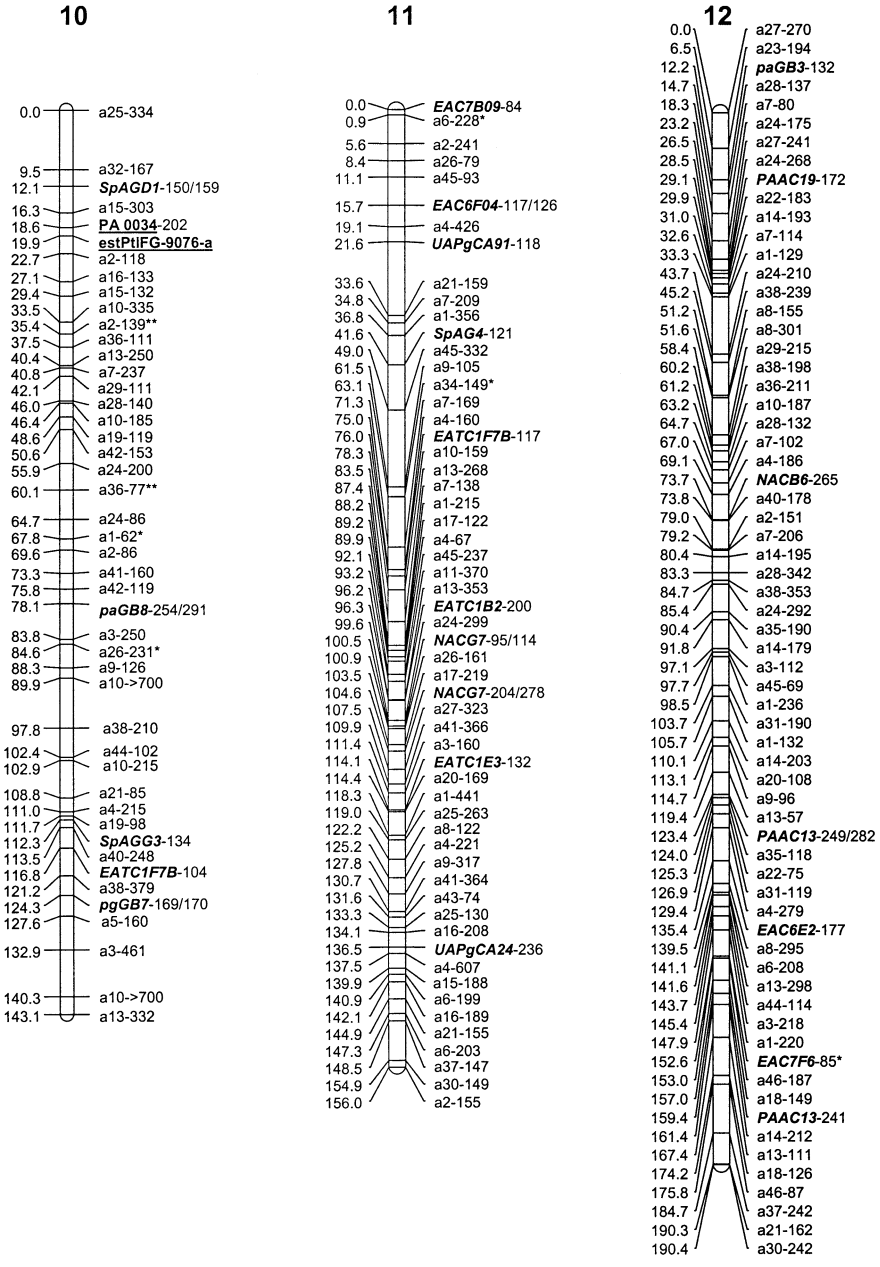
Fig. 1 (continued)



content and order was observed although there were some exceptions; for instance an inversion of EAC7F6 and estPtIFG-RN01F06-a in linkage group 6 of our saturated consensus map and linkage group 1 of the website map. Complete content and order conservation of markers was observed between three linkage groups of our saturated map and the website map. Interestingly, some independent linkage groups, for instance linkage groups G and T of the map of Paglia et al. (1998), could be assigned to a unique linkage group (Lg 8) of our consensus map, suggesting that they could be merged. Conversely, ESTP markers estPtIFG-9076-a and estPpINR-RN01G04-a which belonged to two different linkage groups in our consensus map (Lg 8 and 10) were located on the same linkage group (Lg 11) of the website map. These markers could possibly correspond to paralogous copies of gene families as observed in *Pinus pinaster, P. taeda* and *P. sylvestris* by Chagné et al. (2003) and Komulainen et al. (2003).

Possibilities for interspecific and intergeneric comparison within the Pinaceae were comparatively more limited. Seven ESTP markers mapped on our saturated consensus map were also present in other available maps of *Picea* (*P. glauca*) and *Pinus* (*P. pinaster, P. taeda*) species (Table 3). In most cases however, a unique common marker, insufficient to establish homology, was

Fig. 1 (continued)



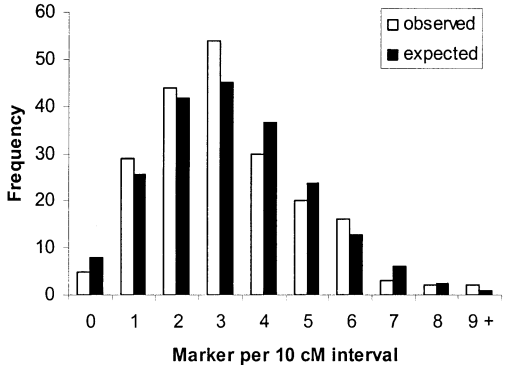


Fig. 2 Comparison of observed and expected frequencies of AFLP markers assuming a Poisson distribution function

found between two linkage groups of the different maps. Only ESTPs estPpINR-AS01C7-a and estPpINRA-AS01H04-a (or estPpINR-PPA8-a), both located on linkage group 6, allowed the establishment of correspondence with linkage group 10 of the *P. pinaster* map of the website.

Discussion

Marker analysis

The present study is the first report of a saturated linkage map for *Picea abies*. The integration of genetic informa-

Table 2 Microsatellite and ESTP markers of the saturated consensus map also present on the other *Picea abies* maps (Paglia et al. 1998; http://www.pierroton.inra.fr/genetics/Picea/, with indication of the corresponding linkage groups

Microsatellite/ESTP markers	Linkage groups		
	This study	Paglia et al. 1998	Website
SpAC03 [b]	1	–	2
EAC6E09 [b]	1	L	12
EstPpINR-ASO1C03-a[a]	2	–	8
EstPtIFG-8732-a[a]	2	–	8
SpAGC2 [b]	2	C	–
EstPpINR-RS01D10-a[a]	3	–	10
EAC7F8 [b]	3	H	10
EAC6B01 [b]	3	–	10
EAC7H7 [b]	5	D04	6
EstPtIFG-739-a[a]	5	–	6
EAC1F7 [b]	5	–	6
SpAC1H8 [b]	5	S	6
SpAGC1 [b]	5	D07	6
EAC1D10 [b]	5	D07	–
EAC7F6-165 [b]	6	–	1
EstPtIFG-RN01F06-a[a]	6	–	1
SpAC1B8 [b]	6	D	1
SpAG2 [b]	6	–	1
EATC2C01 [b]	7	–	15
SpAGH1-87/101 [b]	7	1	14
EstPbnINR-SAM-a[a]	7	–	20
EstPpINR-RNO1G04-a[a]	8	–	11
EAC6E2-97 [b]	8	G	–
EAC7F10 [b]	8	G	7
EAC1G5 [b]	8	T	7
SpL3AG1A4 [b]	8	T	–
EATC1D2 [b]	9	–	3
EstPpINR-COMT-a[a]	9	–	3
EAC1F4 [b]	9	K	3
SpAGD1 [b]	10	B	–
EstPtIFG-9076-a[a]	10	–	11
SpAGG3 [b]	10	B	4
EAC7B9-84 [b]	11	E	–
EATC1B2b	11	–	9
NACG7-204/278 [b]	11	E	–
NACB6 [b]	12	F	5

[a] ESTP marker
[b] Microsatellite marker

tion from two independent parental sources allowed us to build up a consensus genetic map consisting of 12 linkage groups corresponding to the haploid chromosome number of the species. As already pointed out by Remington et al. (1999), Chagné et al. (2002) and Ritter et al. (2002), AFLP technology was confirmed to be an efficient method to saturate genetic maps, especially for species with large genomes as is the case for *P. abies* and for conifers in general. In contrast to several authors (Remington et al. 1999; Young et al. 1999; Cervera et al. 2001; Yin et al. 2003), we did not detect any clustering of AFLP markers in any linkage groups.

Compared to AFLPs, the number of mapped SSR and ESTP markers was insufficient to contribute significantly to the saturation of the map. However, due to their specificity and co-dominance, SSR and ESTP markers were very useful for the integration of the parental maps. Primer availability for the amplification of SSR loci in conifer species still remains low in comparison to major crop species such as maize or rice (maize database, http://www.maizegdb.org; McCouch et al. 2002). Important efforts are currently in progress to reverse this situation (Plomion, personal communication) and the possibilities for using microsatellites in conifer species will undoubtedly increase in the near future. The SSR primer set used in this study yielded simple amplification patterns and generated a high proportion (around 80%) of single locus SSRs, as expected. The remaining 20% were complex multilocus amplification patterns, probably due to the high proportion of repetitive DNA that is characteristic of conifer genomes (Schmidt et al. 2000; Elsik and Williams 2001) in which microsatellite sequences can be embedded. As already noticed in Norway spruce (Scotti et al. 2002b), hard pines (Shepherd et al. 2002) and also in humans (Chakraborty et al. 1997), trinucleotide microsatellites were revealed to be less polymorphic than dinucleotides. This can be explained by differences in the mutation rate, which is inversely related to the length of repeats (Chakraborty et al. 1997).

In contrast to the SSRs, thousands of ESTs have been sequenced in conifers (http://dendrome.ucdavis.edu/Gen_res.htm) and can be a useful source of markers for genetic studies. However our results indicate that the amplification rate decreases when the primers used are derived from Pinaceae genera other than *Picea* (only 10–20% with *Pinus* primers), confirming that an increased phylogenetic distance reduces the amplification success (Perry and Bousquet 1998a, 1998b; Brown et al. 2001). After the amplification step, the level of polymorphism revealed in the mapping population is mainly dependent

Table 3 ESTP markers of the *Picea abies* saturated consensus map also present on the other *Picea* and *Pinus* maps published, with the corresponding linkage groups indicated. The linkage groups are numbered as reported by the authors

ESTP markers	Linkage groups	
	Picea abies map	Other *Picea* and *Pinus* maps
EstPtIF-8732-a	2	8, *P. taeda*; Brown et al. 2001
EstPpINR-PPA7/RSO1G05-a	4	1, *P. pinaster*;Chagné et al. 2003
EstPtIFG-739-a	5	6, *P. taeda*; Brown et al. 2001, Temesgen et al. 2001
EstPpINR-ASO1C7-a	6	10, *P. pinaster*; Chagné et al. 2003
EstPpINR-PPA8/AS01H04-a	6	10, *P. pinaster*; Chagné et al. 2003 5, *P. pinaster*; Ritter et al. 2002
SB29	9	6 from parent M2, *P. glauca* , 6 from parent 80132, *P. glauca*; Gosselin et al. 2002
EstPtIFG-9076-a	10	11, *P. taeda*; Temesgen et al. 2001

on the divergence between the parents, but is also strongly influenced by the detection method used. Resorting to methods allowing the detection of point mutations, such as SSCP or DGGE is generally necessary.

Map length

The length of our consensus map is smaller (2,035 cM) than those of the same species published by Binelli et al. (1994) and Paglia et al. (1998) which reached 3,584 and 2,193 cM respectively, despite a lower number of markers mapped (185 and 447 for these authors, 775 here). These differences in map length can be attributed to the calculation programme used for the construction of each map; Binelli et al. (1994) and Paglia et al. (1998) used MapMaker software, whereas we used JoinMap v3.0. Several authors (Sewell et al. 1999; Vuylsteke et al. 1999; Bradeen et al. 2001; Chagné et al. 2002; Gosselin et al. 2002) who used both software packages on same data sets observed that the maps constructed by MapMaker were longer than those constructed by JoinMap, even when they used the same mapping function (Kosambi). Many differences exist between the JoinMap and MapMaker procedures. In particular, map distances calculated by MapMaker are multipoint maximum-likelihood distances estimated from recombination data for each linkage group, while JoinMap uses a least-squares method to calculate multipoint distances. This is probably a major reason for the observed software effect on map length.

Linkage map and karyotype

Mapping the 5S rDNA to linkage group 2 of the consensus linkage map offered an interesting opportunity to correlate the linkage map and the karyotype. In situ hybridisation studies have been carried out in several *Picea* species. They reported a unique 5S rDNA locus in *P. abies* (Lubaretz et al. 1996) as well as in *P. glauca* and *P. sitchensis* (Brown and Carlson 1997). This 5S rDNA locus was located on homologous chromosomes in the three species, i.e. chromosome II of the *P. abies* and chromosome 5 of the *P. glauca* and *P. sitchensis* karyotypes (Brown and Carlson 1997). Based on this cytogenetic location of the 5S rDNA locus, it was possible to compare linkage group 2 of our consensus map to chromosome II of the *P. abies* karyotype. Correspondence between a linkage group and an individual chromosome could be thus established. Integration of the 5S rDNA in linkage analyses that will be developed for the other *Picea* species mentioned above, as well as in other conifer species with a unique 5S rDNA locus such as *Larix decidua* (Lubaretz et al. 1996) or *Pseudotsuga menziesii* (Amarasinghe and Carlson 1998), would be thus very useful to facilitate extensive comparisons between genetic maps and karyotype.

Comparative genome mapping

To facilitate comparison with other genetic maps, we tested almost all the previously reported SSRs derived from *Picea* species (Pfeiffer et al. 1997; Scotti et al. 2000; 2002a; 2002b; Besnard et al. 2003). This allowed us to map 74 SSR loci, of which 27, located in 11 of the 12 linkage groups of the consensus map, were common to the two other published maps for *P. abies* that included this type of marker (Paglia et al. 1998; http://www.pierroton.inra.fr/genetics/Picea/). Three linkage groups of the web site map http://www.pierroton.inra.fr/genetics/Picea/ even showed a complete conservation of synteny and colinearity of markers with our consensus map.

SSRs can thus be confirmed to be suitable markers for within-species comparative genome mapping. It is important to notice that among the 74 SSR loci of our consensus map, eight originated from *Picea* species others than *P. abies* (e.g. *P. glauca*: Hodgetts et al. 2001; Rajora et al. 2001; Besnard et al. 2003). These transferable SSRs could provide convenient anchor points for extending map comparison to the interspecific level within the genus *Picea*.

Due to their insufficient number on the consensus map, ESTP loci were not totally useful for comparative mapping. However, in contrast, they displayed good transferability to the other *Picea* species tested so far, and also to several species of genus *Pinus* (*P. pinaster, P. taeda*). For this reason, they undoubtedly represent choice markers for further development of comparative genome mapping at the between-species and between-genera levels.

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