germination.

The pathway of transformation of fat into carbohydrates in germinating seed is described by various textbook authors, e.g. by THOMAS (1972), BEEVERS (1980), and HUANG (1987).

During the early stages of germination, the amount of fat and possibly carbohydrates may still be so high that it explains our observation of contamination of the extracted DNA, so detectable amount of DNA is left in the wells and the polymerase chain reaction is not running successfully.

In late stages of germination, nutrients in the form of fat and carbohydrates are removed from the megagametophytes, whereas DNA, which does not have this function, is left relative undisturbed for extraction in a much cleaner form.

The rapid metabolism during seed germination may be the cause for the general finding that megagametophytic DNA was the most sensitive tissue type tested in Norway spruce to utilize in the RAPD assay (SKOV, 1998).

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A Partial Linkage Map of *Picea abies* Clone V6470 Based on Recombination of RAPD-markers in Haploid Megagametophytes

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Abstract

By applying a RAPD assay to the haploid megagametophytes of Norway spruce, a low-density linkage map of a cloned member of the current breeding population has been constructed. The map involves allocation of 61 marker loci to 13 linkage groups as well as 22 markers to 11 single-pair groups; 62 markers remained un-linked. In total, the 82 markers cover 1385 KOSAMBI cM map units. This corresponds to an average distance between marker loci of 23.8 cM (range 2.8 to 57.6). The size of linkage groups varied between 38 cM and 213 cM. In three cases the map order of the markers was judged inconsistent. A data set is provided with multipoint analyses of each linkage group with LOD scores of 'best' and 'best alternative' map in relation to each other. Another dataset is provided with pair-wise map distances within linkage groups based on two-point analyses with the appropriate LOD scores. These data sets should facilitate construction of a 'consensus map' for this species. Perspectives for further developments for mapping of forest trees are outlined.

Key words: Picea abies, linkage maps, RAPD-markers, megagametophytes.

FDC: 165.3; 165.441; 161.6; 174.7 *Picea abies.*

Introduction

Recent development of DNA-markers based on Polymerase Chain Reactions (PCR) has allowed researchers to embark on mapping of the until recently mostly unknown genomes of conifers.

The aim of the present investigation is to test, if RAPD markers (WILLIAMS et al., 1990) are suitable for intermediate to high density genome mapping in our specific organism Norway spruce *Picea abies* L. (KARST.), in which a number of tree improvement programs are operational.

Prerequisites for embarking into such investigations are reliable methods and laboratory protocols to the degree where confidence exists, including the following points, i) DNA extraction, ii) tissue expression, iii) MENDELian or other types of inheritance, and iv) repeatability of observations within and between laboratories. Contradicting evidence has been found concerning the reproducibility of RAPD-markers between laboratories (PENNER et al., 1993). We have, in Norway spruce, investigated this array of points based on our own laboratory conditions (SKOV, 1998a and b) complemented with one other laboratory environment (SKOV, 1998c) and found the behaviour

of these markers to be reliable, providing that strict accordance with protocols is maintained.

Whether the dominant RAPD-markers are suitable for mapping the largely unknown conifer genomes, especially when sampling is carried out on haploid megagametophytes of individual parent trees, has been addressed by a number of investigators, GRATTAPAGLIA et al. (1991) in *Pinus taeda,* NELSON et al. (1993) in *Pinus elliottii,* PLOMION et al. (1995a and b) in *Pinus pinaster,* TULSIERAM et al. (1992) in *Picea glauca,* and BINELLI and BUCCI (1994) in *Picea abies.* These authors generally conclude RAPD-markers are suitable for map construction.

The present investigation uses RAPD-markers expressed in a sample of 80 megagametophytes from one individual, the clone V6470. Further demands on the specific set of markers were, that they should not violate the expected 1:1 segregation in heterozygotic loci in the haploid megagametophytes of the female parent and the markers should also be expressed in the diploid tissue of the same female parent.

The reason for this last demand was, that for later applications for QTL dissection and eventual marker-aided selection, these applications require full expression in both haploid and diploid tissues (O'MALLEY and MCKEAND (1994); GRATTAPAGLIA and SEDEROFF (1994); GRATTAPAGLIA et al. (1995); WELLENDORF and SKOV (1997)).

The principal result of our investigation is a low-density genome map of the specific clone comprising 61 RAPD-markers distributed to 13 linkage groups and 22 further loci in 11 single-pair groups. 62 markers remained unlinked. With the applied sample size, the precision of mapping was occasional insufficient for unambiguous determination of orders of marker loci along the chromosomes.

The conclusion that can be drawn based on our own and the cited investigations is, that provided strict accordance with the developed laboratory assays are maintained, MENDELian segregation takes place, and tissue expression is checked as a current 'quality control', it *is* feasible to apply RAPD-markers for the purpose of genome mapping, subsequent QTL dissection, and marker aided selection. However, for precision-mapping in selected regions, considerably larger sample sizes than 80 megagametophytes used in this study are required.

Table 1. – Applied RAPD markers accepted for the mapping. Distribution to Operon Technologies 10-base random primer series.

Operon series	Opereon no.	No.of usefull markers
A	01	3
	02	2
	06	6
	07	4
	08	6
	11	9
	12	5
	16	4
	17	6
B	02	2
	03	5
	04	4
	05	3
	07	2
	08	7
	11	4
	12	3
	13	4
	18	2
	20	5
C	05	3
	06	3
	07	4
	09	3
	10	2
	13	2
	15	4
	19	3
D	03	3
	05	3
	10	3
	11	3
	14	2
	16	2
	20	5
E	06	2
F	14	5
G	10	3
J	06	4
Total	39	145

Material and Methods

Plant material

The parent clone, V6470, is one of the few clones in the Danish improvement program in Norway spruce which are members of two nuclei breeding populations, each with different breeding objectives, growth rate and resistance against spruce decline, respectively (WELLENDORF et al., 1994). The mapped clone was selected in a Danish spruce plantation of supposed West-continental origin.

The mapping population was composed of 80 megagametophytes from one parent clone rescued from germinating embryos when they were full grown seedlings, just before spontaneous release of the seed coat with the more or less exhausted megagametophyte (SKOV, 1998b).

The applied pedigree of one parent tree and the female haploid megagametophyte with a genotype identical with the gametes from this tree, is termed a 1.5 generation pedigree.

RAPD assay

The applied laboratory procedures are described by SKOV (SKOV, 1998a and b). The assay is built upon the classical sources of information on RAPD (WELSH and MCCLELLAND, 1990; WILLIAMS et al., 1990). The selected primers are listed in *table 1* with information of number of useful markers.

Data collection

Basic observations were recorded as presence or absence of a potential RAPD fragment (band) of 145 loci distributed to 39 primers. The observations were obtained on DNA extractions from the 80 megagametophytes.

Prior to acceptance of the applied set of markers for the mapping procedure, two conditions should be fulfilled as a 'quality control':

i) Expression of the individual marker in diploid tissue of the parent,

ii) X^2 tests of the expected 1:1 segregation among the 80 megagametophytes should be accepted.

Point i) is justified by the requirement to use the mapped markers for QTL investigations where the identity of markers in diploid as well as in haploid tissue is necessary (SKOV, 1998a).

Mapping procedure

(1) General

Most of the mapping in plants is carried out by crossing pure lines of crop species and then studying recombinations in progeny of the F1 generation, i.e. F2 or BC (back cross) generations.

This means that it is possible to map only those loci which differ between the two lines. The parent phases of the markers are known from the marker combination present in the parent lines.

Another situation is faced in the more or less heterozygotic and outcrossing *Pinaceae.* In contrast to the above mentioned situation, the haploid megagametophyte segregation of individual trees can be used as mapping populations, i.e. it is possible to map loci in which the individual parent tree is heterozygotic. As different trees are expected to be heterozygotic in different sets of loci, maps are expected to be more or less specific for individual trees.

Due to the 1.5 generation pedigree, a *priory* knowledge of the linkage phases of the markers of the 'F1' parent and the unknown grandparents were not available in contrast to the pure parental lines normally used in pedigrees of crop species. However, linkage phase of the investigated female parent tree is interpreted as the most common combination of markers in the segregating megagametophytes. Alternative combinations are interpreted as recombinations as a result of crossing-overs.

(2) Preparation of data set

In this investigation the computer program MAPMAKER/EXP 3.0 has been applied (LANDER et al., 1987).

This program is not particularly tailored to haploid material, however, as stated in the manual, apart from the standard progeny types as F2 backcross (e.g. BC1, F2 intercross, F3 intercross, and Recombinant Inbred Lines), other types of crosses can be analyzed because the basic model of recombinations is similar.

In the haploid megagametophyte situation, the F2 backcross (e.g. BC1) option was used. In other words, simulation to a test-cross situation in which a heterozygotic F1 individual is backcrossed to a totally homozygotic recessive line was applied. By doing this in MAPMAKER, all the V6470-phases, which correspond to the simulated heterozygotic F1 individual, appeared to be identical, and consequently linkage between markers in opposite phase was not detected.

To circumvent this problem, all individual observations for each RAPD locus were supplemented with its complement, as shown below:

Original observation	*A01_0750a	HAAA-AHHH
.....		
Complementary obs	*A01_0750b	AHHH-HAAA
.....		

where 'A01_0750a' represents a RAPD locus (primer 'A01'; number of base-pairs '0750' ; phase 'a'), 'H' represents presence of a RAPD band corresponding to a heterozygotic BC individual, 'A' represents absence of a band corresponding to a homozygotic recessive BC individual and '-' represents a missing observation due to failure of the PCR. By this operation, it was possible to construct linkage groups for the 'F1' clone used, where both alternative marker configurations occurred. Consequently additional markers were mapped.

Short indications of the above mentioned procedure has been mentioned by other megagametophyte-mappers (TULSIERAM et al., 1992; NELSON et al., 1993 and 1994; PLOMION et al., 1995b), but was not really substantiated in these papers.

(3) Grouping of markers into linkage groups

Two-point analyses of the whole set of 2 x 145 markers were applied with the GROUP command in MAPMAKER. All pairs of loci with a frequency of recombination - Θ - less than 0.372 and a LOD score exceeding 3.0 were considered linked.

Because of the 'doubled-up' data set, all identified linkage groups were also doubled-up in identical pairs in complementary linkage phases. Arbitrarily, the first of these groups is reported.

(4) Estimation of orders of markers within linkage groups

All possible orders were compared by multipoint analysis and the order which shows the highest likelihood of our observations was chosen. The key point here was then to judge the likelihood of the 'best' order to have given rise to our observations compared to alternative orders. Applying the MAPMAKER procedure 'COMPARE' the LOD-score of the 20 most likely orders are presented. The order with the highest LOD-score was selected as the 'best' estimate. However, the denser the map, the more uncertainty is expected in the estimation of the true order. This is reflected in alternative orders with nearly the same LOD-score. The alternative order with the highest LOD-score compared to the 'best' is presented. If an alternative order has a LOD-score less than 0.5 unit lower than the best one, it is presented as an alternative in the graphic presentation. This corresponds to situations in which an alternative order has more than a $10^{0.5} = 3.16$ fold lower chance of giving rise to our observations.

Because of the large number of possible orders for larger groups (n!/2), the computer used was not able to handle groups larger than 7 members. In those cases the criterion for identifying linkage groups from the total data set was tightened to a LOD-score level of 4.0 and maximum frequency of recombination to 0.30. The members of the resultant smaller linkage group were then ordered as described above, and subsequently attempted expanded by the remaining members identified at the LOD-score 3.0 level. This last operation is done in an iterative way, i.e. the remaining members are attempted inserted in all possible intervals including terminal positions. The positions with the highest LOD-score are considered the 'best' solution; the calculations were carried out by the MAPMAKER procedure 'TRY.'

(5) Mapping of individual linkage groups

For the a *priory* estimated order of markers, the next step was to calculate the maximum likelihood map expressed in cM distances. The KOSAMBI function of frequency of recombinations was applied as map-distances in cM (KOSAMBI, 1944). The KOSAMBI function is termed

$$x / 100 = 1/4 \ln ((1+2y) / (1 - 2y))$$

where x / 100 is the map distance in cM and y is proportion of recombinations. The MAPMAKER procedure 'MAP' calculates the required map distances in the specified map-units based on multipoint analyses, i.e. the original genotype observations for all loci in the sequence are taking into account in estimation of map orders, distances, and map likelihoods. The procedure 'GENOTYPES' further shows the detailed picture of the genotypes of individual megagametophytes and recombinations are identified. Double crossovers are identified, and may be used as an error check of original observations. A final check of the obtained map of the larger linkage groups was performed by the 'RIPPLE' procedure. Here, alternative map sequences are tested against each other in marker neighbourhoods ('windows') of specified size - 5 in our cases.

Within each linkage group, the procedure 'LOD table' supplies pairwise cM distances and corresponding LOD-scores between all member loci. These statistics are based on simple two-point analyses and are required for possible joining with other independently developed maps (STAM, 1993; STAM and OOIJEN, 1995).

Results

1:1 segregation of individual markers

Of the 145 X^2-tests of the expected 1:1 segregation of individual markers, only 4 were significant at the P-level of 0.05 and one was significant at the 0.01 level. According to the classical BONFERRONII multiple test (HOLM, 1979), applicable in situations where multiple tests are performed, the appropriate P-level of the individual tests are p / n ~ 0.05 / 145 = 0.000345. According to this criterion none of the individual 1:1 segregation tests were rejected.

Mapping

No double crossovers were observed. Allocations of 60 markers to the 13 linkage groups are shown in *table 2* along with basic information about linkage phases. Furthermore, the map order and distances are listed as well as the accumulated map distances within each linkage group. Best alternative map is also reported for all linkage groups with the relative LOD-score compared to the 'best' estimated map. This comparison demonstrates the reliability of the estimated maps.

The total map distance across groups accumulated to 1,120 cM. *Table 3* shows the corresponding information on the 22 markers allocated to 11 single pair groups. Here the total map distance accumulated to 265 cM, giving a grand total of 1,385 cM covered by 83 markers. A remaining group of 62 markers showed apparently free recombinations and thereby did not reveal any detectable linkage relations.

The final partial linkage map of V6470 is shown in *figure 1.* Where the 'best' two maps only differ in a LOD-score difference less than 0.55 units, both maps are presented. In three linkage groups, no 2, 3, and 11, alternative sequences of marker loci are presented. In linkage group 2, four alternative sequences were possible, because two pairs could be linked to each terminal end of the group in two possible sequences. Only the 'worst' option is shown in the map with a LOD score only 0.33 units lower than the 'best.'

Pairwise map-distances within linkage groups are reported in the appendix.

Discussion

Coverage of the genome

Recent performed dense mapping in *Pinus* species seems to agree on an approximate total genome size between 1,300 cM and 2,000 cM (GERBER and RUDOLPHE, 1994; PLOMION et al., 1995a and b). In *Picea abies* a recent published map (PAGLIA et

Table 2. – The 13 linkage groups – basic information.
N = total number of successful PCR among the 80 megagamtophyte extractions; Prim_bp / a/b: Prim = primer reference (Operon Technologies), bp = base pairs, a/b = *cis - trans* configuration; Map distances based on multipoint analyses; 'Best' map: Map order which are in best correspondence with the observations. LOD score rel: Likelihood of the observations from the reported map relative to the 'best' map.

Link group	No. of obs	'Best' map				'Best' alternative map			
		Loci / *cis - trans* config	Map distance (Kosambi)		LOD score	Loci / *cis - trans* config	Map distance (Kosambi)		LOD score
	N	Prim_bp / a/b	cM	Σ cM	rel	Prim_bp / a/b	cM	Σ cM	rel
1	77	B08_1495b	•	0,0		B08_1495b	•	0,0	
	77	A06_0740a	49,0	49,0		A06_0740a	48,2	48,2	
	79	A02_1200a	31,9	80,9		C07_0935a	42,9	91,1	
	79	D20_0730b	11,8	92,7		A02_1200a	15,6	106,7	
	73	D05_1100b	7,0	99,7		D20_0730b	11,5	118,2	
	79	D20_1850a	7,0	106,7		D05_1100b	7,0	125,2	
	75	C07_0935a	15,1	121,8		D20_1850a	7,0	132,2	
	78	C15_0700a	42,6	164,4		C15_0700a	36,4	168,6	
	75	B18_1230a	48,4	212,8	0,00	B18_1230a	48,7	217,3	-0,89
2	62	E06_1620a	•	0,0		F14_0805b	•	0,0	*)
	77	F14_0805b	23,7	23,7		E06_1620a	23,9	23,9	
	77	B12_0910a	57,6	81,3		B12_0910a	59,3	83,2	
	74	C07_1785a	13,8	95,1		C07_1785a	13,8	97,0	
	74	D10_1890a	27,3	122,4		D10_1890a	27,3	124,3	
	75	D10_1620b	20,2	142,6		D10_1620b	20,2	144,5	
	75	G10_1100b	6,2	148,8		G10_1100b	6,6	151,1	
	76	B11_0930a	17,9	166,7		D03_0920a	18,1	169,2	
	77	D03_0920a	17,8	184,5	0,00	B11_0930a	18,7	187,9	-0,33
3	79	A16_1030b	•	0,0		F14_1600a	•	0,0	
	76	F14_1600a	23,3	23,3		D03_0605a	14,9	14,9	
	77	D03_0605a	14,3	37,6		J06_0325a	15,6	30,5	
	80	J06_0325a	15,6	53,2		A16_1030b	21,9	52,4	
	78	A08_0625a	23,5	76,7		A08_0625a	26,0	78,4	
	77	A06_0790a	30,0	106,7	0,00	A06_0790a	29,9	108,3	-0,12
4	79	A12_0460b	•	0,0		B05_1260a	•	0,0	
	78	A11_0520b	27,2	27,2		A11_0520b	29,0	29,0	
	75	B05_1260a	28,4	55,6		A12_0460b	27,0	56,0	
	76	F14_1250b	34,4	90,0		F14_1250b	42,6	98,6	
	73	B02_1750a	27,9	117,9		B02_1750a	27,1	125,7	
	76	A08_1120a	31,9	149,8	0,00	A08_1120a	31,9	157,6	-1,05

5	71	B13_1050a	•	0,0		B13_0415a	•	0,0	
	70	B13_0415a	2,8	2,8		B13_1050a	2,9	2,9	
	73	B20_1800b	30,3	33,1		B20_1800b	34,8	37,7	
	76	B11_0780a	30,7	63,8		B11_0780a	30,5	68,2	
	77	B03_0765a	30,3	94,1	0,00	B03_0765a	30,3	98,5	-0,79
6	79	A12_0760a	•	0,0		B05_0840a	•	0,0	
	76	B05_0840a	5,4	5,4		A12_0760a	5,3	5,3	
	75	C05_1540b	28,5	33,9		C05_1540b	32,6	37,9	
	73	C07_1056a	30,8	64,7	0,00	C07_1056a	30,7	68,6	-0,89
7	80	C15_1500a	•	0,0		D11_0960b	•	0,0	
	79	D11_0960b	6,3	6,3		C15_1500a	6,4	6,4	
	73	B20_2000b	21,4	27,7		B20_2000b	23,8	30,2	
	68	B11_0525a	21,4	49,1	0,00	B11_0525a	22,2	52,4	-0,86
8	77	A11_1500a	•	0,0		A11_1500a	•	0,0	
	80	A02_0520a	32,5	32,5		B04_0860a	41,4	41,4	
	76	B04_0860a	9,3	41,8	0,00	A02_0520a	9,1	50,2	-1,44
9	74	A12_0640a	•	0,0		B02_1150b	•	0,0	
	73	B02_1150b	10,5	10,5		A12_0640a	10,3	10,3	
	80	J06_0525b	30,4	40,9	0,00	J06_0525b	36,8	47,1	-1,21
10	77	D03_0570a	•	0,0		D03_0570a	•	0,0	
	76	F14_1330a	31,1	31,1		A17_1210a	48,9	48,9	
	77	A17_1210a	27,3	58,4	0,00	F14_1330a	26,9	75,8	-2,31
11	77	B08_1020b	•	0,0		B08_1020b	•	0,0	
	75	C06_0960b	24,1	24,1		B08_0370a	23,6	23,6	
	70	B08_0370a	18,2	42,3	0,00	C06_0960b	18,1	41,7	-0,02
12	75	C07_0720a	•	0,0		G10_1340a	•	0,0	
	73	G10_1340a	27,4	27,4		C07_0720a	26,8	26,8	
	78	B08_0630a	30,4	57,8	0,00	B08_0630a	38,4	65,2	-1,31
13	76	C05_0945b	•	0,0		B18_0980a	•	0,0	
	75	B18_0980a	7,4	7,4		C05_0945b	6,9	6,9	
	76	C13_1220a	30,2	37,6	0,00	C13_1220a	33,2	40,1	-0,57
1-13 total		Σ 61 loci	**Aver. 23,8**	Σ 1120		61 loci			

*) The order of the two terminal pairs (F14_0805b, E06_1620a) and (D03_0920a, B11_0930a) alternated in 4 possible orders; the reported one alternative order represented the 'worst' alternative of the 4.

Table 3. – The 11 single-pair groups – basic information.
N = total number of successful PCR among the 80 megagametophyte extractions; Prim_bp /a/b: Prim = primer reference (Operon Technologies), bp = base pairs, a/b = *cis - trans* configuration; Map distances based on two-point analyses; LOD score: Likelihood of the observations from the reported map.

Single-pair group	No.of obs N	Loci / *cis - trans* config Prim_bp a / b	Map distance (Kosambi) cM	LOD score
1	79	A01_0750a	•	
	77	B03_0960a	27,0	-41,86
2	77	A06_0570a	•	
	77	B20_1093b	26,6	-40,11
3	79	A07_0780a	0	
	79	A16_1890b	25,0	-41,78
4	79	A12_1600a	•	
	80	D20_1050b	14,3	-37,65
5	79	A16_0970a	•	
	77	A17_1700b	27,0	-41,86
6	77	A17_1400a	•	
	80	C15_1200b	30,6	-42,77
7	78	B08_0880a	•	
	78	B07_1100b	32,5	-43,19
8	79	B20_0540a	•	
	73	D10_2240b	15,6	-35,41
9	74	C06_0615a	•	
	72	C10_0339b	28,0	-36,65
10	74	C06_0845a	•	
	74	G10_1000b	10,4	-30,26
11	73	D05_1000a	•	
	78	D11_1595a	27,9	-38,83
1-11 total		22 loci	Σ 264,9 Aver. 24,0	

al., 1998) based on 447 AFLP and microsatellite loci, 413 markers covered 2,193 cM in 22 linkage groups and 7 single pair groups. The recorded coverage of 1,385 cM in the present investigation falls within the expected range as our map is low-density compared to the best pine and spruce maps. The relative high number of unlinked markers – 62 of 145 tested (43%) – might be a result of the poor coverage of most of the assumed 12 chromosomes.

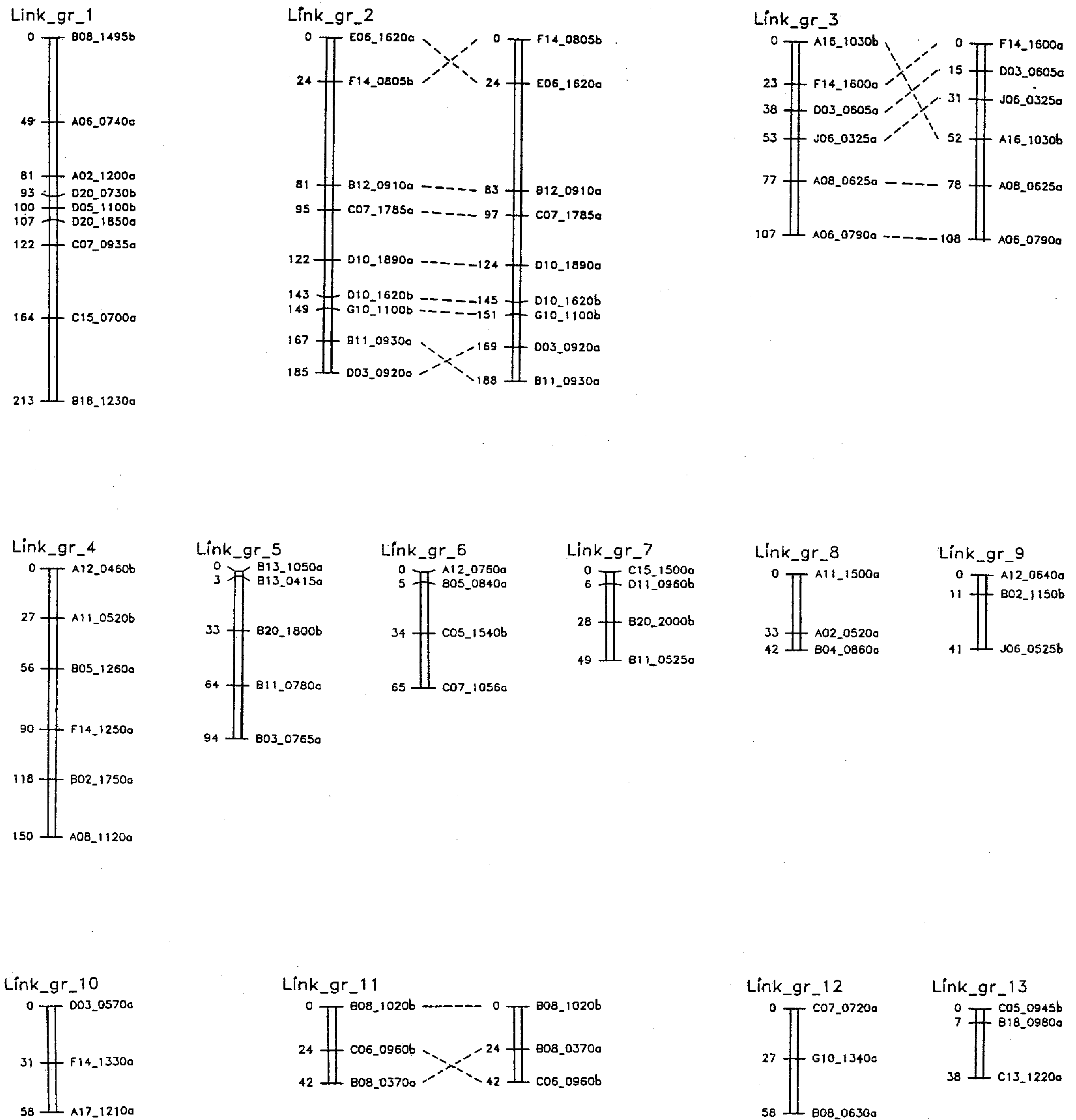

Figure 1. – Partical linkage map of *Picea abies* clone V 6470. The map is based on 145 RAPD markers observed in 80 haploid megagametophytes. Map units are centimorgan (cM) – Kosambi function. The 13 identified linkage groups span 1,120 cM with 61 loci; 11 further single-pair groups (not shown on the figure) span 265 cM with 22 loci, the grand total accumulating to 83 mapped markers spanning 1,385 cM. 62 markers remained unlinked.
In linkage groups 2, 3, and 11, alternative maps are presented which might have given rise to the observations; the statistical criteria for showing alternative maps is that the LOD score is <0.5 units lower than the 'best' map, i.e the 'best' map is less than $10^{0.5}$ = 3.16 times more likely to have given rise to our observations than the alternative map.

According to GRATTAPAGLIA and SEDEROFF (1994) and PLOMION et al. (1995a), it is possible to classify if specific RAPD loci are amplified from more or less repeated regions of the genome. The test determines the copy numbers in the amplified internal sequence between the two recognition sites the RAPD primer has identified. According to these two investigations in *Eucalyptus* and *Pinus* respectively, 89% of the RAPD markers in the pines originate from highly repetitive regions, whereas only 30% of the *Eucalyptus* RAPDs belonged to this category. In other words, the Random Amplified Polymorphic DNA is able to amplify low as well as highly repetitive regions of the conifer genome. Because most of the conifer genome is repetitive (RAKE et al., 1980; MIKSCHE and HOTTA, 1973), the DNA-markers seems to sample the whole genome in a representative way.

Precision of the obtained map

Two sources of statistical uncertainty were encountered: i) allocation of markers to linkage groups and ii) ordering of marker loci within linkage groups.

Concerning allocation of markers to linkage groups, the choice of likelihood level for accepting linkage between pairs of markers reflects a balance between a desire to allocate most markers to linkage groups and the reliability of the results. By choosing a LOD-score of 3.0 for inclusion, 62 out of the 145 markers (43%) remained unlinked. Tightening the LOD-score threshold to 4.0 resulted in 57% unlinked markers. This reflects the old problem of balance between type I and II type errors (SNEDECOR and COCHRAN, 1989).

Concerning the uncertainty of ordering of markers applying the multipoint maximum likelihood analyses within linkage groups, the reported uncertainty reflected in the relative LOD-score of the best alternative map illustrates the problem. Interpretation of one map showing a relative LOD-score one unit higher than an alternative map means that the likelihood of observations originating from the reported map is 10-fold higher than the likelihood of the observations originating from the alternative map (LANDER et al., 1987).

A closer comparison of the basic pairwise distances based on the independent two-point analyses (see appendix) and the maximum likelihood generated multipoint analyses within the reported maps (*Table 2*) demonstrates, how much the multipoint analyses have adjusted the raw pairwise distances.

Conclusion

The conclusion of the first Danish attempt to map the unknown genome of *Picea abies* is that it *is* possible to apply RAPD-markers for genome mapping in this species.

By a relative modest number of 1:1 segregating markers (145) recombining in the haploid megagametophytes of one individual clone, we have identified 13 linkage groups and 11 single-pair groups spanning 1,385 cM (KOSAMBI function). This result is in general agreement with two other published partial genome maps based on the same type of investigation with RAPD-markers in *Picea*: TULSIERAM et al. (1992) in *P. glauca* and BINELLI and BUCCI (1994) in *P. abies* as well as in the much better investigated genus *Pinus*: GRATTAPAGLIA et al. (1991) in *P. taeda,* NELSON et al. (1993) in *P. elliottii,* NELSON et al. (1994) in *P. palustris,* YAZDANI et al. (1995) in *P. sylvestris,* KAYA and NEALE (1995) in *P. brutia* and finally PLOMION et al. (1995a and b) in *P. pinaster.*

Concerning the precision of the presented map, an inconsistency of the sequence of marker loci was observed in several linkage groups. For precision-mapping in specified neighbourhoods of interesting functional genes, considerably larger sample sizes are required. Concerning allocation of markers to linkage groups, a larger sample of markers is required to cover the genome with reliable framework loci and thereby diminish the large proportion of unlinked markers encountered in the present investigation (PAGLIA et al., 1998).

Perspectives for further mapping of forest trees

Concerning application of DNA-markers for mapping purposes, we expect the following more or less parallel lines of activities in the next 5 years:

• Accumulating genome maps, 'consensus maps' (NEALE et al., 1994), by joining separate maps with a number of different marker types and ideally connecting them with chromosomes by applying the Fluorescent In-Situ Hybridization technique. Such maps may be extended to related species by comparative mapping.

• Search for alternative DNA-markers showing either co-dominans (micro-satellites with several alleles per locus) or larger, multiple loci capacity (AFLP - Amplified Fragment Length Polymorphism (VOS et al., 1995)).

• Screening large numbers of DNA-markers for co-segregation with quantitative and qualitative traits applying selective genotyping or bulk segregant analyses (MICHELMORE et al., 1991).

• Mapping of identified markers which co-segregate with the important traits which might be breeding objectives or components of these. The mapping should be put into the 'consensus map.'

• Precision-mapping in neighbourhoods of identified QTL's or other major genes of interest may proceed to a stage where 'chromosome walking' can be realistic with the aim of identifying functional genes.

• Developing early marker-aided selection procedures as integrated components of within-family or within narrow sub-line selection in advanced generation breeding (O'MALLEY and MCKEAND, 1994). Such marker-aided selections might be vegetative propagated by somatic embryogenesis for fast allocation to productive forestry through synthetic seeds (ATTREE and FOWKE, 1993).

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Appendix. – Map-distances within linkage groups based on two-point analyses. Top number cM distance (Kosambi function), bottom number LOD-score. Empty cells indicate map-distances so large, than loci are considered un-linked (LOD-score less than 0.5, roughly corresponding to recombination frequencies greater than 40% (~ 55 cM).

Linkage-group 1

Loci	B08_ 1495b	A06_ 0740a	A02_ 1200a	D20_ 0730b	D05_ 1100b	D20_ 1850a	C07_0 935a	C15_ 0700a	B18_ 1230a
B08_ 1495b	0,0								
A06_ 0740a	49,4 0,96	0,0							
A02_ 1200a	57,2 0,56	31,1 3,42	0,0						
D20_ 0730b	–	37,1 2,29	–	0,0					
D05_ 1100b	–	39,4 1,82	–	7,0 13,79	0,0				
D20_ 1850a	–	47,2 1,16	–	11,6 11,61	7,0 13,79	0,0			
C07_0 935a	54,2 0,67	40,2 1,77	15,3 8,77	13,9 9,55	13,4 9,17	15,3 8,77	0,0		
C15_ 0700a	–	–	–	34,5 2,79	24,3 4,92	36,5 2,43	41,9 1,60	0,0	
B18_ 1230a	–	31,3 3,20	–	52,8 0,76	50,4 0,83	–	53,4 0,69	47,5 1,09	0,0

Linkage-group 2

Loci	E06_ 1620a	F14_ 0805b	B12_ 0910a	C07_ 1785a	D10_ 1890a	D10_ 1620b	G10_ 1100b	B11_ 0930a	D03_ 0920a
E06_ 1620a	0,0								
F14_ 0805b	23,7 4,25	0,0							
B12_ 0910a	–	56,5 0,58	0,0						
C07_ 1785a	–	–	14,1 9,31	0,0					
D10_ 1890a	30,4 2,79	31,9 3,04	28,4 3,97	27,9 3,92	0,0				
D10_ 1620b	–	40,2 1,77	22,8 5,69	18,9 6,91	19,9 6,69	0,0			
G10_ 1100b	–	53,4 0,69	34,0 2,65	18,4 6,71	25,5 4,36	5,8 14,14	0,0		
B11_ 0930a	–	–	41,1 1,72	32,5 2,88	31,3 3,20	20,2 6,48	17,9 7,14	0,0	
D03_ 0920a	–	–	45,4 1,29	34,0 2,65	37,8 2,09	20,2 6,48	15,8 8,31	18,6 7,12	0,0

Linkage-group 3

Loci	A16_ 1030b	F14_ 1600a	D03_ 0605a	J06_ 0325a	A08_ 0625a	A06_ 0790a
A16_ 1030b	0,0					
F14_ 1600a	24,4 5,14	0,0				
D03_ 0605a	19,3 7,11	14,1 9,31	0,0			
J06_ 0325a	21,5 6,49	24,1 5,33	14,7 9,46	0,0		
A08_ 0625a	25,3 4,99	26,1 4,63	19,6 6,90	23,4 5,72	0,0	
A06_ 0790a	–	50,7 0,87	30,2 3,52	38,7 2,10	29,7 3,69	0,0

Linkage-group 4

Loci	A12_ 0460b	A11_ 0520b	B05_ 1260a	F14_ 1250b	B02_ 1750a	A08_ 1120a
A12_ 0460b	0,0					
A11_ 0520b	27,0 4,49	0,0				
B05_ 1260a	43,6 1,44	28,9 3,80	0,0			
F14_ 1250b	42,7 1,56	31,6 3,26	34,0 2,65	0,0		
B02_ 1750a	–	–	–	27,0 4,04	0,0	
A08_ 1120a	–	–	–	52,0 0,78	31,9 3,04	0,0

Linkage-group 5

Loci	B13_ 1050a	B13_ 0415a	B20_ 1800b	B11_ 0780a	B03_ 0765a
B13_ 1050a	0,0				
B13_ 0415a	2,9 17,13	0,0			
B20_ 1800b	34,2 2,36	30,2 3,01	0,0		
B11_ 0780a	–	–	29,9 3,46	0,0	
B03_ 0765a	52,6 0,71	50,4 0,83	41,1 1,65	30,2 3,52	0,0

Linkage-group 6

Loci	A12_0760a	B05_0840a	C05_1540b	C07_1056a
A12_0760a	0,0			
B05_0840a	5,4 15,80	0,0		
C05_1540b	32,2 3,11	27,5 4,09	0,0	
C07_1056a	-	56,6 0,54	29,9 3,46	0,0

Linkage-group 7

Loci	C15_1500a	D11_0960b	B20_2000b	B11_0525a
C15_1500a	0,0			
D11_0960b	6,4 15,69	0,0		
B20_2000b	23,5 5,30	22,2 5,67	0,0	
B11_0525a	31,6 2,98	28,0 3,69	21,9 5,04	0,0

Linkage-group 8

Loci	A11_1500a	A02_0520a	B04_0860a
A11_1500a	0,0		
A02_0520a	32,5 3,17	0,0	
B04_0860a	43,6 1,44	9,3 12,73	0,0

Linkage-group 9

Loci	A12_0640a	B02_1150b	J06_0525b
A12_0640a	0,0		
B02_1150b	10,6 10,43	0,0	
J06_0525b	38,6 2,03	30,8 3,36	0,0

Linkage-group 10

Loci	D03_0570a	F14_1330a	A17_1210a
D03_0570a	0,0		
F14_1330a	30,8 3,36	0,0	
A17_1210a	49,4 0,96	27,0 4,27	0,0

Linkage-group 11

Loci	B08_1020b	C06_0960b	B08_0370a
B08_1020b	0,0		
C06_0960b	25,2 4,77	0,0	
B08_0370a	25,1 4,54	19,1 6,28	0,0

Linkage-group 12

Loci	C07_0720a	G10_1340a	B08_0630a
C07_0720a	0,0		
G10_1340a	27,0 4,04	0,0	
B08_0630a	38,6 2,03	29,9 3,46	0,0

Linkage-group 13

Loci	C05_0945b	B18_0980a	C13_1220a
C05_0945b	0,0		
B18_0980a	7,0 13,79	0,0	
C13_1220a	34,2 2,72	27,5 4,09	0,0