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Genomic analysis in maritime pine (*Pinus pinaster*). Comparison of two RAPD maps using selfed and open-pollinated seeds of the same individual

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Abstract Two genomic maps were constructed for one individual tree of maritime pine, *Pinus pinaster* Ait., using a common set of 263 RAPD markers (random amplified polymorphic DNA). The RAPD markers were chosen from a larger number of polymorphic RAPD fragments on the basis of repeatability and inheritance in a three-generation pedigree. The maps were constructed from two independent mapping samples of 62 megagametophytes (1n) from a self cross and from an open-pollinated cross. The markers were grouped (LOD≥4 ; θ≤0.25) and assigned to 13 major and 5 minor linkage groups. Two framework maps were constructed using the ordering criterion of interval support≥3. Comparison of the two framework maps suggested that the locus order was incorrect for 2% of the framework markers. A bootstrap analysis showed that this error rate was representative for our data set. The results showed that framework maps constructed using RAPD markers were repeatable and that differences in locus order for maps of different genotypes or species could result from chance. The total map distance was 1380 cM, and the map provided coverage of approximately 90% of the genome.

Key words RAPD markers · Linkage map · Map comparison · Locus ordering · *Pinus pinaster*

Introduction

Comparison of genomic maps from different species or genera can provide insight on plant evolution and genome structure (Tanksley et al. 1988; Bonierbale et al. 1988; Gebhardt et al. 1991; Tanksley et al. 1992; Whitkus et al. 1992). In allogamous plant species, map-based comparison of segregation distortion from different crosses of the same individual genotype could reveal chromosomal segments that contain genes affecting fertilization or viability (Gebhardt et al. 1991; Bradshaw and Stettler 1994). Because the same locus order is expected, the comparison of maps from the same individual genotype also provides a way to evaluate the repeatability of genomic map construction. Experimental comparison of maps from the same individual genotype or different genotypes within species has only rarely been reported (e.g., Beavis and Grant 1991).

Two maps from the same individual genotype should closely resemble each other if the experimental methods used to produce the genetic markers and the statistical methods for constructing the genomic maps are sufficiently rigorous (i.e., repeatable). The comparison of genomic maps depends upon the accurate determination of locus order. The "ordering problem" is difficult because of the large number of possible locus orders (n!/2 for n loci) and because customary likelihood ratio tests cannot be carried out (reviewed by Ott 1991). Framework maps are constructed for a chosen subset of markers ordered with an interval support≥3, a widely employed criterion (e.g., Reiter et al. 1992; Kesseli et al. 1994; Grattapaglia and Sederoff 1994) recommended by Keats et al. (1991). Interval support is obtained by subtracting the log likelihood for the linkage group with the best locus order from the log likelihood for the same linkage group with a different local order (usually alternative permutations of three adjacent markers). The order for framework loci should be more certain than the order of closely linked loci on a comprehensive genomic map (no local support criteria for locus order), but framework locus order probably depends on the grouping criteria and the algorithm used.

Here we report a comparison of two maps that were constructed for one individual of maritime pine (*Pinus pinaster* Ait. 2x=2n=12) using RAPD (random amplified polymorphic DNA) markers (Williams et al. 1990; Welsh and McClelland 1990). The only genetic markers known in this

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species before this study were proteins revealed by two-dimensional gel electrophoresis (Bahrman and Damerval 1989; Gerber et al. 1993). We used a replicated design involving four sets of 31 different individuals to choose RAPD markers that were highly repeatable and easily scored in megagametophytes. A map was constructed with two sets of 31 megagametophytes from a self family (SELF map) and compared to a map constructed with two sets of 31 megagametophytes from an open-pollinated family (OP map). Conifers are believed to have a large number of recessive embryonic lethal genes that could result in segregation distortion (Sorensen 1967; Strauss and Conkle 1986). The comparison of two maps constructed for the same individual provided an opportunity to test the reliability of markers and the robustness of the linkage groupings, as well as to screen for segregation distortion. The individual that was mapped (twice) is part of a breeding program for genetic improvement of maritime pine in France and is an F_1 hybrid between the Landes and Corsican races. The genetic markers could be used to introgress stem straightness and good branching habit from the Corsican race into the widely planted Landes race if these traits are oligogenic.

Materials and methods

Plant material and DNA extraction

DNA samples were prepared from needles of the Corsican and Landes grandparents (accessions C10 and L146, respectively) and the inter-racial hybrid parent (accession H12), as well as from the megagametophytes of selfed and open-pollinated seeds from H12. The seeds were germinated following standard methods. After emergence and just before the seed coat was cast off, the megagametophyte was collected from the seedling and freeze-dried or stored at −80 °C. Megagametophyte tissue frozen in liquid N_2 was ground to a fine powder in a 1.5-ml microfuge tube. Freeze-dried needles (4 g) of both grandparents and the hybrid parent were ground under liquid N_2 using a prechilled mortar and pestle and transferred to 1.5-ml microfuge tubes. DNA was then extracted using the CTAB method of Doyle and Doyle (1987). The DNA extracted from these older needle samples was purified further by centrifugation in a CsCl-ethidium bromide density gradient. The pine DNA was diluted to a working concentration of approximately 1 ng/μl by comparison with the fluorescence of lambda DNA concentration standards on an ethidium bromide-stained agarose gel.

DNA amplification by polymerase chain reaction (PCR)

The method of Williams et al. (1990) was used to PCR-amplify polymorphic DNA fragments to be used as genetic markers. The volume of the reaction mixture was 15 μl and contained 8 mg/ml non-acetylated bovine serum albumin. The mixture was covered with 50 μl of mineral oil, and amplification were carried out in 96-well microtitre plates using a MJ Research PT-100 thermal cycler (MJ Research, Watertown, Mass.). The DNA fragments were separated by standard electrophoretic methods on 2% horizontal agarose 1× TBE gels containing 0.2 mg/ml ethidium bromide. Gels were videographed (Stratagene Eagle Eye) under UV illumination, and images were printed with a thermal printer. Primers were purchased in kits (OP-A through OP-Z) from Operon Technologies (Alameda, Calif.).

Primer screening

A total of 520 arbitrary ten-base primers were screened for polymorphisms using needle DNA samples from both grandparents (C10 and L146) and the F_1 hybrid (H12). Primers that produced DNA fragments present in one grandparent and in the F_1 parent, but absent in the other grandparent, were chosen for mapping.

Identification of RAPD markers

RAPD polymorphisms that are good genetic markers should be easily repeatable in PCR amplification reactions carried out on different days. We carefully screened for RAPD polymorphisms that were repeatable across four replicate sets of 31 independent megagametophytes. Two sets comprised the SELF mapping sample, and two sets comprised the OP mapping sample. This replicated design allowed us to choose RAPD polymorphisms that were repeatable across replicates within and between mapping samples. Some markers had co-migrating bands or were difficult to classify as presence or absence in some of the replicates. Each photo was scored twice and the individual phenotypes compared. When two scores disagreed, the lane was scored as missing for that sample.

Linkage analysis of RAPD markers

The linkage relationships of the markers were analyzed with MAPMAKER (Lander et al. 1987) version 2.0 for the Macintosh provided by S. Tingey (DuPont, Wilmington, De.). The genetic model for conifer megagametophyte segregation data for individual trees is analogous to a testcross with the parental linkage phase unknown (O'Malley et al. 1986). The MAPMAKER Macintosh HAPLOID model assumes that all markers are in the coupling phase and consequently does not recognize linkages for markers in repulsion. The assignment of coupling and repulsion phases is arbitrary for a testcross model, and repulsion phase linkages can be detected by analyzing recoded data (i.e., presence recoded to absence, and vice versa) together with the original data set. Analysis of the combined data yielded twice the expected number of linkage groups, corresponding to the two homologs for each chromosome. The two homologous groups contained the same markers in the same exact locus order. Markers were assigned to linkage groups using a LOD≥4.0 and recombination fraction of $\theta \leq 0.25$. The order of the markers was approximated using FIRST ORDER (a matrix correlation procedure). Framework maps were then constructed by comparing the likelihood of all permutations of all adjacent triplets using RIPPLE. Individual markers were dropped from each linkage group until a marker sequence was obtained that had an order at least 1000 times better than other orders (i.e., log likelihood difference≥3). The markers that were dropped were placed on the framework map as accessory markers and located to the closest framework markers (Fig. 1). Recombination fractions were converted to map distances using the Kosambi mapping function.

Comparative mapping between the SELF and the OP maps

Statistical evaluation of differences in locus order is intractable due to the large number of possible orders when more than a few loci are considered. A comparison of two genomic maps therefore must assume loci that have the same locus order on both maps. Homogeneity of individual two-point recombination fraction estimates was tested using a G-statistic approach implemented in GMENDEL 2.0 (Liu and Knapp 1992). This test can be expressed as follows:

$G^2_{\text{homogeneity}} = G^2_{\text{SELF}} + G^2_{\text{OP}} - G^2_{\text{POOL}}$, where the G-statistics are 1 *df* tests for the independent assortment of a pair of markers in the SELF, OP, and POOL maps. For a global test that combined all intervals (i.e., summed the G-statistics), *P* values were approximated following Beavis and Grant (1991) and Lander and Botstein (1989). The significance level on the whole experiment is approximated by $\alpha \approx 1 - (1-\alpha')^i$, where α' is the nominal significance level for each interval, and i is the number of intervals.

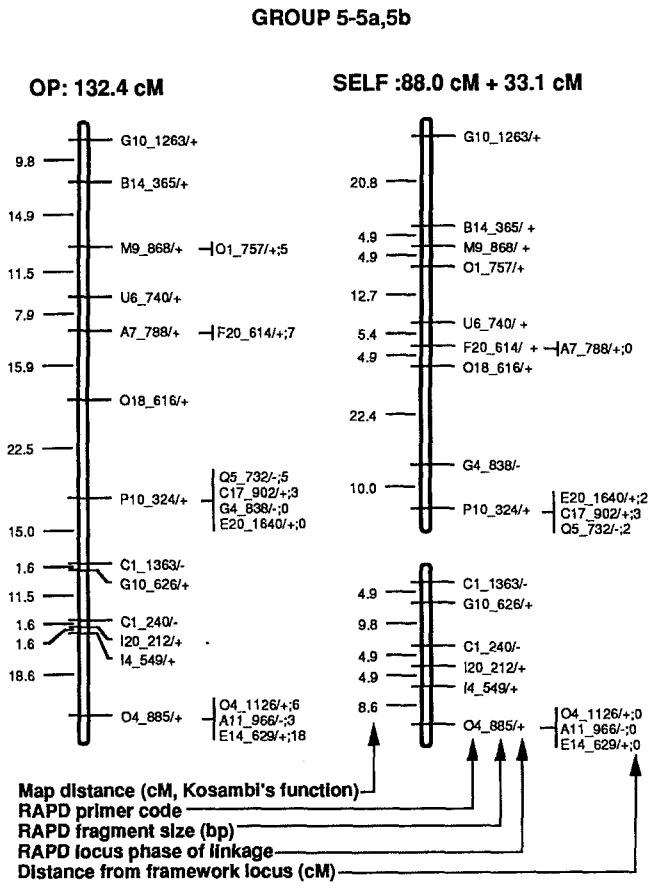

Fig. 1 Linkage group 5, generated with megagametophytes of open-pollinated seeds (OP) and megagametophytes of selfed seeds (SELF). RAPD loci are listed to the *right* and recombination distances (cM) are listed to the *left*. Framework markers have been ordered with an interval support ≥3. RAPD markers that could not be ordered with similar confidence are listed to the *right* of the framework markers with the cM distance to the closest framework locus. RAPD marker loci are identified by the OPERON primer code, their estimated size in base pairs, and the grand-parental origin: + denotes markers inherited from the Corsican grandparent, – denotes markers inherited from the Landes grandparent

The distribution of markers for the whole genome follows a Poisson distribution under the expectation of random location. The observed and expected distributions of markers was compared for 20-cM and 25-cM intervals. A chi-square test for the departure from a Poisson distribution was computed for six classes or group of classes containing at least five observations. The parameter of the Poisson distribution was estimated as the mean number of markers per 20- and 25-cM interval length.

Results

Screening for RAPD polymorphisms

RAPD polymorphisms that should segregate in the megagametophytes of the F_1 hybrid individual were identified by screening with genomic DNA samples taken from needles of the two grandparents and the F_1 individual. RAPD fragments that were present in only one of the two grandparents and also present in the F_1 should be coded by a heterozygous locus in the F_1. Of the 520 oligonucleotide primers that were screened, 31 (6.0%) failed to amplify any DNA fragments, 387 (74.4%) did not yield any polymorphisms and 102 (19.6%) amplified at least 1 scorable polymorphism (146 polymorphisms in total).

Identification and inheritance of RAPD markers

The 102 primers that revealed polymorphisms among the two grandparents and the F_1 parent were used to amplify DNA fragments from megagametophytes of the F_1 individual. RAPD reactions yielded a total of 374 DNA fragments that showed polymorphisms in at least one of the four replicate sets of 31 different individuals. RAPD fragments that amplified in only one replicate set were dropped from further analysis. Nonrepeatable polymorphisms were typically faint bands and had a molecular weight of more than 2000 bp or less than 200 bp. There were 303 RAPD polymorphisms scored in both replicate sets of the SELF mapping sample and 289 in both replicate sets of the OP mapping sample. Some of the RAPD polymorphisms were repeatable only in the SELF mapping sample and some were repeatable only in the OP mapping sample, but most (263) were repeatable between the two mapping samples and these were used as markers for mapping. The similarity index (Sorensen 1948) for the two lists of repeatable polymorphisms for the SELF and OP mapping samples was 88.3. The screening of the grandparents and the F_1 parent yielded only 146 candidate polymorphisms, but more polymorphisms were detected from the segregation analysis because heterozygous and homozygous dominant grandparental phenotypes could not be distinguished.

The segregation ratio of most RAPD polymorphisms did not depart significantly from 1:1, the expected Mendelian ratio in megagametophytes. Polymorphisms that showed the strongest departures from the 1:1 segregation ($P<0.002$) were later shown to involve co-migrating polymorphic bands. There were six such cases specific to the SELF mapping sample, and two cases specific to the OP mapping sample. From the 263 repeatable polymorphisms identified as genetic markers common to both SELF and OP mapping samples, 7 markers out of 526 showed significant departure from the 1:1 segregation ($0.002 \leq P \leq 0.01$). The number of departures from 1:1 was close to that expected due to chance, and the departures did not repeat between the two mapping samples. RAPD fragments ranged in size from 194 bp to 2326 bp, with an average of 874±416 bp. There were 7 putative codominant markers. The 102 primers identified by screening ultimately yielded 2.6 markers per primer.

Linkage analysis and locus ordering

Grouping and ordering of markers were carried out using a LOD≥4.0 and $\theta \leq 0.25$. Of 263 markers, 251 markers were assigned to 13 large linkage groups (Fig. 2A), with the remaining 12 assigned to five doublets and triplets (not

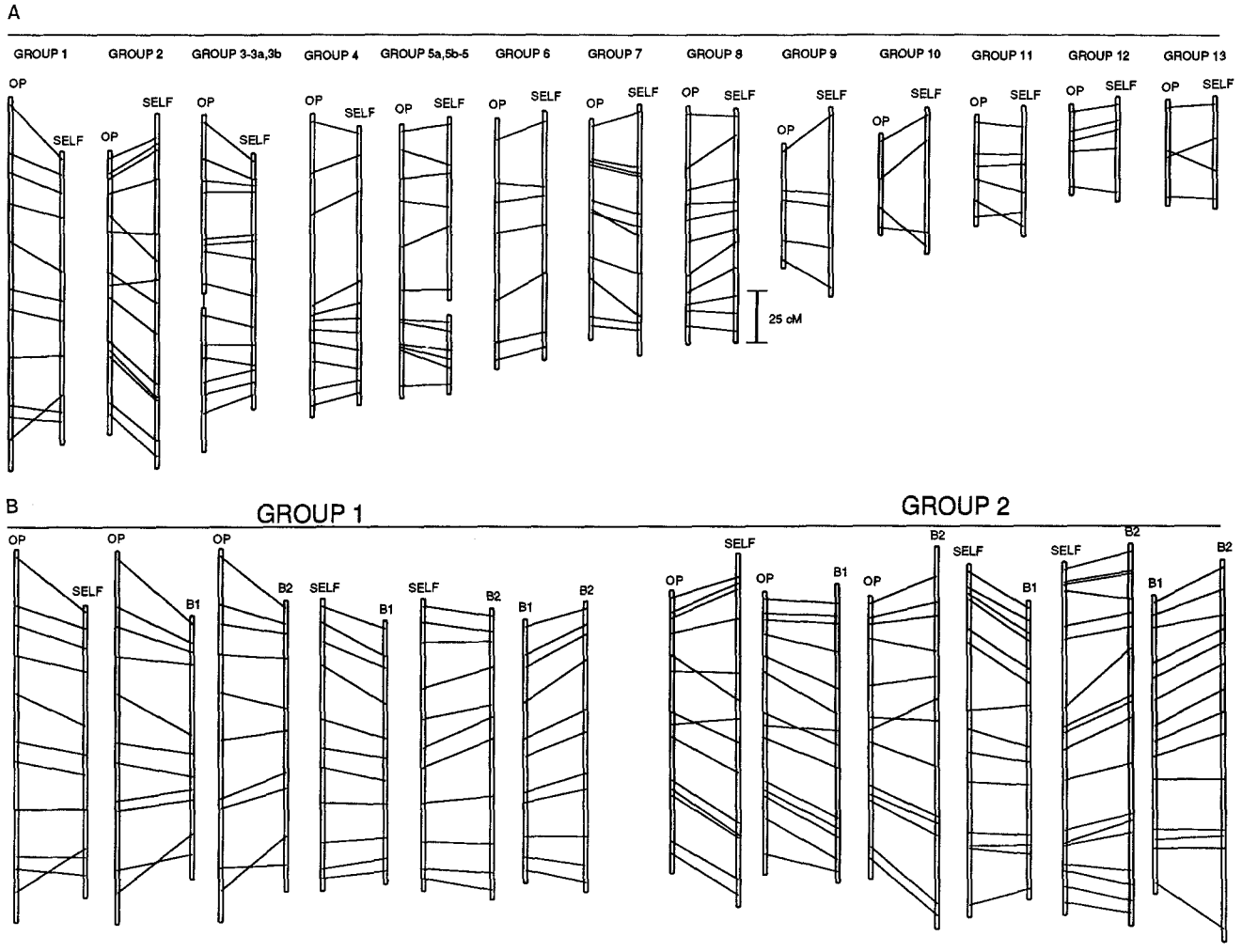

Fig. 2 **A** Ordering comparison of common framework RAPD loci between SELF and OP maps for the 13 major linkage groups of maritime pine hybrid 'H12'. **B** Ordering comparison of common framework RAPD loci for linkage group 1 and 2 among the SELF, OP, and two bootstrap samples (B1 and B2) constructed with 62 randomly chosen megagametophytes. The *connecting lines* indicate the positions of corresponding loci (129 for panel A and 152 for panel B)

shown). When the grouping criteria were relaxed (LOD $\geq$4.0, $\theta \leq 0.30$), groups 3.a and 3.b in the OP map were joined (indicated by a faint line on Fig. 2A), as they were already in the SELF map. Group 5 in the OP map was divided into groups 5.a and 5.b in the SELF map. The grouping criteria would have to be relaxed to LOD$\leq$1.5 and $\theta \geq 0.40$ to join 5.a and 5.b. The linkage group assignments were generally stable for 3$\leq$LOD$\leq$6, with $\theta \leq 0.25$. Using the RIPPLE command, we constructed framework maps for the SELF (173 markers) and OP (152 markers) mapping samples using an interval support $\geq$3. Tightly linked markers were dropped one by one until the framework criterion was met. These "accessory" markers were generally located $\theta \leq 5$ cM from the closest framework marker. The locus order of the framework maps obtained with MAPMAKER 2.0 (matrix correlation method) and with GMENDEL 2.0 (simulated annealing, $\theta \leq 0.25$ and $P \leq 0.0001$) were almost identical. The exceptions were permutations of closely linked markers.

For the framework maps, the average spacing between markers was 9.0±5.8 cM in the SELF and 10.3±6.3 cM in the OP, with a maximum gap between consecutive markers of 26.1 cM and 25.9 cM, respectively. The size of the large linkage groups ranged from 33.1 cM to 183.2 cM, with an average size of approximately 96 cM. The mean number of markers per 20- or 25-cM interval was 3.5 and 4.2, respectively, including both accessory and framework markers. A chi-square (5 *df*) goodness-of-fit test for departure from a Poisson distribution provided no evidence that the markers were clustered (P<0.35).

Homogeneity of recombination fraction

Homogeneity of recombination fraction was tested for 2 marker pairs adjacent to an apparent break between linkage groups 5.a and 5.b that occurred in the OP map but not in the SELF map (Fig. 1). The pairwise combinations of the 4 markers (G4_838/– and P10_324/+ against C1_1363/– and G10_626/+) were tested for departure from homogeneity among the SELF and OP mapping samples

Table 1 G-statistics test (df=1) for homogeneity of recombination fraction among the selfed (SELF) and open-pollinated (OP) mapping samples for four RAPD markers flanking a break in linkage group 5 (G_{SELF} and G_{OP} G-statistics for linkage for the SELF and OP mapping samples, respectively, G_{homo} G-statistics for homogeneity, R recombination fraction)

RAPD markers		P10_324	C1_1363	G10_626
G4_838	G_{SELF}	38.40***	0.40	1.00
	R	1.94	45.88	56.65
	G_{OP}	61.00***	29.40***	25.80***
	R	0.00	14.99	16.96
	G_{homo}	1.20	11.50***	8.20**
P10_324	G_{SELF}		2.80	1.70
	R		59.01	41.67
	G_{OP}		33.20***	26.70***
	R		14.76	16.66
	G_{homo}		8.40**	7.90**
C1_1363	G_{SELF}			49.60***
	R			4.92
	G_{OP}			55.00***
	R			1.70
	G_{homo}			0.10

** Significant at the 0.005 level; *** Significant at the 0.001 level

(Table 1). These markers segregated in Mendelian proportions in both populations and had no missing data. A significant departure from homogeneity (P<0.005) among OP and SELF was observed between marker pairs flanking the break. By means of the G-statistics provided in GMENDEL, 174 other intervals that were flanked by the same pairs of markers and that had the same exact locus order in the SELF, OP and POOL maps were evaluated for departures from homogeneity. Only 2 intervals (1 in group 7 and 1 in group 8) showed a departure from homogeneity (0.005<P<0.01). None of these departures would be significant using a nominal significance level of α'=0.0003 computed for α=0.05 and i=174, as suggested by Beavis and Grant (1991).

Genome size estimation

The total map distance was estimated following Hulbert et al. (1987). For the maritime pine marker data, the number of informative meioses per map was 62. The number of framework loci was n=173 and n=152 for the SELF and OP maps, respectively. Linked markers were determined by a minimum LOD threshold of T=5.0 and a recombination fraction of $\theta \leq 0.25$. According to Chakravarti et al. (1991), we set the parameter X of Hulbert et al. (1987) to the maximum cM distance between linked markers: 26.1 cM and 25.9 cM for the SELF and OP map, respectively. The TWO-POINT command of MAPMAKER was used to determine the number K of informative marker pairs each within X cM and linked with LOD score≥T. The total distances estimated by this method were 1336 cM (K=581) for the SELF map and 1357 cM (K=438) for the OP map. The same calculation taking into account the 263 mapped markers gave an estimate of 1223 cM (K=1470) and 1236 cM (K=1444) for the SELF and OP map, respectively.

Discussion

Repeatability, quality, and segregation of RAPD markers

RAPD markers provided a fast, efficient, and reliable way to construct genomic maps in maritime pine. The two genomic maps were constructed over a period of 6 months for an individual tree using megagametophytes from open-pollinated seeds (OP map) and from selfed seeds (SELF map). RAPD markers for genomic mapping were chosen on the basis of repeatability, inheritance, and expression using genomic DNA from needles. The markers segregating in megagametophytes could be detected in the diploid tissue of both the F_1 individual and its two parents, as well as in the F_2 progeny, with a few exceptions. These RAPD polymorphisms should be valuable genetic markers for future mapping studies provided that care is taken to use identical conditions for carrying out the PCR protocols, as noted by Penner et al. (1993).

There were no unusual mapping problems for the RAPD markers in maritime pine, in contrast to those described in lettuce by Kesseli et al. (1994). The data quality for the RAPD framework maps appeared to be high; the observed and expected numbers of "double recombinants" were similar. Misclassification of band phenotypes results in apparent "double recombination" events (discussed by Ott 1991). Assuming the order of framework markers was correct, we estimated the frequency of double recombinants by multiplying together the recombination fraction for adjacent intervals on the framework maps. For a sample of 2 large linkage groups, there were no significant differences between the observed and expected numbers of apparent double recombinants, and none of them had ambiguous band phenotypes.

The RAPD markers that we identified showed few cases of segregation distortion in the SELF and OP mapping samples, so there was no evidence for genetic load in the F_1 individual.

Framework map comparison

Tests for homogeneity of recombination fraction over all markers (for regions where locus order was identical) did not reveal evidence of heterogeneity. One marker pair, however, showed a significant departure from independent assortment (i.e., linkage) for one map, but not for the other map. Homogeneity of recombination fraction was rejected for this pair of markers and resulted in a large linkage group in the OP map being "broken" in the SELF map (Fig. 1). The analysis of an additional 40 megagametophytes for this marker pair confirmed independent assortment in the SELF and linkage in the OP, suggesting that the original result of no linkage for the SELF mapping sample was not spurious. We could not find a biological explanation for an increased recombination fraction in the SELF versus the OP map for that marker pair.

The locus order for the two maritime pine framework maps was different for 7 out of 129 direct comparisons

(Fig. 2A). The lines connecting common framework markers crossed because the locus order was different for one of the two maps. There were no missing data for these markers. To ascertain whether this observation was representative for our data, a statistical technique called "bootstrapping" (Efron 1982) was used to resample the data from our experiment and to generate "new" maps to compare with the original pair. Two new sets of 62 gametes were sampled with replacement from the pooled 124 megagametophytes data set (SELF plus OP mapping samples). Two new framework maps were constructed for linkage groups 1 and 2, and these were compared pairwise with the original maps for these groups (Fig. 2B). The number of differences in locus order for 152 comparisons was seven. Thus, our observation of order differences based on the comparison of two framework maps appeared to be representative of the variation in locus order that can be expected. Each difference suggested that the order for one of the two maps was mistaken (i.e., one difference=1/2 mistake per map). For framework maps constructed by the criteria we used, our result suggests that the locus order will be incorrect for approximately 2% of loci. Bootstrapping experiments using genomic mapping data could be a valuable tool to help understand practical aspects of locus ordering, but framework map construction methods are laborious and have not yet been reduced to a simple set of algorithms.

The maritime pine RAPD maps were constructed for the genetic analysis of quantitative traits, thus the accuracy of locus order and the marker density is adequate for our purposes (Darvasi et al. 1993). A 2% error rate could be important for map comparisons where differences in locus order suggest genetic rearrangements. For example, in humans, Higgins et al. (1990) reported a case where the order determined by physical mapping was different from the order inferred by genetic mapping. For a comparison of genomic maps of maize and sorghum, Whitkus et al. (1992) attributed 9 out of 14 differences in locus order to chromosomal rearrangements, with the remaining 5 cases (3% error rate) attributed to uncertainties in locus ordering methods. Thus, our result confirms their suspicion that a small number of locus order differences should be expected by chance due to map construction methods. Accurate locus order is also important for gene isolation by map-based cloning. A better quality of locus order could have been obtained either by increasing the sample size while holding the number of framework markers at approximately 150, or by holding the sample size at 62 and raising the interval support criterion for choosing framework loci.

Genome size and map coverage

Pine has 12 metacentric chromosomes (Sax and Sax 1933). Total map distance for pine can be estimated from the number of chiasmata per bivalent (2.5) observed in pine pollen mother cells (Saylor and Smith 1966). Total map distance corresponds to half of the number of chiasmata (Ott 1991), so the total map distance of pine should be approximately 1500 cM. Both RAPD maps for maritime pine had 13 large linkage groups plus 5 small groups of 2–3 markers. The framework linkage maps for this maritime pine F_1 individual had a total map distance of approximately 1380 cM. The RAPD maps, however, were determined from meioses from megaspore formation, and recombination rates between markers could differ for pollen and megaspores (Moran et al. 1983).

The 263 RAPD markers common to both maps appeared to provide nearly complete coverage of the maritime pine genomic map. The addition of 41 markers unique to the SELF sample and 26 markers unique to the OP sample expanded the total map length by only 3% (42.8 cM) and 2% (28.5 cM), respectively. The 5 small linkage groups could account for perhaps 200 cM of map distance if each map included 20 cM at both ends of these groups where linkages with other markers or genes could be detected. A total map length of approximately 1380+200 cM closely agrees with the estimate from cytological studies and suggests that the map was approximately 90% complete.

Genomic map coverage was also evaluated assuming that markers are randomly distributed on a 1500-cM map. According to Lange and Boehnke (1982), 110 markers are needed to cover a genome of 1500 cM with $P \geq 0.95$ and $d \leq 20$ cM. For a genomic map of 263 markers randomly located on a genomic map of 1500 cM, an additional marker will be located within 20 cM of an existing marker with $P \leq 0.998$. Although the marker distribution appeared to be random, showing no significant departure from Poisson expectations in a goodness-of-fit test, the maritime pine map had 5 small linkage groups, suggesting that the map was not so complete or that our method of evaluating randomness was weak. The method of Hulbert et al. (1987) provided an estimate of total map length (1288 cM) that was closer to the observed length (1380 cM).

Genomic analysis in maritime pine

In forestry, restriction fragment length polymorphism (RFLP) markers have been used for studies of genome structure and evolution as well as for analysis of quantitative genetic variation (Neale and Williams 1991; Groover et al. 1994; Devey et al. 1994). Forest trees are generally genetically heterogeneous and highly outcrossed, thus anonymous markers such as RAPD can be readily detected despite their dominant pattern of inheritance. RAPD markers are an efficient first step towards establishing a genomic maps for previously unstudied species (Tulsieram et al. 1992; Nelson et al. 1993; Grattapaglia and Sederoff 1994). Our result demonstrates that a high quality genomic map that covers 90% of the genome can be constructed from RAPD markers. Additional genetic markers (e.g., isozymes, proteins, RFLPs, sequence-tagged sites) will be needed to establish synteny with other species. This genomic map of maritime pine will facilitate quantitative trait dissection studies and marker-assisted breeding. The RAPD map can be supplemented by known and unknown genes to further characterize the maritime pine genome.

As a part of our mapping project, 27 protein loci have been located on the maritime pine map described in this paper (Plomion et al. 1995).

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