Theor Appl Genet (2000) 100:1249–1256

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An integrated genetic map of *Populus deltoides* based on amplified fragment length polymorphisms

Received: 10 September 1999 / Accepted: 3 November 1999

Abstract Amplified fragment length polymorphism (AFLP) is an efficient molecular technique for generating a large number of DNA-based genetic markers in *Populus*. We have constructed an integrated genetic map for a *Populus* backcross population derived from two selected *P. deltoides* clones using AFLP markers. A traditional strategy for genetic mapping in outcrossing species, such as forest trees, is based on two-way pseudo-testcross configurations of the markers (testcross markers) heterozygous in one parent and null in the other. By using the markers segregating in both parents (intercross markers) as bridges, the two parent-specific genetic maps can be aligned. In this study, we detected a number of non-parental heteroduplex markers resulting from the PCR amplification of two DNA segments that have a high degree of homology to one another but differ in their nucleotide sequences. These heteroduplex markers detected have served as bridges to generate an integrated map which includes 19 major linkage groups equal to the *Populus* haploid chromosome number and 24 minor groups. The 19 major linkage groups cover a total of 2,927 cM, with an average spacing between two markers of 23. 3 cM. The map developed in this study provides a first step in producing a highly saturated linkage map of the *Populus deltoides* genome.

Key words AFLP · Heteroduplex · Intercross marker · Linkage map · *Populus deltoides* · Testcross marker

Communicated by P.M.A. Tigerstedt

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Introduction

Genetic linkage maps have become a powerful tool for studying important biological phenomena in many forest trees (Liu and Furnier 1993; Bradshaw et al. 1994; Grattapaglia and Sederoff 1994; Marques et al. 1998; Remington et al. 1999). A complete linkage map can be used to efficiently identify key quantitative trait loci (QTL) that affect plant growth, development, heterosis and genotype × environment interaction (Bradshaw and Stettler 1995; Grattapaglia et al. 1995) and to explore genome evolution between lineages (Devey et al. 1999). The map is also essential to manipulate and utilize these QTL through marker-assisted selection or gene cloning.

Poplars are one of the most intensively studied forest tree species because of their importance as a fiber or biofuel source and a model research organism (Stettler et al. 1996). The potential of poplars for these endeavors would be further enhanced by a detailed linkage map of the poplar genome. Previous mapping attempts were limited due to an inadequate number of polymorphic markers, relying mainly on allozyme polymorphisms (Liu and Furnier 1993). The current development of molecular techniques has made it possible to identify nucleotide-level DNA sequence polymorphisms between individuals, which can provide a virtually unlimited source of genetic markers for the construction of detailed linkage maps (Williams et al. 1990; Vos et al. 1995).

In plants and animals with short generation times, an efficient mapping strategy is to utilize homozygous parental strains and recombinant inbred lines produced by multiple generations of sib-mating or self-fertilization (Burr and Burr 1991). In spite of its many advantages this strategy is difficult in a species with long generation times, such as forest trees (6 – 30 years). However, forest trees generally have high heterozygosity, and hybridization between or within species can thus lead to tremendous segregation due to gene recombination. Through extensive breeding efforts in the past decades, a number of hybrid populations have been established in many species, and these provide invaluable materials for ge-

netic mapping as long as the appropriate modification of current statistical algorithms for linkage analysis is made.

Simple sequence repeats (SSRs) appear to be ideal markers for constructing highly dense mouse and rice maps because of their high level of heterozygosity, wide dispersal and abundance throughout the genome and transferability across strains or species. However, the development of a set of single-locus SSRs that adequately cover the genome requires extensive resources of time and money. For those uncharacterized species, such as forest trees, in which only limited resources are available, a better strategy for generating molecular markers to cover the genome is to initially choose the alternative methods that are currently more feasible. One such method is amplified fragment length polymorphisms (AFLPs), which does not require extensive DNA manipulations but can detect high levels of heterozygosity, informative in nearly all organisms (Cervera et al. 1996; Marques et al. 1998; Paglia et al. 1998; Remington et al. 1999). The AFLP technique utilizes the polymerase chain reaction (PCR) to amplify restriction fragment length polymorphisms (Vos et al. 1995). The high levels of variability detected with this method enable large numbers of informative loci to be rapidly analyzed even in crosses involving relatively closely related strains.

We report an AFLP-based linkage map of *Populus* constructed by a backcross progeny derived from the intraspecific cross of *P. deltoides*. The map represents a useful framework of markers by which to characterize the recombination patterns and marker distribution for the poplar genome.

Materials and methods

The mapping pedigree

An intraspecific hybrid pedigree was derived from two different "elite" clones of *Populus deltoides* Bartr., both selected from a natural population in North America. Clone I-69, female, was crossed with clone I-63, male, to generate an F_1 family at the Chinese Academy of Forestry. One of the F_1 hybrids, designated C-135, was chosen to backcross with its male parent I-63. The backcross progeny were maintained at a nursery site near the Chinese Academy of Forestry, of which 93 individuals were selected to construct a genetic linkage map for *Populus*.

The AFLP protocol

Genomic DNA was isolated from frozen leaf tissues of the two original parents, the F_1 parent, and the 93 backcross hybrids following the DNeasy Plant Mini procedure developed by QIAGEN. The DNA preparations were quantitated by comparing the fluorescence intensities of ethidium bromide-stained samples to those of λ-DNA standards on 0.8% agarose gels.

The AFLP method was performed following Remington et al. (1999) who modified the approach of Vos et al. (1995) for the automatic analysis of AFLPs. Templates for AFLP reactions were prepared using 500 ng poplar DNA for restriction digests with *Eco*RI and *Mse*I and for the ligation of adapters. The restriction-ligation (RL) mixture was diluted 1:10 in deionized water prior to preamplification.

Preamplification was conducted using standard AFLP *Eco*RI (E) and *Mse*I (M) primers containing selective nucleotides E+A and M+C. The reaction mixtures consisted of 20-µl volumes for preamplification contained 5 µl diluted RL mixture as template, 1.2 U *Taq* polymerase (Boehringer), 30 ng E primer, 30 ng M primer, 10 mM TRIS-HCI pH 8.3, 1.5 m*M* $MgCl_2$, 50 m*M* KCI and 0.2 m*M* each of all four dNTPs. PCR amplification consisted of 28 cycles of a 30-s denaturation at 94°C, 30-s annealing at 60°C and a 60-s extension at 72°C.

Selective amplifications were performed using various combinations of E and M primers, both with three selective nucleotides (E+3/M+3). Reaction mixtures were as described above for preamplification, except that 5 µl of 1:100 dilutions of the preamplification products were used as template, and only 5 ng of infrared dye (IRD)-labeled E primer (Li-Cor) was used. PCR amplifications were conducted with 36 cycles of a 30-s denaturation at 94°C, 30-s annealing at 60°C and 60-s extension at 72°C. The annealing temperature, which was 65°C for the first cycle, was reduced by 0.7°C for each of the next 12 cycles and was 56°C for the remaining 23 cycles.

Scoring of AFLP fragments

AFLP reaction products were resolved on denaturing gels containing 6% or 7% Long Ranger polyacrylamide (FMC), 7.5 *M* urea and 1 × TBE (89 m*M* TRIS, 89 m*M* boric acid, 2 m*M* EDTA). Loading buffer (10 µl) consisting of 95% deionized formamide, 20 m*M* EDTA, pH 8.0, and 1 mg/ml bromophenol blue (USB) was added to each selective amplification product prior to gel loading. This mixture was heated at 94°C for 3 min, then quickly cooled on ice before 1.5 µl of each sample was loaded on the gel. IRD-labeled molecular-weight markers (Li-Cor) were loaded in two lanes as a standard. Electrophoresis was carried out on Li-Cor 4000L automated sequences using 1 × TBE running buffering, with run parameters of 2000 V, 35 mA, 70 W, signal channel 3, motor speed 3 or 4, 50°C plate temperature and 16-bit pixel depth for collection of TIFF image files.

Polymorphic fragments were scored by eye in the TIFF image files using RFLPscan version 3.0 (Scanalytics). Automatic detection thresholds were set at the maximum level to minimize the number of automatically scored fragments, and polymorphic fragments were scored electronically by the user. The software automatically assigned molecular weights to fragments, binned the corresponding fragments from different samples representing single polymorphisms and generated reports of fragment presence/absence strings for each sample. These reports were converted into mapping software formats using a spreadsheet program.

Map construction

Chi-square tests were performed to check whether individual markers segregated following Mendelian ratios. The linkage map was constructed using MAPMAKER version 3.0 (Lincoln et al. 1992). Markers were first grouped using a minimum LOD score of 4.0 and maximum recombination (θ) value of 0.25. For each linkage group, markers were ordered by using a minimum LOD score of 3.0 and a maximum θ of 0.40 using the First-Order command. The ordered marker sequences were confirmed using the Ripple command. Markers ordered with low confidence were placed again using the Try command. New merkers were placed at appropriate positions of the maps with the Place command. Linkage maps were generated with the Map command using the Kosambi map function. The dataset was duplicated and recorded to allow the detection of linkage of markers in repulsion phase. Possible errors or double crossovers were checked by the Genotype command before map construction.

Results

Inheritance of AFLP markers

Twenty-two AFLP primer combinations amplified the original parents, F_1 parent and backcross hybrids, which led to a total of 523 polymorphic bands (Table 1). The feature of the TIFF image generated by the Li-Cor automated sequencer system, on which polymorphic bands were scored, is shown in Fig. 1. The number of segregating AFLP fragments was strongly associated with the composite of the primer selective extensions, with a range of 14 to 40 scored polymorphisms per primer pair. The size of the AFLP fragments ranged from approximately 40 to 400 bp. Of the 523 polymorphic markers detected, 408 (78%) segregated in a 1:1 ratio and 81 (15%) in a 3:1 ratio, whereas the other 34 (7%) showed segregation distortion.

Different segregation patterns in the backcross progeny between markers can be determined from parental band types. Assume two alleles, *A* and *a*, at a marker locus. A possible 3 genotypes for each of the two parents are combined to generate nine cross types and 15 genotypes for the F_1 hybrids (Table 2). Of all the possible backcross types, 4 are not segregating, 4 are segregating but not scorable, and the remaining 7 are both segregating and scorable. However, the 7 scorable types display different segregation patterns, with 4 segregating 1:1 and 3 segregating 3:1.

Of the 1:1 segregating markers we detected many more that were only heterozygous in the original male

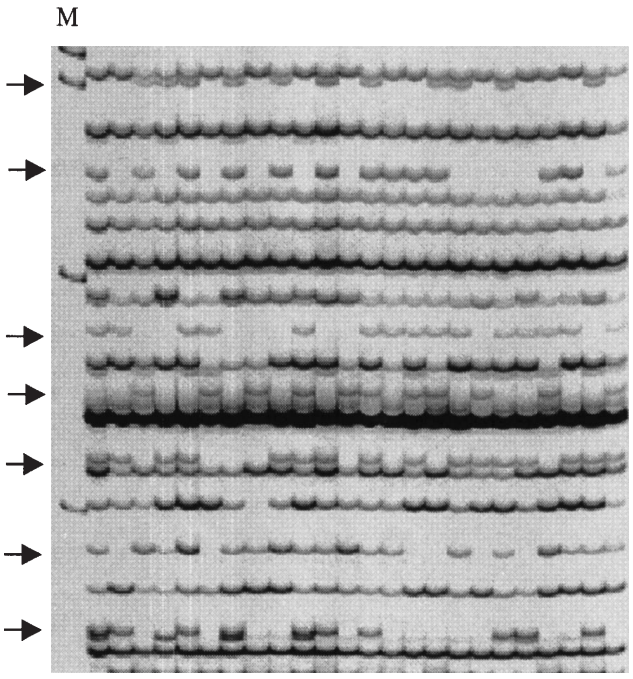

Fig. 1 A portion of a TIFF image for AFLP products amplified by primer pair E+CAA/M+ATA. Each lane is labeled with regard to the STR Marker (*M*), the original parents (I-69 and I-63), F_1 female parent (C-135) and a portion of the backcross progeny. Polymorphic markers are identified by the fragments that are present in some samples but absent in others (*arrows*)

Table 1 Number of AFLP amplification products generated with 22 different primer combinations

Eco +3	Mse +3	Number of polymorphic bands	Size range of AFLP fragments	Number of markers with different segregation patterns		
				1:1	3:1	Distorted
AAC	AAC	20	45 – 341	16	3	1
	AAG	26	47 – 486	24	1	1
	AAT	16	92 – 691	14	1	1
	AGA	14	59 – 404	11	0	3
	AGC	18	44 – 904	16	2	0
	AGG	22	37 – 474	18	4	0
	AGT	19	39 – 475	15	2	2
CAA	AAC	32	35 – 702	27	3	2
	ACC	25	35 – 600	20	5	0
	ACT	22	42 – 649	18	3	1
	AGA	31	46 – 617	29	2	0
	AGC	24	35 – 641	16	5	3
	AGG	37	35 – 627	32	3	2
	AGT	18	36 – 385	11	4	3
	ATA	22	55 – 394	19	3	0
CCT	AAC	22	32 – 469	17	2	3
	ACC	16	24 – 549	11	4	1
	ACT	21	34 – 465	16	4	1
	AGA	14	34 – 356	7	4	3
	AGT	30	39 – 574	21	4	5
	ATC	34	27 – 470	21	11	2
	ATG	40	36 – 737	29	11	0
	Total	523	24 – 904	408	81	34

Table 2 Segregation of AFLP amplification products in the backcross progeny from outbred parental genotypes I-69 and I-63

Cross type	Original parent		Hybrid progeny			
	Female (I-69)	Male (I-63)	F_1 (C-135)	Backcross (C-135 × I-63) and band pattern		Remarks
1	*AA* (+)	*AA* (+)	*AA* (+)	*AA* × *AA*	++++++++++++	Not segregating
2a	*AA* (+)	*Aa* (+)	*AA* (+)	*AA* × *Aa*	++++++++++++	Not scorable
b			*Aa* (+)	*Aa* × *Aa*	+++++++++ – – –	3:1
3	***AA* (+)**	***aa* (–)**	***Aa* (+)**	***Aa* × *aa***	**++++++ – – – – – –**	**1:1**
4a	*Aa* (+)	*AA* (+)	*AA* (+)	*AA* × *AA*	++++++++++++	Not segregating
b			*Aa* (+)	*Aa* × *AA*	++++++++++++	Not scorable
5a	*Aa* (+)	*Aa* (+)	*AA* (+)	*AA* × *Aa*	++++++++++++	Not scorable
b			*Aa* (+)	*Aa* × *Aa*	+++++++++ – – –	3:1
c			***aa* (–)**	***aa* × *Aa***	**++++++ – – – – – –**	**1:1**
6a	*Aa* (+)	*aa* (–)	***Aa* (+)**	***Aa* × *aa***	**++++++ – – – – – –**	**1:1**
b			*aa* (–)	*aa* × *aa*	– – – – – – – – – – – –	Not segregating
7	*aa* (–)	*AA* (+)	*Aa* (+)	*Aa* × *AA*	++++++++++++	Not scorable
8a	*aa* (–)	*Aa* (+)	*Aa* (+)	*Aa* × *Aa*	+++++++++ – – –	3:1
b			***aa* (–)**	***aa* × *Aa***	**++++++ – – – – – –**	**1:1**
9	*aa* (–)	*aa* (–)	aa (–)	*aa* × *aa*	– – – – – – – – – – – –	Not segregating

parent (154) compared to those only heterozygous in the F_1 female parent (77), possibly suggesting high heterozygosity for the original parent I-63. The majority of the 1:1 segregating markers heterozygous for I-63 had no bands in the original female parent, I-69 (cross type 8b, Table 2), suggesting that I-69 may be fixed for many markers. A few 1:1-segregating markers heterozygous in the F_1 parent, C-135, and detected to have no bands in the original female parent I-69 were excluded from further analyses because they do not follow the expectation by cross types 3 and 6a (Table 2). We also detected 105 markers segregating 1:1 in the backcross despite the fact that both the F_1 parent and original parent had no bands and 72 markers segregating 1:1 although both parents had bands.

The 1:1 segregation of the non-parental bands may result from heteroduplex formation generated by base pairing between complementary single strands derived from the different parental duplex molecules during genetic recombination (Ayliffe et al. 1994). This can be inferred from the segregating pattern of the heteroduplex as described in Fig. 2. Consider a primer pair amplifying an AFLP band from the backcross DNA that is not amplified from either of the parental DNAs (Fig. 2, band *x*). But the same primer pair detected two other polymorphisms between the parental DNAs (Fig. 2, band *y* and *z*). Band *y* was amplified from DNA of C-135 and not from I-63 DNA, while for band *z* a reciprocal pattern of amplification was observed amongst the parental DNAs. Both bands *y* and *z*, inherited by the backcross, were expected to segregate in a 3:1 ratio, consistent with dominantly inherited products. Meanwhile, if they are allelic, bands *y* and *z* should show a co-segregation ratio of 1:2:1. Unlike these two bands, however, the segregation pattern of band *x* follows a 1:1 ratio, with individuals having associated bands *y* and *z* (heteroduplex) as the heterozygote class and the null individuals comprising one of the two homozygote classes. The segregation of the heteroduplex results from a physical interaction between the two alleles from both parents.

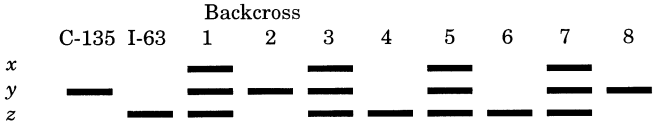

Fig. 2 Diagram of non-parental heteroduplex formation between allelic AFLP products from both parents (C-135 and I-63)

The 1:1 segregation of the markers with bands in both parents may be due to segregation distortion between two heterozygous parents, C-135 and I-63. If a normal Mendelian segregation pattern was followed, the markers heterozygous in both parents should not significantly deviate from the 3:1 ratio, as found for 15% of the polymorphic markers (Table 1). One possible explanation for those intercross markers deviating from 3:1 in the progeny is segregation distortion, a phenomenon commonly detected in forest tree mapping projects (T. Bradshaw, personal communications).

Linkage map

Because the markers segregating in alternative parents (called "testcross" markers) cannot be linked with each other using a full-sib family, the pseudo-testcross mapping strategy has been developed to construct two genetic maps specific to different parents (Grattapaglia and Sederoff 1994). However, these two different maps can be merged into a composite map by using those markers that are heterozygous in both parents (called "intercross"

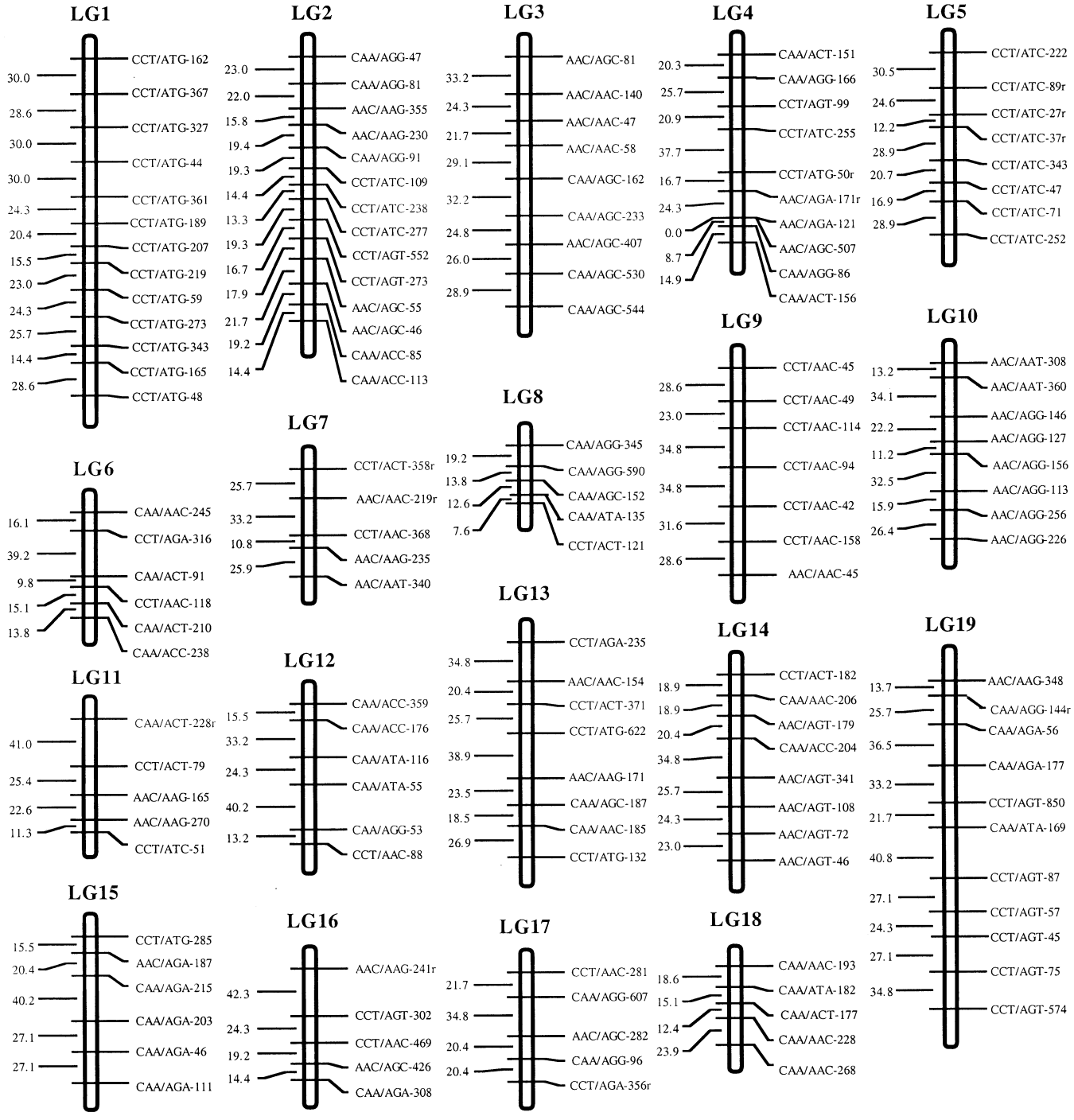

Fig. 3 An integrated linkage map for a backcross population of *Populus deltoides* constructed from AFLP markers. Maker names ending with *r* are in reverse linkage phase to those not so designated

markers) (Barreneche et al. 1998; Maliepaard et al. 1998). Such merging cannot be well performed when the intercross markers are dominant. Simulation experiments showed that dominant markers are very limited in producing a precise and unbiased estimate for recombination frequency (Maliepaard et al. 1997). In this study, a number of 1:1 segregating heteroduplex markers resulting from the interaction of alleles from both parents were detected which represent the nature of the intercross markers. As a result, we only used the heteroduplex markers as bridges to integrate the two parent-specific maps.

We first combined the testcross markers segregating in I-63 and the heteroduplex markers to construct a framework map and the testcross markers segregating in C-135 and the same heteroduplex markers to construct a second linkage map. Based on their relative positions in the second map, the testcross markers segregating in C-135 were then placed beside their closest heteroduplex

markers in the framework map. Thus, an integrated linkage map comprises the following three markers: (1) the testcross markers heterozygous in I-63 and (2) heterozygous in C-135 and (3) the intercross heteroduplex markers segregating due to the interaction of alleles from both I-63 and C-135.

At a LOD score of 4.0 and recombination frequency ≤ 0.25, 154 testcross markers segregating in I-63 and 105 intercross heteroduplex markers were assigned into 43 groups, with 19 major groups (Fig. 3) and 24 minor groups (five triplets and 19 doublets), while 69 markers remained unlinked. The same criteria were used to construct the second map using 77 testcross markers heterozygous in C-135 and the same heteroduplex markers. Only 8 of the testcross markers could be unambiguously placed in the framework map. Each of the 19 major linkage groups was ordered at a LOD score of 3.0. These 19 linkage groups covered 2,927 cM, with an average distance between 2 markers of 23.3 cM. The size of the linkage groups ranged from 53 cM to 295 cM, with an average of 154 ± 69 cM. The existence of these minor linkage groups and unlinked markers indicates that there are many large gaps with few markers (Kesseli et al. 1994).

We have tested the extent of marker clustering in each of the 19 major linkage groups using a non-parametric nearest neighbor analysis (Clark and Evans 1954). The ratio of the observed to expected distance to the nearest neighboring marker was estimated to examine the loci of a linkage group for "patchiness". We did not detect significant clustering of genetic markers on the linkage groups because the ratio was either greater than 1.0 (regular or even marker distribution) or equal to 1.0 (random distribution) for each linkage group (results not shown). Only when the ratio is less than 1.0, is the distribution of markers is not random (Clark and Evans 1954).

The AFLP markers amplified by the same primer pair were not randomly distributed on the map; many fragments amplified by the same AFLP primer pair were mapped on the same linkage group. For example, linkage groups 1 and 5 included all markers derived from E+CCT/M+ATG and E+CCT/M+ATC, respectively. In linkage group 9, most of the markers were amplified by E+CCT/M+AAC. In many other linkage groups, the markers from a particular primer pair were prevalent. Markers amplified by E+CAA/M+AGT and E+CCT/M+ACC were not assigned to any linkage group.

Discussion

AFLP and individual tree maps

Forest trees are a group of plants with many unique biological properties, such as long generation cycles, high genetic load and high heterozygosity. As a result of these properties, traditional quantitative genetic approaches have been very limited in providing an understanding of the genetic architecture of a natural or managed population of forest trees (Namkoong and Kang 1990). Genetic analysis using new molecular marker techniques provides a powerful approach to understand the organization and distribution of genetic resources in forest trees.

The AFLP technique developed by Vos et al. (1995) is robust and reliable for constructing a genetic map and identifying important loci for growth, resistance and adaptation in forest trees (Cervera et al. 1996; Marques et al. 1998; Paglia et al. 1998; Remington et al. 1999). AFLP markers assay the presence/absence of restriction enzyme sites and sequence ploymorphisms adjacent to these sites. Briefly, three crucial steps are followed to obtain AFLP markers: (1) digestion of genomic DNA with two different enzymes, such as *Mse*I (frequent-cutter enzyme) and *Eco*RI (rare-cutter enzyme); (2) ligation of adapter oligonucleotides to the restriction ends; and (3) selection of fragments by two successive PCR-based amplification steps using primers complementary to the adapter oligonucleotides with additionally one to three selective nucleotides.

The high yield of information achieved with AFLP markers makes it an extremely efficient tool for the analysis of informative testcross configurations compared to other anonymous marker systems (e.g., randomly amplified polymorphic DNA, RAPD), despite the increase in technical complexity. AFLP markers detect more point mutations per reaction than RAPD or restriction fragment length polymorphism (RFLP). They can identify tenfold the number of informative markers per analysis than RAPD markers (Grattapaglia and Sederoff 1994; Marques et al. 1998). The AFLP assay is sensitive to single base changes that cause the loss or gain of restriction sites, many kinds of rearrangements and changes in the DNA sequence recognized by the selective nucleotides.

Other advantage of AFLP markers is that in some cases different alleles belonging to a single AFLP locus can be identified from different parents (van Eck et al. 1995) and that the recombination of these alleles leads to the formation of heteroduplex, where non-complementary but similar strands anneal and then migrate more slowly on gels than the parental DNA fragments (Fig. 2; Davis et al. 1995; Antolin et al. 1996; Novy and Vorsa 1996). The identification of heteroduplex alleles descended from both parents (allelic bridges) is essential for the integration of the separate maternal and paternal linkage maps to one genome map. These heteroduplex markers segregating 1:1 are very informative but not intensive computationally for bridging the testcross markers heterozygous in alternative parents (Maliepaard et al. 1997). The 1:1 segregating heteroduplex alleles are recognized to represent a low fraction of the AFLP markers (van Eck et al. 1995). Although it may not be actually a case based on our findings in this study, this disadvantage can be largely compensated by a large number of markers generated by the AFLP technique.

Map construction

The genetic linkage map of *Populus deltoides* now comprises 198 AFLP markers. There are 19 large linkage groups of 53 cM or more in length and 24 smaller linkage groups. The 19 major linkage groups, whose total map distance (2,927 cM) is similar to the expected length (2,800 cM) estimated based on the physical size and recombination pattern of the *Populus* chromosomes (Bradshaw et al. 1994), is a first step in producing a highly saturated linkage map of the genome. However, the map has still not coalesced into the 19 chromosomes of *Populus* because of many minor linkage groups and unlinked markers, which suggests both that the AFLP markers identified are not randomly sampled throughout an entire chromosome (Paglia et al. 1998) and the existence of hot spots of recombination (Kesseli et al. 1994). To bridge these gaps we shall analyze the segregation of terminal and unlinked markers in expanded backcross populations as well as target by bulked segregant analysis markers to these regions.

Of all the polymorphic markers scored, 7% exhibited segregation distortion due to genetic load in *Populus deltoides*. Similar or higher percentages of distorted markers were detected in *Eucalyptus* (8–9%, Verhaegen and Plomion 1996), *Pinus* (14–15%, Kubisiak et al. 1995) and *Quercus* (18%, Barreneche et al. 1998). In these studies, there was a general trend towards a clustering of distorted markers on the linkage groups. However, distorted markers were not mapped in this study because an appropriate use of these markers for linkage analysis relies upon the genetic mechanism of segregation distortion. We have not tested possible mechanisms, as did Bradshaw and Stettler (1994) in the interspecific hybrids between *P. trichocarpa* and *P. deltoides*. In addition, we excluded those dominant markers which segregate in both parents for linkage analysis because these markers provided limited information for precisely estimating recombination frequency (Maliepaard et al. 1997).

The identification of optimal marker orders represents a significant issue for genetic mapping. Assembling loci into linkage groups is relatively easy for a marker analysis. However, the precise gene order becomes progressively more difficult to determine as the number of informative meioses becomes limiting; “final” orders are often only slightly more likely than alternatives. However, it is not critical to order all loci precisely. We are especially interested in a correct marker order in regions of typical interest, such as those flanking disease resistance genes. Several strategies can be used to solve mapping ambiguities in these regions. For example, we may increase the size of a mapping population and analyze those individuals that are recombinant in a particular region (Kesseli et al. 1994). Another significant issue that hampers all aspects of map development is experimental error such as misscored individuals or contamination. Errors inflate the number of apparent recombinants and expand map distance; this is especially severe when markers are tightly linked. Double crossovers and possibly misscored individuals or loci can be identified by specific commands in the current computer software (e.g. MAPMAKER, Lincoln et al. 1992).

Targeting regions to increase map density and bridge gaps

The average recombination distance between two neighboring markers was higher than that reported in previous mapping studies by Bradshaw et al. (1994), Grattapaglia and Sederoff (1994) and Marques et al. (1998) who demonstrated a high level of recombination suppression. One of the major differences between our cross and previous crosses was the evolutionary distance between the parents. Our study was an intraspecific cross of two selected clones originally from wide populations of *P. deltoides* in North America, while many of the previous estimates of recombination suppression were based on the recombination frequencies of interspecific hybrids, which have been shown to have more meiotic abnormalities (Bradshaw and Stettler 1994; Bradshaw et al. 1994). However, as shown by simulation experiments, wide marker spacings of 20 or even 50 cM may be optimal to initially scan the genome for QTL mapping (Darvasi et al. 1993). Also, from a statistical point of view, relatively even-spaced framework markers in our 19 major linkage groups provide a favorable property for QTL scanning throughout the entire genome. In this study, we found strong evidence for the accumulation of markers on a particular linkage group amplified by the same primer pair (see also Young et al. 1998).

The current genetic map can be made more dense by adding more markers genome wide. However, a more efficient approach is to develop bulked segregant analysis (BSA) to target markers to genomic regions of particular interest, usually those flanking disease genes (e.g., Michelmore et al. 1991). With the aid of BSA, we can identify and include additional molecular markers linked to the target genes. The identification of a region containing an unclustered downy mildew resistance gene *Dm13* of lettuce represents a good example of this technique (Kesseli et al. 1994). The BSA technique can be used to bridge the gaps existing in the map by linking those co-segregated markers to form a larger genetic group. Through the construction of a consensus map, this technique can also map genes that do not segregate in an original mapping population by combining information from multiple crosses.

Acknowledgments We thank Prof. R. R. Sederoff for kindly providing laboratory space for some of the experiments conducted in this study and Dr. D. M. O’Malley and Dr. D. L. Remington for helpful discussions and guidance on the experiments and analyses. This work is partially supported by grants from the Department of Forestry, China, and the National Institutes of Health (GM 45344) and by the NCSU-Industrial Biotechnology Associates.

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