FRAMEWORK LINKAGE MAPS OF *PINUS RADIATA* D. DON BASED ON PSEUDOTESTCROSS MARKERS

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ABSTRACT

Using a double pseudotestcross strategy, we have constructed framework linkage maps of two *Pinus radiata* D. Don trees (850.055 and 850.096) with RAPD, AFLP and SSR markers. These maps consist of 235 and 194 markers respectively, which mapped to 20 and 21 groups with three or more markers. Maps were constructed using a support-for-order criterion of LOD 3.0 for final order of framework markers, and covered 1413.7 and 1144.0 (Kosambi) cM each, equating to an estimated 85 and 77 % of genome covered within 10cM of a marker. Depending upon threshold LOD for declaring linkage, estimates of total map length for 850.055 ranged between 1927.2 and 2068.1 cM (Kosambi), and 1902.8 to 1998.1 cM for 850.096. These map lengths were not significantly different, indicating no differences in recombination rates between male and female gametophytes. These estimates of total map length were similar to other *Pinus* species, providing further evidence of an overall conservation in recombination rate in this genus. These framework maps will serve as a basis for adding new codominant markers, which in turn should further applications such as QTL detection, molecular breeding, and candidate gene mapping.

Keywords: Framework linkage map, *Pinus radiata*, pseudotestcross, molecular breeding, AFLP, RAPD, microsatellite.

INTRODUCTION

In recent years a considerable number of linkage maps have been reported for a host of economically important species. Motivation for constructing such maps has been fuelled in large part by the prospect of molecular breeding. Many maps have been constructed using marker technologies that may ultimately be replaced with newer, more fit-for-purpose marker systems. For forest trees, dominant PCR-based markers such as RAPDs and AFLPs have been extensively used for linkage mapping. However, codominant marker systems such as simple sequence repeats (SSRs) and expressed sequence tags (ESTs) are more likely to be useful in tree improvement, as breeding programs predominantly use multiple unrelated and highly heterozygous parents for both advanced-generation breeding, and seed production for establishment of commercial plantations. Thus, as we move toward operational implementation of marker-assisted selection, a framework map should provide a suitably robust structure on which to place new markers with some degree of confidence.

KEATS *et al.* (1991) recommended statistical guidelines for constructing framework maps. For example, to accept a specific locus order, a log-likelihood difference of 3.0 ('support for order') is recommended, i.e., the accepted order is 1000 times more likely for the data provided compared to the next best (evaluated) order. Such guidelines have been applied for constructing genetic linkage maps of a number of economically important forest tree species including *Pinus pinaster* (PLOMION *et al.* 1995), *P. taeda* (WILCOX 1995 unpubl. PhD thesis), *P. strobus* (ECHT & NELSON 1997), *Eucalyptus grandis* and *E. europhylla* (GRATTAPAGLIA & SEDEROFF 1994) and *E. globulus* and *E. teretecornis* (MARQUES *et al.* 1998). An alternative method has been propsed by LIU (1998) and used by REMINGTON *et al.* (1999) to construct a genomic map of *P. taeda*.

We have constructed a framework map, initially for the purposes of detecting quantitative trait loci controlling variation in commercially important traits in radiata pine (WILCOX *et al.* 1997, KUMAR *et al.* 2000), but also for the purpose of providing a suitably robust framework into which we can place new codominant markers.

P. radiata pine is widely recognised as a commer-

cially versatile species, with uses ranging from provision of fibre for various pulp and paper products, to moderate-high value sawn timber. This, in addition to it's comparatively fast growth rates and silvicultural flexibility, has made it one of the most commercially important conifer species worldwide. While native to coastal California and some outlying islands, it is mostly planted as a timber species in southern hemisphere regions with temperate maritime climates (New Zealand, and parts of Australia and Chile), as well as some (limited) plantation areas in the northern hemisphere (e.g., Spain). There are a number of active radiata pine breeding programs, particularly in Australasia, where some have been in existence for 50 years (eg., SHELBOURNE *et al.* 1986), with well demonstrated genetic gains (eg. CARSON *et al.* 1999). The commercial importance of this species, combined with the successful application of breeding and propagation technologies, has no doubt contributed to interest in developing molecular breeding technologies.To this end, a range of linkage maps have been constructed for this species (reviewed by WILCOX 1997), and a consensus map between radiata and loblolly pines (*Pinus taeda* L.) has also been constructed (DEVEY *et al.* 1999). Here, we report two framework linkage maps based on dominant markers that will serve as templates for adding new codominant markers for the purposes of both molecular breeding and comparative mapping.

MATERIALS AND METHODS

Plant Materials

A population of 93 18 year-old trees was randomly chosen in 1996 from a single full-sib family which had been planted in a production plantation as part of a genetic gains experiment (CARSON *et al.* 1999). The parent trees, 850.055 and 850.096, were originally selected on the basis of superior size and form in 1950 from among trees planted in forest plantations. These two trees have been subjected to numerous genetic evaluations, primarily via progeny tests (SHELBOURNE *et al.* 1986), and tree 850.055 has been recognised as an outstanding genotype for growth and form, as well as having fibre properties suitable for thermomechanical pulping (CORSON *et al.* 1989), despite lower-than-average wood density (NYAKUENGAMA *et al.* 1998). In this study, 850.055 was used exclusively as the cone (female) parent, and 850.096 was used as the pollen parent. Progeny trees chosen for this study had been planted in Compartment 1210, Kaingaroa Forest, in the Bay of Plenty region of New Zealand, at an original spacing of 711 trees per hectare. These trees had not been subjected to any silvicultural selection, although a low level of mortality was evident when trees were originally chosen for this study (CARSON *et al.* 1999).

DNA extraction and generation of marker data

Needle tissue was collected from each tree by shooting needle clusters from live branches using a 12 gauge shotgun, as live crowns were generally at least 8 meters above ground at the time of sampling. Genomic DNA was extracted either by a modified CTAB protocol (CATO & RICHARDSON 1996), or using a FastDNA kit (BIO 101, Vista, CA) with a FastPrep FP120 machine (Savant Instruments, Holbrook, NY) as per manufacturers' specifications. Three different marker systems were used to generate genotypic data: random amplified polymorphic DNA (RAPD, WILLIAMS *et al.* 1990), amplified fragment length polymorphisms (AFLP, VOS *et al.* 1995), and simple sequence repeats (SSR, WEBER 1990).

For RAPD markers, primers used to genotype the mapping population were chosen after screening the two parents plus six progeny for bands segregating as pseudotestcross markers (i.e., present in one parent, segregating in the progeny, and absent in the other parent). Primer screening and genotyping were carried out as described by KUANG *et al.* (1999). For the RAPD markers, the two parents plus 87 offspring were genotyped with 72 pre-screened ten base-pair primers. Parents which were included as a control to confirm segregation patterns. For AFLP markers, the method adapted for use in radiata pine as described by CATO *et al.* (1999) was used to generate all genotypic data. The parents and a further six offspring were genotyped for AFLP markers, in addition to the 87 that had been genotyped with RAPD markers (ie., 93 offspring). A total of 57 primer combinations were used (CATO *et al.* 1999). SSR genotypes were determined for the parents and the same 87 individuals as the RAPD markers. SSR marker genotypes were determined using a total of ten primer pairs: two pairs identified by SMITH and DEVEY (1994) and eight pairs described by FISHER *et al.* (1998), including one which amplified two loci. FISHER *et al.* (1998) provides a full description of PCR conditions, electrophoresis and segregation and transmission patterns for the SSR loci. Genotypes were manually assigned (and independently verified) from either gel or autorad images.

Sizes of polymorphic RAPD fragments as well as images of AFLP autoradiographs and primer sequences are available on request from the senior author.

Segregation ratios were calculated using a chi-square goodness-of-fit method as implemented in Qgene 2.30 (NELSON 1997). Marker loci with segregation ratios differing from expected 1:1 ($p < 0.001$) were

noted, but not initially eliminated from linkage analysis on the basis that biologically-based segregation distortion in this species has previously been reported (KUANG *et al.* 1999), and because distorted markers in backcrosses could nonetheless be mapped without biasing estimated recombiation frequencies (BAILEY 1961, KUANG *et al.* 1999).

Map Construction

Linkage maps were constructed using a strategy where one of the two outcrossed parents is heterozygous for a marker locus and the other parent is homozygous, so that one set of progenies can be used to construct linkage maps of each parent independently. Because both parents are heterozygous for different loci (except some of the codominant SSR – see below), it is possible to construct linkage maps of both outbred parents using the same set of progeny if there are sufficiently large numbers of markers (e.g., GEBHARDT *et al.* 1990, BARZEN *et al.* 1992, ECHT *et al.* 1994, GRATTAPAGLIA & SEDEROFF 1994). As most of the SSR primer pairs amplified loci that were fully informative (both parents heterozygous and segregating for 3 or 4 alleles per locus), progeny genotypes were scored as pseudotest-cross markers for each parent, and analysed along with the other markers in the same data set.

Data sets were constructed and analysed separately for each parent. The Macintosh OS version of Mapmaker/Exp (LANDER *et al.* 1987), Mapmaker Macintosh 2.0, was used for linkage analysis and map construction. Because this software assumes phase-known data generated from inbred lines, our phase-unknown genotypic data had to be 'inverted' by exchanging genotypic scores for every locus so that repulsion phase linkages could be detected and recombination fractions estimated (e.g., NELSON *et al.* 1993). Markers appearing to segregate as pseudointercross loci were not included in this analysis.

To construct preliminary partial linkage maps of each parent, markers were grouped using a maximum recombination fraction of 0.28 and a minimum log-likelihood-of-odds (LOD) ratio of 4.5. Marker orders for groups with seven markers or less were determined by comparing all possible orders, and accepting the most likely order. For groups with eight or more markers, we used the FIRST ORDER command in Mapmaker Macintosh 2.0 to determine the most likely order. A framework map of each group was then constructed by dropping markers from groups where the most likely order was less than 1000 times greater than the next most likely local order as determined using the RIPPLE function in Mapmaker Macintosh. In this step, marker orders were permuted three markers at a time and the log likelihood difference between the initial and alternative order calculated. Where a particular order was less than 1000 times more likely, a marker was dropped on the basis of either (a) spacing, or (b) deviation from triangular equality. The effect of dropping a marker was evaluated using the DROP MARKER command in Mapmaker. The order of the remaining markers was then re-evaluated as above. This process was repeated for every marker dropped until the order of the remaining markers was 1000 times more likely than any other local order. For groups with seven markers or less, the log-likelihood difference between the final and next most likely order was checked using the COMPARE function, rather than FIRST ORDER, to ensure that the order was the best possible order for all candidate framework markers.

Once linkage groupings and framework marker orders were evaluated, further analyses were undertaken to merge syntenic linkage groups. Two approaches were used: an analytical method, and a comparative method using an existing linkage map. For the analytical method, data sets were constructed consisting only of terminal markers from the framework linkage groups, plus unlinked markers. Putative groups were then determined by lowering thresholds to a maximum recombination fraction of 0.35 and a minimum LOD ratio of 4.0. Any merged groups were then re-ordered using all framework markers from those groups. Merged groups were only accepted as being syntenic when the resulting order was consistent with orders prior to merging, and where the condition of triangular equality was satisfied. Therefore, we did not accept merges where the closest linkage involved at least one non-terminal framework marker, i.e., the minimum recombination fraction between markers from the two groups had to involve terminal markers from both groups. The second approach used to merge syntenic groups was based on an existing RAPD and SSR map of tree 850.055 (KUANG *et al.* 1999) that was generated from megagametophytes derived from self-pollinated seed. This method was applied to the (outcross-derived) map of 850.055 only. Here, markers common to both maps were identified, and syntenic groups were decided on the basis that at least one framework or accessory marker was common to both maps. RAPD markers generated by the same primer and of similar size (+/– 50 base pairs) in the selfed and outcrossed mapping populations were considered to be the same marker allele.

Genome Length and Map Coverage

Total map length was estimated by using Method 3 of CHAKRAVARTI *et al.* (1991), which is a modification of

a maximum-likelihood method described by HULBERT *et al.* (1987), where total map length is estimated from the ratio of the number of total marker pairs to the number of marker pairs at or exceeding a pre-set LOD threshold for linkage, multiplied by the map distance corresponding to the maximum observed recombination at or exceeding the pre-set LOD threshold. These distances were modified for regions not accounted for at the termini of linkage groups using the adjustment described by REMINGTON *et al.* (1999). Map distances were based on the Kosambi mapping function (KOSAMBI 1944).

Confidence intervals for the estimated map lengths were determined by simulation. Here, the total number of markers for each map was randomly assigned to 12 chromosomes with a genome size equal to the estimated length as described above. The observed number of pairwise linkages and total map lengths were then calculated for the simulated map according to the criteria described above. The process was repeated 40 000 times for each map/LOD threshold combination. Empirical confidence intervals were the values corresponding to the 5th and 95th percentiles of the distribution of simulated map lengths.

In order to compare results with other *Pinus* species we used the approach of ECHT and NELSON (1997) where we estimated map length by varying LOD thresholds (3.0, 4.0, 5.0) using only loci with less than 15% missing data.

Map coverage was estimated using two methods: firstly the proportion of the genome covered *within* linkage groups was calculated simply by dividing the area covered within linkage groups by the estimated total map length. The second method estimated the proportion of the genome covered within 10 cM of a marker based on the assumption of random marker distribution:

$$E(c) = 1 - e^{-2dn/L}$$

where $E(c)$ = expected coverage within d (=10) cM of a marker, n = number of markers, and L is the estimated total genome length (LANGE & BOEHNKE 1982, REMINGTON *et al.* 1999).

RESULTS

A total of 235 markers were mapped in 850.055 and 194 for 850.096 (Table 1). The majority of loci on both maps were AFLP markers (67–71%), with RAPD markers the next most frequent, and microsatellite markers the least abundant (Table 1). For 850.055, there were 20 groups following the merging analysis (see below), with the number of markers per group ranging from 3 to 31 (Figure 1a). One further group consisted of only two markers. Of the 235 marker loci, 22 were not linked to any other marker at the thresholds described above. For 850.096, the map consisted of 21 groups ranging between 3 and 22 markers per group (Figure 1b). A further two groups contained only two markers. Of 194 markers, 28 were not linked to any other marker. The proportion of framework markers (of total markers) was similar between maps: 51.0 % for 850.055 and 47.4 % for 850.096. The total map distance covered within linkage groups was 1413.7 (Kosambi) cM for 850.055, and 1144.0 cM for 850.096. Map densities were also similar: 11.8 cM between

Table 1. Marker quantities by type for each map.

Marker type	Parent	
	850.055	850.096
SSR	11	9
RAPD	57	56
AFLP	167	129
Total	235	194

Table 2. Estimated map lenghts and coverage corresponding to different LOD thresholds.

Parent	LOD threshold	Maximum observed theta (cM Kosambi)	Number of loci (<15 % missing data)	Number of locus pairs with theta > LOD threshold	Estmated map length (cM Kosambi)	95 % confidence interval (cM)	
						upper	lower
850.055	3	34.1	188	556	1927.2	1713.8	2132.1
	4	30.6	188	474	2068.1	1843.7	2284.7
	5	26.7	188	420	2061.2	1835.7	2281.9
850.096	3	35.0	143	322	1972.1	1756.4	2177.2
	4	29.9	143	289	1902.8	1694	2104.8
	5	27.1	143	253	1998.1	1777.9	2207.8

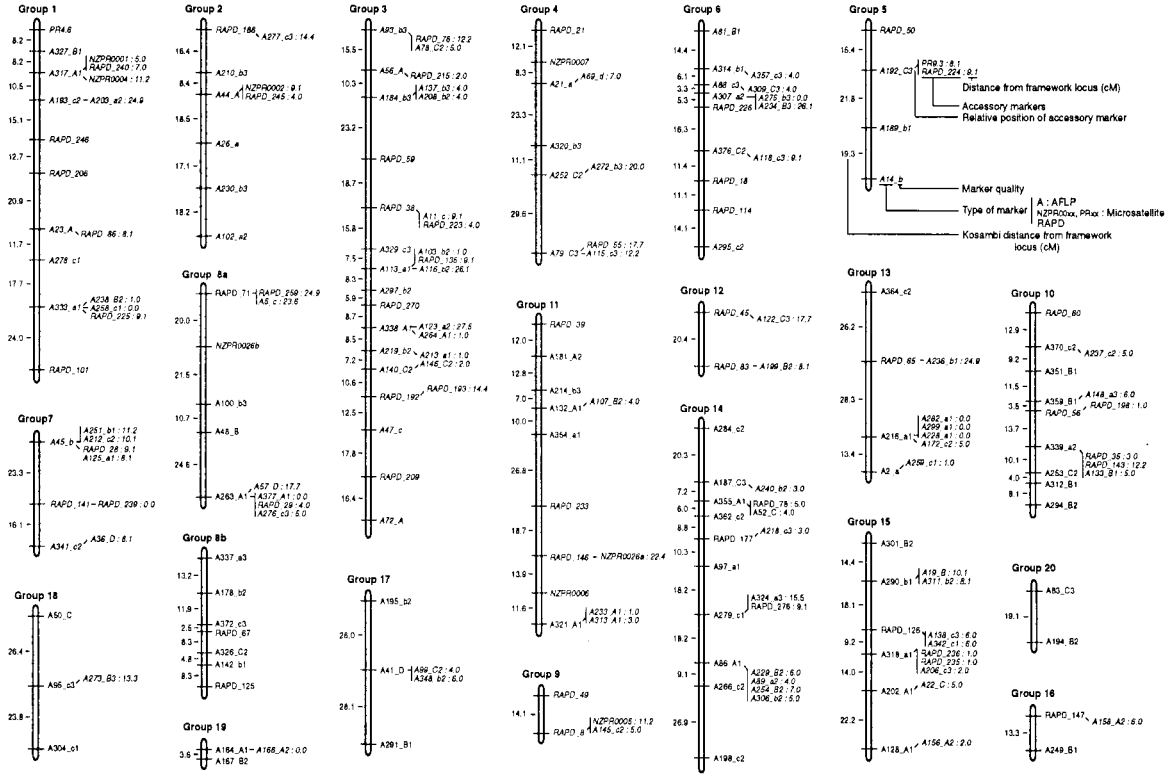

Figure 1a. Framework linkage map of tree 850.055.

markers on the 850.055 map and 12.4 cM on the 850.096 map.

Map lengths were calculated using a subset of markers (< 15 % missing data) for different LOD thresholds as recommended by ECHT and NELSON (1997), using a maximum likelihood method (HULBERT et al. (1987), CHAKRAVARTI *et al.* (1991), REMINGTON *et al.* (1999)). Estimates of total map length were similar for both parents (Table 2), ranging from 1927 to 2068 cM for 850.055, and 1902 to1998 cM for 850-.096. Map lengths of the two parents were not significantly different, irrespective of LOD threshold used (Table 2).

Merging of linkage groups was investigated using two approaches: an analytical approach, and direct comparison with a previously constructed map (described above). The analytical method resulted in two sub-groups being merged on each maps: linkage group 11 on the 850.055 map, and linkage group 17 on the 850.096 map. For the 850.055 map we were able to merge one further group (8a and 8b, Figure 1a) based on markers from a previously constructed map of the same parent (Kuang *et al.* 1999), where each group had at least one common framework marker.

Genome coverage within linkage groups was slightly greater for 850.055 (68.3–73.4 % compared to 57.3 – 60.2 % for 850.096). Similarly, the proportion of within-group genome coverage to within 10cM of a marker (ie., takes into account teminii of linkage groups) was also greater for 850.055 than 850.096 (83.8–85.8 % compared to 76.1–77.8 %) (Table 2).

Both parents had markers with segregation ratios significantly different from expected 1:1 (comparison-wise $p < 0.001$). For 850.055, there were a total of 11 distorted markers (4.7 %) and for 850.096, 7 (3.6 %) markers were distorted. For 850.055, 7 of the 11 distorted markers were not linked with any other marker. Of the remaining four, two were adjacent framework markers on linkage group 2, and the other two markers mapped to different groups. For 850.096, six of the eight distorted markers were linked with other markers. Of these, three made up an entire group (linkage group 19), indicating another possible region of distortion. The remainder mapped to different linkage groups. All seven of the unlinked distorted markers on the 850.055 map exhibited segregation ratios that were not significantly different from 3:1 segregation ratios (comparison-wise $p < 0.05$). Likewise, both of the unlinked, distorted markers on the

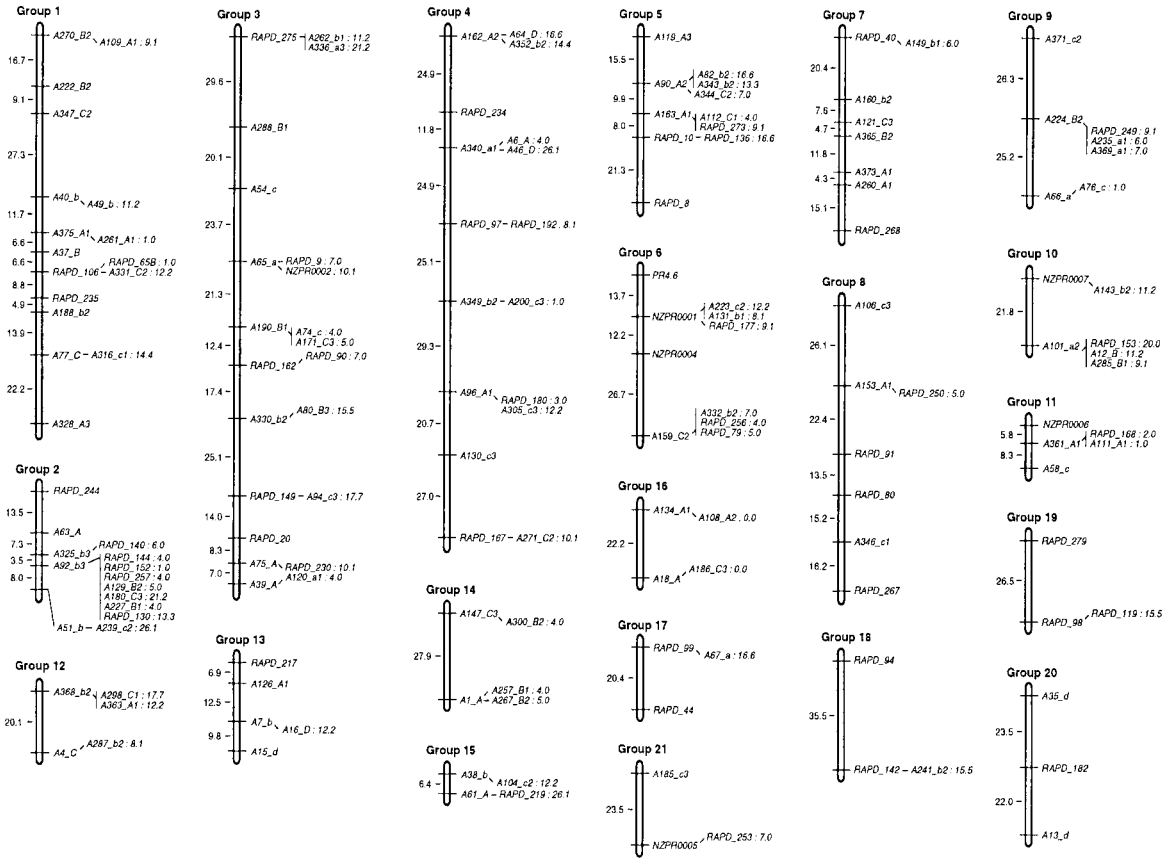

Figure 1b. Framework linkage map of tree 850.096.

850.096 map were also consistent with 3:1 segregation ratios.

The small number of SSR markers enabled us to identfy six putative syntenic groups on the two maps (Table 3). For example, Group 1 on the 850.055 map contained three linked SSR markers. The same three markers were also linked on the 850.096 map (Group 6).

DISCUSSION

We have presented here two linkage maps constructed using standardised criteria for choosing framework markers and their corresponding orders. Because these maps will be used to place new markers and to judiciously choose subsets of new markers for further experiments, the structure must be suitably robust. Comparison of framework maps constructed using the same criterion may provide some valuable (empirical) insights. We compared the map of 850.055 described here with that constructed from megagetophytes of seeds from self-pollination from the same parent (KUANG *et al.* 1999). Because the outcross map was constructed from predominantly AFLP markers, only a subset (46) of RAPD and SSR markers were common to both maps. In only one case, linkage group 3 on the outcross map (= linkage group 1 on the self map), did

Table 3. Syntetic linkage groups in the two maps as determined with SSR marker loci.

Marker	850.055 group	850.096 group
NZPR001	1	6
NZPR002	2	3
NZPR0004	1	6
NZPR0005	9	21
NZPR0006	11	11
NZPR0007	4	10
NZPR0026a	11	UN[1]
NZPR0026b	8	UN[1]
NZPR0035	UN	NS[2]
PR 4.6	1	6
PR 9.6	5	NS[2]

[1] UN = unlinked; [2] NS = homozygous in this parent.

a linkage group consist of three common framework markers on both maps. In this case, order was identical (data not shown). In a far more comprehensive study, PLOMION *et al.* (1995) showed that relatively few differences in order (equating to approximately 2% error per map) were found when constructing independent framework maps of the same tree, again from comparison of self and outcross-derived populations. This level of error, while greater than implied by the LOD 3 criterion, seems reasonable for the intended purpose of these maps. However, the likelihood ratio approach used in this study does not account for alternative possible models (orders) weighted by the probabilities of observing these data given these alternative orders. Therefore these results should be treated with some caution, as orders presented here may not in some cases be the correct order, and that the evidence for the given order may be overestimated using a likelihood approach.

Other approaches to framework map construction have been reported. REMINGTON *et al.* (1999) used a resampling approach to determine empirical confidence intervals for given orders based on methods described by LIU (1998). WILCOX (1995 unpubl. PhD thesis) compared this approach with that used in the present study for construction of framework linkage maps, and found that the resampling method identified 151 out of 199 markers (of 314 total) initially chosen using the likelihood approach described here. The order of the 151 common markers was identical. In a similar comparison for two *Eucalyptus* AFLP maps, MARQUES *et al.* (1998) reported approximately 50% of framework markers were identified by both methods, with approximately 12% difference in locus order.

The genome length estimates reported here are similar to those reported in several independent studies for radiata pine (WILCOX 1997, KUANG *et al.* 1999), indicating a map length of approximately 2000cM (Kosambi). Genome length estimates derived from independent and unrelated parents were not significantly different, indicating no significant differences in recombination rates between male and female gametophytes. This result is consistent with that of DEVEY *et al.* (1996) for the same species, but differs from a more limited study based on isozyme loci by MORAN *et al.* (1983). The result also differs from GROOVER *et al.* (1995) and SEWELL *et al.* (1999) who found genome size estimates for the male gametophyte to be significantly larger for loblolly pine. However, WILCOX (1995, unpubl. PhD Thesis) and REMINGTON *et al.* (1999) reported larger map lengths based upon female megagametophytes for loblolly pine, but with greater genome coverage and more marker loci than the studies reported by GROOVER *et al.* (1995) and SEWELL *et al.* (1999).

By estimating genome length using the standardized approach recommended by ECHT and NELSON (1997), we found that the estimated genome lengths for these radiata pine trees were not significantly different to *P. palustris*, *P. pinaster* and *P. strobus* (ECHT & NELSON 1997), as well as male gametophyte maps of *P. taeda* (Sewell *et al.* 1999). These results are consistent with those of ECHT and NELSON (1997) that map length does not differ among taxonomic subsections within the genus *Pinus*.

For this study, map distances were estimated using the Kosambi (KOSAMBI 1944) mapping function. The adequacy of this function was not validated for these data although EMEBIRI (1997 unpubl. PhD thesis) found Kosambi's function fitted the data better than HALDANE's (1919) for radiata pine, similar to *P. taeda* (WILCOX 1995 unpubl. PhD thesis). Whether or not the Kosambi mapping function is the most suitable for these data is yet to be determined, given the range of alternative approaches (see LIU 1998), and is a topic for further study. In the context of our perceived use of these maps (adding new markers and selecting subsets for QTL detection), choice of mapping function is less relevant than applications such as map-based cloning.

A number of markers on both maps were identified with distorted segregation ratios. On both of the maps, a single region contained several distorted, linked markers. For 850.055, we were able to make some limited comparisons with the map constructed by KUANG *et al.* (1999). Because of the paucity of common markers, we were unable to determine if the distorted region in the outcross map reported here also exhibited segregation distortion in the self map. On the genome-wide basis, the widespread segregation distortion reported by KUANG *et al.* (1999) for the self map was not evident on the outcross map described here, suggesting most of the distortion reported by KUANG *et al.* (1999) is likely to be associated with inbreeding. In particular, severe distortion associated with the *Sdpr* locus was not evident in either outcross map providing further evidence that *Sdpr* is associated with inbreeding. The underlying reason for the distorted regions on both of the outcross maps reported here is not known, and is a topic for further investigation.

Some of the unlinked markers exhibiting aberrant segregation ratios may be due to non-allelic polymorphisms of very similar size that were not resolved by electrophoresis. Such markers, if unlinked, would appear to segregate as a single marker on a gel with a 3:1 segregation ratio. Indeed all of the markers that were both unlinked and were significantly distorted from expected 1:1 segregation ratios, were consistent with 3:1 segregation ratios.

Both maps had more linkage groups than the number of chromosomes per haploid nucleus 12 (20 and 21 linkage groups for 850.055 and 850.096 respectively, compared with 1C = 12 chromosomes). This result is not dissimilar to other studies in this species: for example, DEVEY *et al.* (1996) reported 22 linkage groups with 96 progeny genotyped with 208 loci. Furthermore, for *Pinus taeda* L., which has a similar map length and same number of chromosomes, REMINGTON *et al.* (1999) genotyped over 500 markers to get the same number of linkage groups as haploid chromosomes. In their study, a similar number of offspring (96) was used to that in the study reported here (87–93). Given the similarity in map length between the two species, a similar number of markers may be necessary in *P. radiata* to reduce the number of linkage groups down to equal to the haploid chromosome number.

Highly multi-allelic codominant marker systems are becoming the tool of choice for marker-assisted breeding programs in conifers, particularly as selection in many unrelated pedigrees will be required. We are currently using the maps reported here to place new markers and select subsets for genotyping an unrelated QTL detection population. Coverage is therefore a critical issue, and the maps described here have a significant proportion of the genome covered, therefore they should provide sufficient coverage with which to add and select new markers. The construction of a suitably robust framework map will mean increased confidence in the position of new markers, and therefore a more judicious choice of which of these markers to use for future studies. Furthermore, we envision that these framework maps will ultimately lead to construction of a single map consisting of only codominant markers, and that this map will be used for marker assisted breeding in radiata pine.

We are now in the process of using these framework maps to place additional SSR loci and polymorphism associated with expressed genes (CATO *et al.* 2001). These codominant markers will allow us to undertake cost-effective molecular breeding (across multiple pedigrees), as well as comparative mapping with other pine species, and ultimately candidate gene mapping.

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