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Linkage mapping and genome length in eastern white pine (*Pinus strobus* L.)

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Abstract Haploid linkage analysis of eastern white pine, *Pinus strobus* L., was carried out using mainly RAPD markers and microsatellite, or simple-sequence-repeat, markers. Ninety one loci mapped to 12 linkage groups of three or more markers. The resulting framework genome map, the first for a soft pine species, contained 69 markers. The map covered 58% of the estimated genome length of 2071 cM(K), with a 95% confidence interval of 1828–2242 cM(K). A systematic comparison of linkage data from eastern white pine, longleaf pine (*P. palustris* Mill.) and maritime pine (*P. pinaster* Ait.), gave genome-length estimates for all three species very close to either 2000 cM(K) or 2600 cM(H), depending on whether the Kosambi(K) or Haldane(H) map functions, respectively, were employed. Differences among previous pine genome-length estimates were attributed to the divergent criteria used in the methods of estimation, and indicate the need for the adoption of uniform criteria when performing genome-length estimates. Current data suggest that members of the two pine subgenera, which diverged during the late Mesozoic era, have highly conserved rates of recombination.

Key words Genome mapping · Map length · Pines · RAPD · Microsatellite DNA

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Introduction

Eastern white pine (*Pinus strobus* L. 2n = 2x = 24) is the only native 5-needled pine in eastern North America. It is a long-lived, ubiquitous, pioneer species that is naturally outcrossing, but is more tolerant of inbreeding than most other pines (Johnson 1945; Fowler 1965; Franklin 1970). Its abundance has been decreasing for the past 4000 years (Jacobson and Dieffenbacher-Krall 1995), hastened in recent times by extensive logging at the turn of the century. As a timber resource, *P. strobus* is still highly valued, but re-establishment efforts are hampered in many areas by seedling and sapling mortality resulting from white pine blister rust infections. This rust can infect all North American 5-needled pines, which as a group are known as white pines of the subsection *Strobi* (following the classification of Little and Critchfield 1969). Mature stands have not yet been subject to epidemic outbreaks of the disease, as has occurred among sugar pine (*P. lambertiana*, Dougl.) stands in California, but the threat is a concern among landowners and foresters. A white pine blister rust resistance gene has been identified and genetically mapped in sugar pine (Kinloch 1992; Devey et al 1995). Unlike many pines of the subsection *Strobi*, *P. strobus* and *P. lambertiana* do not intercross, thus limiting comparative mapping studies of resistance genes to the use of linked homologous marker loci.

The abundant phenotypic variability (Buchert 1994; Beaulieu and Simon 1995) and high heterozygosity (Eckert et al. 1981; Beaulieu and Simon 1994; Echt et al. 1996) found in *P. strobus* indicate that significant gains in growth, timber quality, and stress-tolerance traits could be realized from selection and breeding. Forest-tree improvement programs are expected to benefit most when the co-dominance and high variability of simple sequence repeat (SSR, or microsatellite) markers are exploited for marker-aided selection and breeding (Grattapaglia and Sederoff 1994; Devey et al. 1996). Genetic linkage information in *P. strobus* has been

limited to isozyme markers (Eckert et al. 1981; Beaulieu and Simon 1994), but SSR markers are being developed for *P. strobus* (Echt et al. 1996).

We report here a RAPD/SSR framework linkage map for *P. strobus*. RAPDs were used to provide genomic linkage information for five SSR loci and a single sequence-tagged-site chitinase pseudogene. RAPD markers quickly provide linkage information (Welsh and McClelland 1990; Williams et al. 1990), especially in conifers where haploid megagametophytes can be used for genotyping (Guries et al. 1978; Tulsieram et al. 1992). If RAPD and SSR loci are randomly distributed in the pine genome, then the early use of RAPDs in map construction can reduce overall efforts needed to achieve complete map coverage using SSR loci.

Pines in general have very large nuclear genomes, with 1C estimates ranging from 19 to 27 pg (Wakamiya et al. 1993; Valkonen et al. 1994; O'Brien et al. 1996). Based on linkage data, genome-length estimates for hard pines range from 1288 cM for *P. pinaster* (Plomion et al. 1995 a) to 3000 cM for *P. elliotii* (Nelson et al. 1993). If reported differences among genome-length estimates have a biological basis it would indicate dramatically different recombination rates within the genus, whose origin dates back at least 130 million years (Millar 1993). We re-examined some of the hard pine linkage data and report systematic comparisons of genome-length estimates between two unrelated hard pines and *P. strobus*, a soft pine.

Materials and methods

DNA isolation

DNA was extracted from 72 *P. strobus*, clone P-18 (open pollinated), megagametophytes using a procedure adapted from Guillmaut et al. (1992). Each megagametophyte was homogenized in 100 mM sodium acetate, 50 mM sodium chloride, 50 mM EDTA, 1.4% SDS, 0.5% polyvinyl pyrolidone and 60 mM cysteine, pH 5.3. The homogenate was incubated at 55°C for 30 min, followed by the addition of a 0.5 vol of 3.5 M potassium acetate, pH 5.3. The chilled mixture was cleared by centrifugation, transferred to a fresh tube, and the DNA precipitated in isopropanol. The DNA was re-suspended in 10 mM Tris-Cl, 1 mM EDTA, pH 8, treated with RNase, and extracted twice using Strataclean resin (Stratagene Cloning Systems). The concentration of the purified DNA was measured by fluorometry. Average yields were 14 μg of DNA per seed. For SSR analysis only, 10 ng of each DNA template were transferred into 96-well, V-bottom plates, then dried in a food dehydrator at 50°C, and stored at −20°C until used for PCR.

Marker analysis

RAPD analysis was carried out as described by Nelson et al. (1994) using 10-mer primers obtained from both Operon Technologies, and the Biotechnology Laboratory at the University of British Columbia. The primers we used were selected as those most likely to reveal polymorphisms among coniferous species (C. D. Nelson and W. L. Nance, unpublished), and are available as a set from the UBC Biotechnology Laboratory. RAPD loci names denote the supplier (OP = Operon, BC = British Columbia) and the name of the primer used, followed by the approximate marker size in base pairs. SSR loci were named and analyzed as described by Echt et al. (1996). Segregation data for a chitinase pseudogene, *Pschi* 1, (GenBank accession No. U57409), were obtained using single-strand-conformation polymorphism (SSCP)-gel analysis of a 253-bp PCR fragment amplified with the primer pair CGTGTTCGATGTTGTGGTCAGCAAAGAG and ATTTGGCTACCATGACCAACGT.

Chi-square values for 1:1 segregation data were calculated using the Yates' correction for continuity (by subtracting 0.5 from the absolute value of the difference between the observed and expected classes). The null hypothesis for non-Mendelian inheritance was rejected when $P > 0.05$. Linkage analyses for the haploid data were done with the MAPMAKER program (Lander et al. 1987), version 2.0 for Macintosh (generously provided by S. Tingey, DuPont Inc). Marker phenotypes were reciprocally coded for each locus so that the program could calculate coupling- and repulsion-phase linkages from the arbitrary linkage-phase data (Nelson et al 1993; Plomion et al. 1995 a). Subsequent map construction followed established principles and recommendations (Keats et al. 1991; Plomion et al. 1995 a) for framework mapping. After loading the file as "haploid" data, loci were assigned to linkage groups based on a lod score (log of likelihood odds) ≥ 4.0 and an r (recombination fraction, or theta) ≤ 0.30. A preliminary order of loci within each linkage group was assigned by the "First Order" matrix-correlation method, and final orders, determined with the help of the "Ripple" function, had an interval support ≥ 3. As individual unsupported loci were dropped from a framework group, the order and interval support of the remaining loci were re-checked. After a minimized set of framework loci was obtained for a group, dropped markers were added back into likely intervals, one at a time, and re-evaluated to confirm their lack of suitability as framework loci. Unsupported loci were included in the final map by listing them as ancillary markers at the side of their linkage group (Plomion et al. 1995 b). As recommended by Keats et al. (1989) and Ott (1991), map distances derived using the Haldane (1919) map function were expressed as cM(H), while those using the Kosambi (1944) map function were expressed as cM(K).

Genome-length and map-coverage estimations

Genome lengths, G, were estimated using the method-of-moment estimator, $E(G) = 2MX/K$ (Hulbert et al. 1988), where M is the number of informative meioses, X is the maximum observed map distance among the locus pairs above a threshold lod, Z, (Chakravarti et al. 1991) and K is the number of locus pairs having lod values at or above Z. The values used for Z were 3, 4 and 5. Since all meioses were informative in the haploid mapping population, $M = n(n-1)/2$, where n is the number of loci analyzed. The values of X and K were obtained from an inspection of the list of values generated by the "lods . . . " function of MAPMAKER. When using the "lods . . . " function, the upper limit of r was set to 0.5 so that all locus pairs above the given lod limits could be found. Values for X were obtained using both the Haldane and Kosambi map functions. The confidence interval for G, $I_\alpha(G)$, was calculated from the equation, $I_\alpha(G) = E(G)(1 \pm n_\alpha K^{-1/2})^{-1}$, where $n_\alpha = 1.96$ for an α of 5% (Gerber and Rodolphe 1994).

The expected genome map coverage, $E(C_n)$, was calculated from the equations,

$$E(C_n) = 1 - P_{1,n},$$

and

$$P_{1,n} = \frac{2R}{n+1}\left[\left(1-\frac{X}{2G}\right)^{n+1} - \left(1-\frac{X}{G}\right)^{n+1}\right] + \left(1-\frac{RX}{G}\right)\left(1-\frac{X}{G}\right)^{n}$$

(Bishop et al. 1983), where R is the number of chromosomes ($R = 12$), X is the maximum cM distance when $Z = 4$ (and is dependent on the map function employed), and G is either 2600 cM(H) or 2000 cM(K), depending on whether the Haldane or Kosambi map functions, respectively, were evaluated. Observed genome map coverage, C_O, was calculated from the equation, $C_O = G_F + X(L - R)$, where G_F is the total cM length of the framework map, X is that used for determining $E(C_n)$, and L is the total number of linkage groups, pairs and unlinked loci. This method for calculating map coverage takes into account the maximum distance, X, of detectable linkages to unlinked loci and the ends of linkage groups, as well as the reduction in coverage, $1/2X$, expected at the ends of chromosomes (Nelson et al. 1994).

Analysis of other pine species

For systematic comparisons of white pine to other pine species, phenotype-segregation data were obtained from outside laboratories and analyzed as described above. RAPD data for *P. pinaster* were kindly provided by Christophe Plomion (INRA, France) and were generated as described in Plomion et al. (1995 a, b). The RAPD data for *P. palustris* were those used by Nelson et al. (1994). For all data sets, loci that had greater than 15% missing phenotype scores were not included for mapping or genome-length analyses. The number of megagametophytes used, m, was 80 for *P. palustris* and 94 for *P. pinaster*.

Results

Eight megagametophytes from the *P. strobus* clone P-18 were screened using 288 10-mer primers to find candidate segregating RAPD markers. The full mapping population of 72 megagametophytes was then genotyped using 96 selected primers, and segregation data were obtained for 97 RAPD markers produced by 76 of the primers. The attrition of primers used to obtain segregation data resulted from certain RAPD marker phenotypes being poorly reproducible or difficult to score. Segregation data were obtained also for five SSR loci and for *Pschi*1, a chitinase pseudogene. An example of the segregation of SSR alleles is shown in Fig. 1.

Of the 103 loci used in the subsequent linkage analysis, 91 mapped to 12 linkage groups of three or more loci, plus five pairs of linked loci. The resulting framework map length was 745 cM(K) (Fig. 2). The average marker density of the framework map was 14.0 $\pm$ 5.9 cM(K) ($\pm$ average deviation from the mean). If map distances were calculated using the Haldane map function then the framework map length was 996 cM(H) for an average marker density of 16.3 $\pm$ 7.6 cM(H). Sixty nine (67%) of the linked markers were placed on the framework map, while 12 markers remained unlinked, including the *Pschi* 1 locus. One locus, BC579_725, could not be placed to a linkage group at lod ≥ 4, but was included as a framework locus because it had close linkage to RPS84 in group 2 (lod = 3.6, $r = 0.26$), and had an interval support ≥ 3. Only three loci deviated from Mendelian inheritance; the chi-square value for locus BC620_700 in linkage group 1 was 7.35 ($0.005 < P < 0.01$), that for locus BC580_800 also in group 1 was 4.13 ($0.04 < P < 0.05$), while that for the framework locus BC348_1900 in group 9 was 4.56 ($0.03 < P < 0.04$).

Genome lengths of *P. strobus* were estimated for a range of Z values, and are given in Table 1. Values of $E(G)$ for longleaf pine (*P. palustris*) and maritime pine (*P. pinaster*) are also given in Table 1. For all pines examined, the average estimated genome lengths were very close to either 2600 cM(H) or 2000 cM(K). When $Z = 4$, the 95% confidence intervals for $E(G)$ were 1772–2485 cM(K) for *P. strobus*, 1828–2242 cM(K) for *P. palustris*, and 1880–2084 cM(K) for *P. pinaster*. The proportion of missing phenotype scores, relative to the total number of marker phenotypes evaluated, was highest for the *P. strobus* data at 5.4% (402/7416), followed by 5.1% (663/12 880) for the *P. palustris* data, and was least for the *P. pinaster* data at 2.1% (604/28 670).

Framework maps were also assembled for *P. palustris* and *P. pinaster*. A summary of map lengths and statistics for all three species is given in Table 2, with

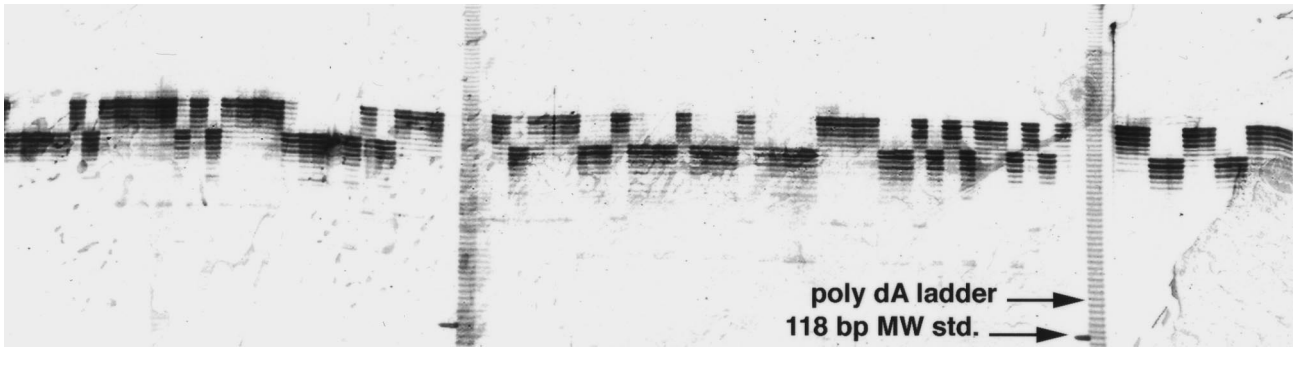

Fig. 1 Segregation of two alleles from the $(AC)_n$ locus RPS84, in a silver-stained denaturing polyacrylamide gel. The allele sizes of 147 bp and 153 bp correspond to the *second band* from the top of the fragment ladders. Band-stuttering artifacts are common with dinucleotide repeats. Seventy five megagametophyte phenotypes are shown

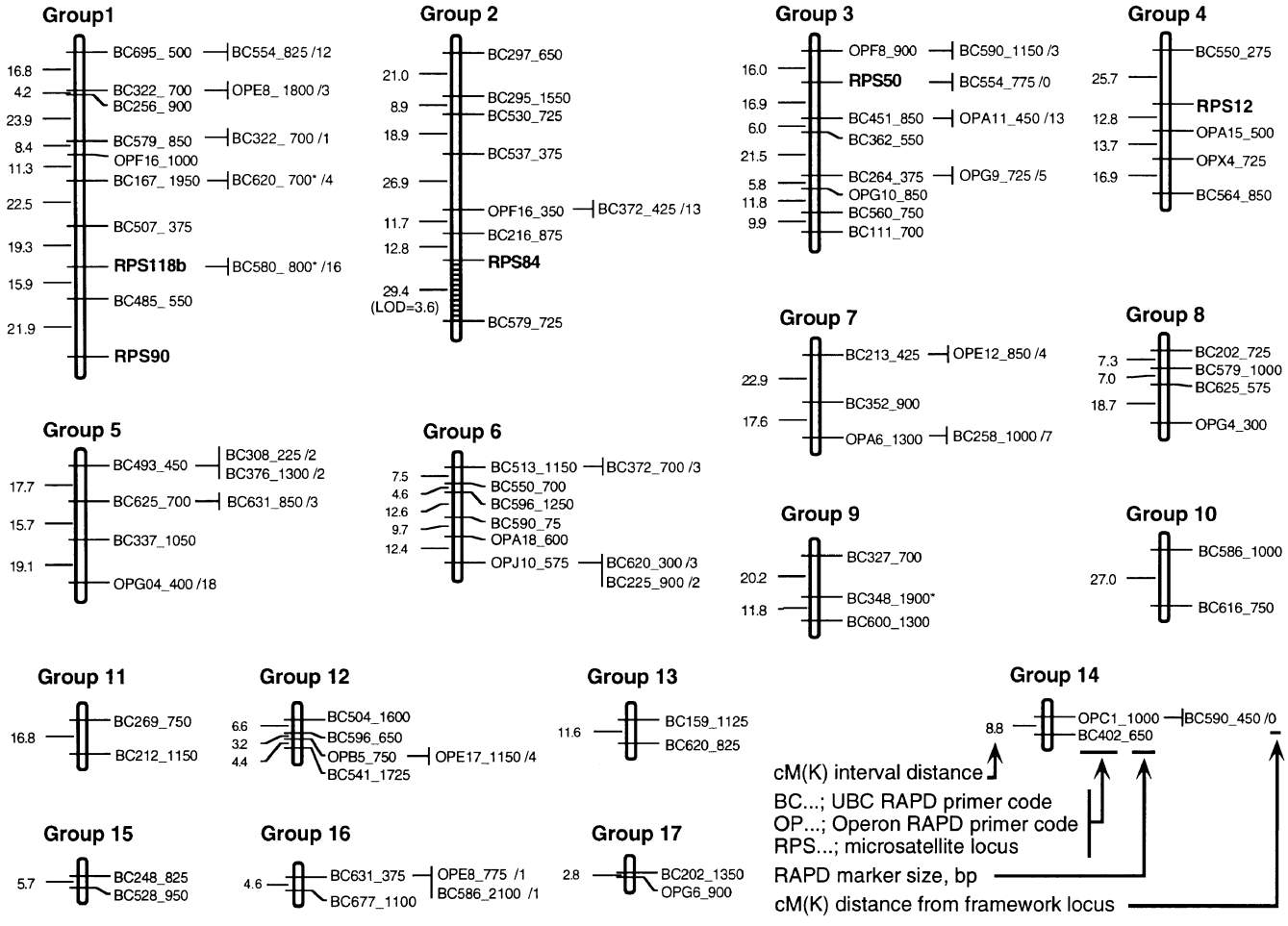

Fig. 2 Framework linkage map of eastern white pine clone P-18. Loci that could not be ordered with an interval support ≥ 3 are listed to the far right of each group, along with their distances to the nearest framework locus. Map distances were derived using the Kosambi map function. *Asterisks* denote loci that deviated from Mendelian inheritance (see text)

comparisons of their expected and observed percentage genome coverages presented in Table 3. As expected, the most complete coverage was provided for *P. pinaster*, for which 305 loci were analyzed.

Discussion

White pine genome mapping

The 12 chromosomes of *P. strobus* (MacPherson and Filion 1981) were represented in the genome map by a total of 17 linkage groups and pairs, plus 12 unlinked loci. The 103 loci provided only about 60% coverage of the genome. With the current sampling of 72 meioses in the mapping population, an additional 130 markers would be needed for an expected genome coverage of 95%, although the observed map coverage would probably be less (see discussion below).

The five SSR loci were mapped to four linkage groups, but no linkages to the chitinase pseudogene, *Pschi*1, were found. Based on a two-point linkage analysis in a different *P. strobus* tree (C. Echt, unpublished), *Pschi*1 was closely linked to a functional Class-II chitinase gene, *Pschi*4 (GenBank accession No. U57410). Genomic linkage analysis of *Pschi*4 was not possible because of lack of variation at the locus within the P-18 mapping population.

Of the 103 loci analyzed, only three did not exhibit the expected 1:1 phenotypic segregation ratio, which is about the frequency expected to arise by chance (type-1 errors), given the level of significance employed ($P < 0.05$). The data thus indicate little genetic load in this wind-pollinated species. This is consistent with observations that eastern white pine has no decrease of seed set or seed fill in self-pollinated cones when compared to out-crossed cones (Johnson 1945; Fowler 1965; Franklin 1970). Our segregation results are similar to those for RAPD markers among maritime pine megagametophytes (Plomion et al. 1995 a). In contrast, 10% of RAPD markers segregating among slash pine (*P. elliottii*) megagametophytes deviated from

Table 1 Estimated pine genome lengths and corresponding K and X values, calculated for different map functions

Map function	Species (m; n)[a]	$E(G)$, cM (K; X)			Average $E(G)$
		$Z = 3$	$Z = 4$	$Z = 5$	
Haldane	*P. strobus* (72; 103)	2770 (157; 41.4)	2600 (137; 33.9)	2491 (124; 29.4)	2620
	P. palustris (80; 161)	2618 (429; 43.6)	2569 (373; 37.2)	2592 (320; 32.2)	2593
	P. pinaster (94; 305)	2742 (1623; 48.0)	2634 (1461; 41.0)	2476 (1337; 35.7)	2617
Kosambi	*P. strobus* (72; 103)	2135 (157; 31.9)	2071 (137; 27.0)	2025 (124; 23.9)	2077
	P. palustris (80; 161)	2000 (429; 33.3)	2017 (373; 29.2)	2077 (320; 25.8)	2031
	P. pinaster (94; 305)	2057 (1623; 36.0)	2005 (1461; 31.6)	1956 (1337; 28.2)	2006

[a] m is the number of megagametophytes, or meioses, genotyped; n is the number of loci

Table 2 Observed genome map coverages and statistics for three pines

Species	cM(H)	cM(K)	# Groups + pairs	# Framework loci	# Unlinked loci
P. strobus	1574	1204	12 + 5	69	12
P. palustris	1914	1562	13 + 2	98	4
P. pinaster	2141	1845	13 + 0	179	0

Table 3 Expected and observed percent genome map coverages for three pines

Species	% Coverage		
	$E(C_n)$[a]	C_O(H)[b]	C_O(K)[c]
P. strobus	72	60	58
P. palustris	88	74	79
P. pinaster	99	82	92

[a] Values are independent of the map function used
[b] Based on framework map coverage using the Haldane map function
[c] Based on framework map coverage using the Kosambi map function

Mendelian inheritance (Nelson et al. 1993), as did 7% of RAPD markers among longleaf pine (*P. palustris*) megagametophytes (Nelson et al. 1994). Accordingly, both of these Southern pine species carry a genetic load, as shown by the reduced seed fill and germination rates that result from self-crossing (Franklin 1970).

Genome-length estimates

The method of estimating genome length described by Hulbert et al. (1988) is widely used because it is easy to calculate from readily available genome-mapping data and statistics. Chakravarti et al. (1991) presented evidence, however, that the Hulbert method tends to over-estimate genome length, especially when there is missing-phenotype data. When estimating G using values of X that are theoretically determined from the formula $Z = n\{r \log 2r + (1 - r)\log[2(1 - r)]\}$, they found that the over-estimation is pronounced. When X is determined empirically, they found $E(G)$ is more accurate, yet still somewhat inflated. For the three sets of segregation data analyzed in the present study, however, $E(G)$ values increased only slightly when X was determined theoretically. Values of X calculated from the preceeding formula, using $Z = 3$, 4 or 5, increased by less than 1.5 cM(H) from our observed values (Table 1), and the resulting averaged $E(G)$ values increased by less than 2.5% (data not shown). These small differences between theoretical and observed values for X stand in contrast to the results of Chakravarti et al. (1991). Since their study only considered a maximum of 40 loci, we suggest that the larger numbers of loci reported here render negligible the differences between using theoretically or empirically determined X values for estimating G, even when there is a modest proportion of missing-phenotype data.

Genome length may also be estimated from the number of chiasmata observed per chromosome. Plomion et al. (1995 a) estimated a pine genome length of 1500 cM based on prior cytogenetic observations of 2.5 chiasmata per bivalent. From human chiasma data, the sex-averaged estimate for the human genome is 3300 cM (see Ott 1991); yet the human recombination

Table 4 Published estimated genome lengths for three pines, based on the Hulbert estimator

Species	$E(G)$	Reference
P. elliottii	3000 cM(H)	Nelson et al. (1993)
	2350 cM(H)	Kubisiak et al. (1995)
P. palustris	2350 cM(H)	Kubisiak et al. (1995)
	2600 cM(H)	Nelson et al. (1994)
P. pinaster	2000 cM(H)	Gerber and Rodolphe (1994)
	1290 cM(K)	Plomion et al. (1995 a, b)

minimization linkage map is 4000 cM(K) (Murray et al. 1994) while current integrated human high-density framework maps are over 4500 cM(K) (Cooperative Human Linkage Center, http://www.chlc.org). When compared to genome lengths determined by linkage data, chiasma-derived estimates thus appear to underestimate genome length, and should be regarded as a lower bound (Ott 1991).

Published estimates of G for *P. palustris* and *P. pinaster*, based on the Hulbert estimator, are presented in Table 4. The close agreement among $(E)G$ values obtained in our study under standardized criteria (Table 1) indicate that previously reported differences have no biological basis, but arose from the divergent criteria used in calculating the estimates. The criteria we followed were: to exclude individual loci having over 15% missing-phenotype scores, to determine K without limiting r, to not base estimates on only framework markers, and to specify the map function used to obtain X, and thus $E(G)$.

Using simulated data, Kubisiak et al. (1993) concluded that up to 15% total missing-phenotype data could be tolerated before the structure of framework maps became noticeably affected. This upper limit also may hold true for obtaining accurate genome-length estimates. The original complete set of data for *P. palustris* ($m = 80$, $n = 174$) had 9.4% missing-phenotype scores, and gave an estimate of 2597 cM(H) for $Z = 3$. This estimate differs little from that derived from data having 5.1% missing-phenotype scores (Table 1). The complete set of *P. pinaster* data ($m = 124$, $n = 434$), however, had 27% missing-phenotype scores and gave an estimate of 3364 cM(H) for $Z = 3$, which is substantially larger than the value derived from data having only 2.1% missing-phenotype scores (Table 1).

While, at first, it may expected that pines would have similar genome lengths, it should be kept in mind that the three species we examined belong to different subgenera, and are separated by at least 130 million years of evolution (Millar 1993). In all, it is surprising that $E(G)$ values agreed as closely as they did, and suggests a highly conserved genomic rate of recombination among all pines.

Map coverage

Theoretical map coverage was greater than observed framework map coverage for all three species (Table 3). Although these differences became less pronounced with greater numbers of mapped loci, and were minimized by use of the Kosambi map function, they were never resolved. The differences between expected and observed values may indicate a non-random distribution of RAPD markers in pine genomes, or may simply result from the process of framework map construction, where typically only 60–67% of linked loci were retained on the framework maps. Assuming RAPD loci were randomly distributed, the Kosambi map function provided more accurate genome-length estimates than did the Haldane function (Table 3).

Map utility

Similar recombination rates among diverse pines should benefit comparative mapping studies, provided that enough homologous markers can be found. Homologous RAPD markers among species, or even among populations within species, are difficult to find. Preliminary results indicate that most polymorphic *P. strobus* SSR primer pairs amplify polymorphic loci from other white pines like *P. lambertiana*, but not from the more distantly related hard pines (C. Echt and P. Marquardt, unpublished). Markers that have higher interspecies sequence homology, such as cDNA RFLPs or STSs, will be needed to establish broad syntenic linkage relationships among divergent pine taxa. Because of their multi-allelism and informativeness across populations, however, SSR markers should be most useful for marker-assisted breeding and selection programs (see Grattapaglia and Sederoff 1994). Until sufficient numbers of SSR, or similarly informative, markers are available for constructing full-coverage genome maps, RAPD-based framework maps, such as the one presented here, can be used to determine the map position of extant SSR markers. Once a full-coverage *P. strobus* SSR framework map is constructed, it should be useful for comparative mapping and breeding applications among all white pines.

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References

Beaulieu J, Simon JP (1994) Inheritance and linkage relationships of allozymes in *Pinus strobus* L. Silvae Genet 43:253–261

Beaulieu J, Simon JP (1995) Variation in cone morphology and seed characters in *Pinus strobus* in Quebec. Can J Bot 73:262–271

Bishop DT, Cannings C, Skolnick M, Williamson JA (1983) The number of polymorphic DNA clones required to map the human genome. In: Wier BS (ed) Statistical analysis of DNA sequence data. Marcel-Dekker, New York, pp 181–200

Buchert GP (1994) Genetics of white pine and implications for management and conservation. For Chron 70:427–434

Chakravarti A, Lasher LK, Reefer JE (1991) A maximum-likelihood method for estimating genome length using genetic linkage data. Genetics 128:175–182

Devey ME, Delfino-Mix A, Kinloch BB, Neale DB (1995) Random amplified polymorphic DNA markers tightly linked to a gene for resistance to white pine blister rust in sugar pine. Proc Natl Acad Sci USA 92:2066–2070

Devey ME, Bell JC, Smith DN, Neale DB, Moran GF (1996) A genetic linkage map for *Pinus radiata* based on RFLP, RAPD, and microsatellite markers. Theor Appl Genet 92:673–679

Echt CS, May-Marquardt P, Hseih M, Zahorchak R (1996) Characterization of microsatellite markers in eastern white pine. Genome 39:1002–1008

Eckert RT, Joly RJ, Neale DB (1981) Genetics of isozyme variants and linkage relationships among allozyme loci in 35 eastern white pine clones. Can J For Res 11:573–579

Fowler DP (1965) Effects of inbreeding in red pine, *Pinus resinosa* Ait. IV. Comparison with other northeastern *Pinus* species. Silvae Genet 13:170–177

Franklin EC (1970) Survey of mutant forms and inbreeding depression in species of the family *Pinaceae*. USDA Forest Service Research Paper 1–21

Gerber S, Rodolphe F (1994) An estimation of the genome length of maritime pine (*Pinus pinaster* Ait). Theor Appl Genet 88: 289–292

Grattapaglia D, Sederoff R (1994) Genetic linkage maps of *Eucalyptus grandis* and *Eucalyptus urophylla* using a pseudo-testcross: mapping strategy and RAPD markers. Genetics 137:1121–1137

Guillmaut P, Maréchal-Drouard L (1992) Isolation of plant DNA: a fast, inexpensive, and reliable method. Plant Mol Biol Rep 10:60–65

Guries RP, Friedman ST, Ledig FT (1978) A megagametophyte analysis of genetic linkage in pitch pine. Heredity 40:309–314

Haldane JBS (1919) The combination of linkage values, and the calculation of distances between the loci of linked factors. J Genet 8:299–309

Hulbert S, Ilott T, Legg EJ, Lincoln S, Lander E, Michelmore R (1988) Genetic analysis of the fungus, *Bremia lactucae*, using restriction length polymorphism. Genetics 120:947–958

Jacobson GL Jr, Dieffenbacher-Krall A (1995) Insights from the past: white pine and climate change. J For 93:39–42

Johnson LPV (1945) Reduced vigor, chlorophyll deficiency and other effects of self-fertilization in *Pinus*. Can J Res 23c:145–149

Keats B, Ott J, Conneally M (1989) Report of the committee on linkage and gene order. Cytogenet Cell Genet 51:459–502

Keats BJB, Sherman SL, Morton NE, Robson EB, Beutow KH, Cann HM, Cartwright PE, Chakravarti A, Francke U, Green PP, Ott J (1991) Guidelines for human linkage maps: an international system for human linkage maps (ISLM, 1990). Genomics 9:557–560

Kinloch BB (1992) Distribution and frequency of a gene for resistance to white pine blister rust in natural populations of sugar pine. Can J Bot 70:1319–1323

Kosambi DD (1944) The estimation of map distances from recombination values. Ann Eugen 12:172–175

Kubisiak TL, Nelson CD, Stine M (1993) Simulation study of linkage map construction with missing and mis-scored RAPD data. In: Proc 22nd Southern Forest Tree Improvement Conference, June 14–17, 1993, Atlanta, Georgia. Publication #44 of the Southern Forest Tree Improvement Committee, pp 241–248

Kubisiak TL, Nelson CD, Nance WL, Stine M (1995) RAPD linkage mapping in a longleaf pine × slash pine F_1 family. Theor Appl Genet 90:1119–1127

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newburg L (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181

Little EL, Critchfield, WB (1969) Subdivisions of the genus *Pinus* (Pines). USDA For Serv, Misc Pub 1144

MacPherson P, Filion WG (1981) Karyotype analysis and the distribution of constitutive heterochromatin in five species of *Pinus*. J Hered 72:193–198

Millar CI (1993) Impact of the eocene on the evolution of *Pinus* L. Ann Mis Bot Gard 80:471–498

Murray JC, Buetow KH, Weber JL, Lundwigsen S, Scherpbier-Heddema T, Manion F, Quillen J, Sheffield VC, Sunden S, Duyk GM, Weissenbach J, Gyapay G, Dib C, Morressette J, Lathrop GM, Vignal A, White R, Matsunami N, Gerken S, Melis R, Albertsen H, Plaetke R, Odelberg S, Ward D, Dausset J, Cohen D, Cann H (1994) A comprehensive human linkage map with centimorgan density. Science 265:2049–2054

Nelson CD, Nance WL, Doudrick RL (1993) A partial genetic linkage map of slash pine (*Pinus elliottii* Engelm. var. *elliottii*) based on random amplified polymorphic DNAs. Theor Appl Genet 87:145–151

Nelson CD, Kubisiak TL, Stine M, Nance WL (1994) A genetic linkage map of longleaf pine (*Pinus palustris* Mill.) based on random amplified polymorphic DNAs. J Hered 85:433–439

O'Brien IEW, Smith DR, Gardner RC, Murray BG (1996) Flow cytometric determination of genome size in *Pinus*. Plant Sci 115:91–99

Ott (1991) Analysis of human genetic linkage. The Johns Hopkins University Press, Baltimore

Plomion C, O'Malley DM, Durel C-E (1995 a) Genomic analysis in maritime pine (*Pinus pinaster*). Comparison of two RAPD maps using selfed and open-pollinated seeds of the same individual. Theor Appl Genet 90:1028–1034

Plomion C, Bahrman N, Durel C-E, O'Malley DM (1995 b) Genomic mapping in *Pinus pinaster* (maritime pine) using RAPD and protein markers. Heredity 74:661–668

Valkonen JPT, Nygren M, Ylonen A, Mannonen L (1994) Nuclear DNA content of *Pinus sylvestris* (L.) as determined by laser flow cytometry. Genetica 92:203–207

Tulsieram LK, Glaubitz JC, Kiss G, Carlson JE (1992) Single-tree genetic linkage mapping in conifers using haploid DNA from megagametophytes. Biotechnology 10:686–690

Wakamiya I, Newton RJ, Johnston JS, Price HJ (1993) Genome size and environmental factors in the genus *Pinus*. Am J Bot 80:1235–1241

Welsh J, McClelland M (1990) Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res 18:7213–7218

Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV (1990) DNA polymorphisms amplified by arbitrary pimers are useful as genetic markers. Nucleic Acids Res 18:6531–6535