Linkage map of birch, *Betula pendula* Roth, based on microsatellites and amplified fragment length polymorphisms

M. Pekkinen, S. Varvio, K.K.M. Kulju, H. Kärkkäinen, S. Smolander, A. Viherä-Aarnio, V. Koski, and M.J. Sillanpää

Abstract: The first genetic linkage map for silver birch, *Betula pendula* Roth, was constructed by using a pseudo-testcross mapping strategy and integration of linkage data from 3 unrelated 2-generation pedigrees. The map is based on the genetic inheritance and segregation of 82 amplified fragment length polyhmorphisms and 19 microsatellite markers, and was constructed by simultaneously comparing the performance of CRI-MAP and OUTMAP packages. The analysis revealed 16 linkage groups, and the total map coverage is 1561 cM (Kosambi units). Average map distance between adjacent markers is 15.5 cM. Linkage groups range between 6 and 18 loci and from 81.2 to 326.5 cM; the remaining 9 linkage groups consist of 2 or 3 loci ranging from 6.3 to 42.4 cM. The uncertainty of the map is illustrated with sensitivity analysis. This initial map can serve as a basis for developing a more detailed genetic map.

Key words: Betula pendula, linkage map, microsatellite, AFLP, CRI-MAP, OUTMAP.

Résumé : La première carte génétique du bouleau commun, *Betula pendula* Roth, a été produite via une stratégie de pseudo-testcross et par intégration des données provenant de 3 pedigrees bigénérationnels non-apparentés. La carte s'appuie sur l'hérédité et la ségrégation de 82 marqueurs AFLP et 19 microsatellites. Elle a été produite tout en comparant les performances des logiciels CRI-MAP et OUTMAP. Cette analyse a produit 16 groupes de liaison dont l'étendue est de 1561 cM (unités Kosambi). La distance moyenne entre marqueurs adjacents est de 15,5 cM. Les groupes de liaison comprennent entre 6 et 18 locus et mesurent entre 81,2 et 326,5 cM. Les 9 autres groupes de liaison comprenaient 2 ou 3 locus et totalisaient 6,3 à 42,4 cM. L'incertitude de la carte est illustrée au moyen d'une analyse de sensibilité. Cette carte initiale peut servir d'assise pour développer une carte plus détaillée.

Mots clés : *Betula pendula*, carte de liaison, microsatellite, AFLP, CRI-MAP, OUTMAP.

[Traduit par la Rédaction]

Introduction

Genetic linkage maps are necessary tools for genome analysis and breeding applications in forest trees. A high-density map can provide information to locate genes, facilitate marker-assisted selection, and clarify the biological basis of complex traits (Grattapaglia and Sederoff 1994; Sewell et al. 1999).

In this paper, we present the first genetic linkage map, based on microsatellites and amplified fragment length polymorphism (AFLP) markers, for silver birch (*Betula pendula* Roth). The genus *Betula* comprises 35–60 species of deciduous, wind-pollinated trees and shrubs native to the north temperate and Arctic regions. Silver birch is an economically important hardwood species in Northern Europe, providing valuable raw material for pulp, paper, furniture, and plywood industries.

Genetic linkage maps in plants are usually constructed using segregating populations obtained from crosses between inbred lines. Such populations are generally not available for forest trees because of time constraints (Grattapaglia and Sederoff 1994). In conifers, haploid megagametophytes have allowed direct analysis of linkage and construction of genetic maps (Nelson et al. 1994; Echt and Nelson 1997; Travis et al. 1998; Hurme et al. 2000). Available pedigrees for the majority of angiosperm outbred tree species, including the silver birch, generally involve only 2 parents and their full-sibs or maternal half-sib families. Combining information from multiple pedigrees or populations can be used to construct a single consensus map to represent a given species. An advantage of consensus maps is that a

Received 24 November 2004. Accepted 12 April 2005. Published on the NRC Research Press Web site at http://genome.nrc.ca on 5 August 2005.

Corresponding Editor: J.B. Bell.

M. Pekkinen. Department of Applied Chemistry and Microbiology, P.O. Box 27, FIN-00014, University of Helsinki, Finland.
S. Varvio, H. Kärkkäinen, S. Smolander, and M.J. Sillanpää.[1] Department of Mathematics and Statistics, P.O. Box 68, FIN-00014, University of Helsinki, Finland.
K.K.M. Kulju. Nationaal Herbarium Nederland, Universiteit Leiden Branch, P.O. Box 9514, 2300 RA Leiden, The Netherlands.
A. Viherä-Aarnio and V. Koski. Finnish Forest Research Institute, Vantaa Research Centre, P.O. Box 18, FIN-01301, Vantaa, Finland.

[1]Corresponding author (e-mail: mjs@rolf.helsinki.fi).

 doi: 10.1139/G05-031

larger number of loci can be placed onto a single map. These maps have been constructed for a number of plant species. Recently, a consensus map was constructed for loblolly pine (*Pinus taeda* L.) on 2 outbred 3-generation pedigrees (Sewell et al. 1999) and for spruce (*Picea abies* Karst.) using sibships of megagametophytes (Bucci et al. 1997).

Materials and methods

Plant material

Initial screening for suitable *B. pendula* pedigree material, which was done among existing birch breeding materials, resulted in 24 families. From these, 4 Mendelian families were selected. These included 1 half-sib and 2 full-sib families, which were crosses between trees of different provenances in Finland and Latvia, Estonia or Russia (Fig. 1). In total, 127 trees (including 7 parents) represented the mapping population. The crosses were made by the Finnish Forest Research Institute, and study material was collected from the Institute's clone collection at Paimio (parents of the progeny tests), progeny test No. 1043/1 at Saarijärvi (62°46′ N, 25°38′ E, altitude 160 m) in middle Finland, and test No. 1176/1 at Loppi (60°39′ N, 24°22′ E, altitude 135 m) in southern Finland. The progeny tests were long-term field trials established in the late 1980s as a part of birch breeding research in Finland.

Genetic markers

AFLP markers were developed using AFLP kit modules for small genomes according to the AFLP Plant Mapping Protocol (Applied Biosystems/Perkin Elmer, Foster City, Calif., USA). The PCRs were carried out in a PTC-100 thermocycler (MJ Research Inc., Waltham, Mass.) and analyzed by ABI 377 Sequencer (Applied Biosystems/ Perkin Elmer, Foster City, Calif., USA). Dominant AFLP markers were named using the selective nucleotides in the EcoRI primer, followed by the selective nucleotides in the MseI primer, and then by the molecular size of the DNA fragment. The reproducibility of AFLPs was tested using DNA of the parents in 4 independent experiments, and the amplified fragments were subjected to electrophoresis in 2 different gels. The methods to obtain microsatellite markers from size-selected genomic DNA of *B. pendula* and primer sequences for microsatellite amplification are given in Kulju et al. (2004).

Distortion tests

AFLP markers scored as dominant were analyzed in the mapping population by using a pseudo-testcross strategy (Grattapaglia and Sederoff 1994). In other words, only those marker loci at which 1 parent was heterozygous and the other a recessive homozygote in at least 1 family were selected. Since multiple families were considered, markers varied in the number of informative families. A χ^2-test was performed for each locus to determine whether the inherited alleles of the offspring were in compliance with the Mendelian segregation ratios. The test was executed over all 4 families simultaneously. Because of the unusual family structure (the first 2 families having a common mother, the last 2 being independent), this was implemented in Java. The between-families χ^2 test statistic was calculated as a sum of within-family quantities. In the families having a common mother the χ^2 statistic was first calculated for the fathers separately and then for the mother in the 2 families combined. The degrees of freedom at each marker were determined as the number of possible genotype classes — 1 summed over the 2 independent families and over the families sharing a mother.

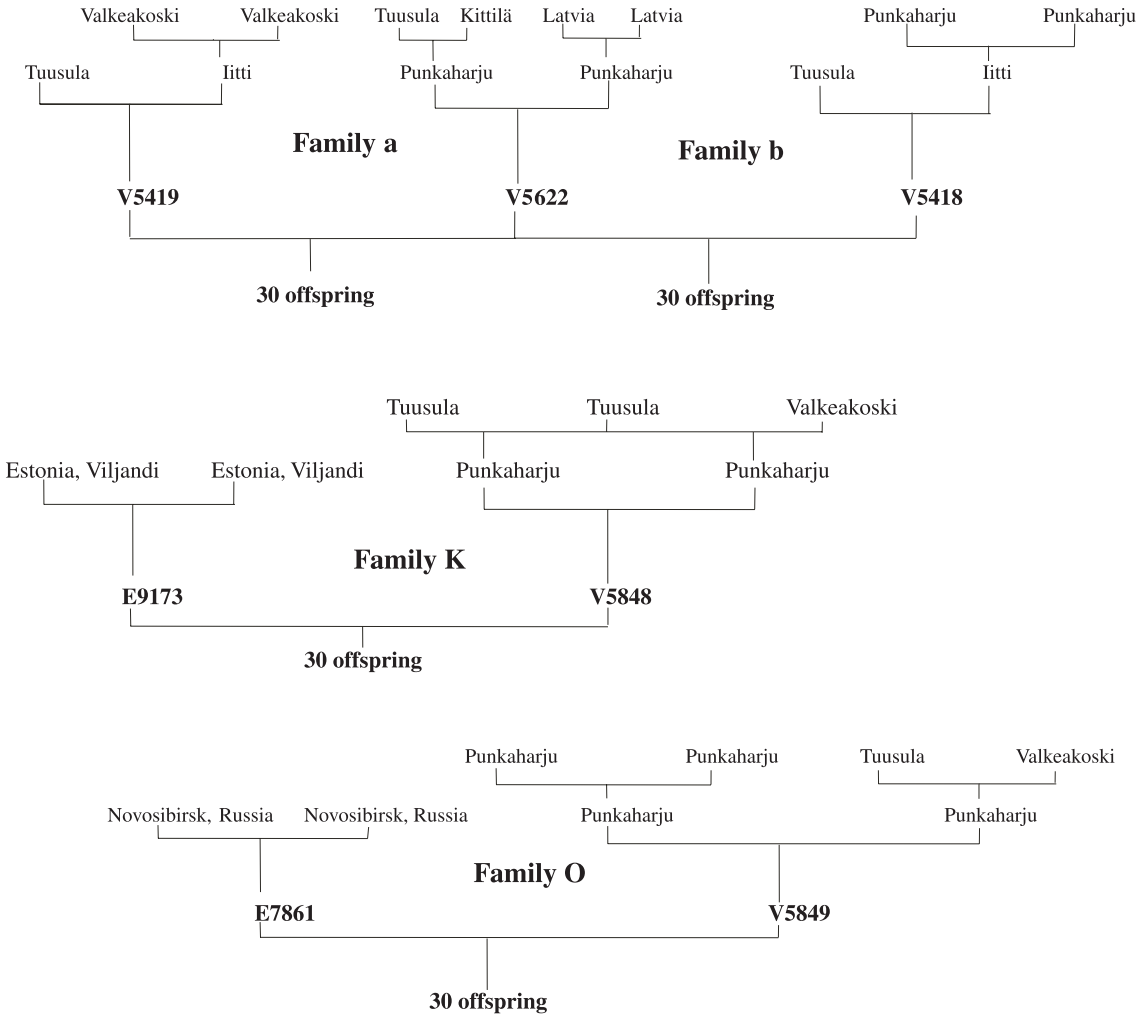
Map construction

Linkage analysis was performed using the CRI-MAP version 2.4 (Green et al. 1990), which allows simultaneous analysis of several families. (Note that a very recent paper of Hu et al. (2004) follows a similar idea.) The map was constructed from 3 unrelated families, that is, 2 unconnected and 2 connected families. Kosambi's mapping function was used to estimate map distances in centimorgans (cM). Parental linkage phases were assumed to be unknown in this data set. In the calculation of the logarithm of the odds (LOD) score, all families were considered simultaneously, and a maximally stringent LOD threshold was selected such that it still produced stability and clustering. First, linkage groups were formed using a 2-point LOD score of 2.2 as a threshold for framework markers, which formed the backbone of the map. Accessory markers that were not placed in linkage groups at the initial stringency (LOD ≥2.2) were added subsequently if they showed linkage only to marker(s) within a single group and with LOD scores ≥1.6.

Locus ordering was performed by using CRI-MAP with exhaustive enumerating algorithm so that both sexes were considered to be equal. Ordering of large linkage groups containing more than 8 loci was carried out stepwise, so that the small number of additional loci were ordered in each round with respect to the given fixed order obtained in the previous round. The competence of these orders was finally checked by reanalyzing all such "suborders" that were obtained from the original order by flipping 2 adjacent markers at a time.

However, it is well known that this kind of stepwise ordering strategy for large linkage groups, employed here with CRI-MAP, is not optimal. Therefore, the optimality of these CRI-MAP orders was further examined by using the OUTMAP computer program (Ling 1999; Butcher et al. 2002), which uses more sophisticated optimization methods for simultaneous locus ordering. OUTMAP requires that the ordering of loci be executed in each family separately. The CRI-MAP linkage groups were used because information in a single family was not sufficient for grouping. All 3 ordering algorithms of the OUTMAP package were used for linkage groups with 10 or more marker loci. The algorithms 2-opt, 3-opt (Johnson 1990), and simulated annealing (Kirkpatrick et al. 1983) are all among the most famous general local optimization algorithms, which have usually been successfully applied for difficult combinatorial optimization problems. Provided that the putative OUTMAP order for a single family differed from the original order created by CRI-MAP, the new order was used in CRI-MAP as a fixed backbone map. The loci not segregating in the family in question were then placed to the backbone map to compare the LOD scores of the original map and the new one using a LOD-score of 1.9 or higher as a threshold. Multipoint link-

Fig. 1. Pedigree structure of *Betula pendula* families from crosses of the Finnish Forest Research Institute. Names refer to the geographical origin or the location of selection of the parent trees used in crossings.



age analysis for given fixed order was then performed with CRI-MAP in distance estimation.

Genome map sizes were estimated using the method-of-moments estimator (method 3 in Chakravarti et al. 1991; Hulbert et al. 1988). We obtained the estimates by considering the number of significant pairwise linkages between the 84 unique loci assuming threshold LOD values from 1.6 to 2.2.

Results

Marker development

AFLP technique involves selective PCR amplification of restriction fragments generated after total digestion of the genome. It does not require prior sequence knowledge and allows 50–100 loci per gel lane to be obtained simultaneously (Vos et al. 1995). We used 10 primer combinations for screening the mapping population (Table 1). Seven primer combinations were useful and revealed a total of 145 presence/absence type of polymorphisms. The number of amplified fragments per primer combination varied between 59 and 125, with a mean of 89 fragments. Fragment sizes ranged from 50 to 500 bp, and 24% of amplified fragments were polymorphic in the mapping population.

Screening of 2 genomic libraries resulted in 33 potential microsatellite clones. We estimate that there are GA repeats in about every 360 Kbp and CA repeats in about every 1.2 Mbp in the silver birch genome. The cumulative genome density for the 2 different microsatellite motifs tested is approximately 1 microsatellite sequence motif every 216 Kb. This is in agreement with results previously obtained in plants (Morgante and Olivieri 1993; Lagercrantz et al. 1993; Taramino et al. 1997). The sequenced microsatellites showed approximately the same proportion of perfect and compound repeats. CA repeats showed a distinct predominance (81%) of compound repeats. Imperfect repeats were relatively rare for both types of repeats.

Informativeness of AFLP markers

Marker informativeness is considered here as the number of informative families (and each informative family has 2 × 30 informative meioses) at the marker. From 145 AFLP markers, there were 90, 41, and 14 markers that were simultaneously informative at 1, 2, or 3 families of 4 (half-sib family was considered as 2 separate families in this calcula-

Table 1. Numbers of amplified fragment length polymorphism amplification products generated with 9 different primer combinations.

Eco +2	Mse +3	Number of polymorphic bands
AA	CTA	10
AC	CAC	28
AC	CAG	27
AC	CAT	16
AG	CAA	18
AT	CTG	—
TA	CTT	16
TA	CAT	—
TC	CAT	30

Note: Total number of polymorphic bands is 145.

tion), respectively. Altogether, there were 36, 61, 77, and 40 markers in family a, b, K, and O (Fig. 1), respectively, that had informative mating type (heterozygote × homozygote) at the marker. Moreover, 20 of these markers were simultaneously informative in families a and b (which together are a half-sib family).

Segregation distortion

In all, 17 of the markers showed significant ($P < 0.001$) distortion from the Mendelian expectation (distorted markers are denoted with an asterisk in the map). Almost one-third (32%) of the markers departed from the expectation at the $P < 0.05$ level. The proportion of distorted loci was similar in microsatellite and AFLP markers. The most significantly distorted loci are clustered together in the map. In particular, a large distorted region was found in linkage group 1.

Map construction

The 164 marker (19 microsatellite and 145 AFLPs) genotypes were determined for mapping population: 101 of the 164 markers (60%) showed linkage to at least 1 other marker at LOD ≥1.6. The total map distance contained within the 16 linkage groups was 1561 cM (Kosambi units), with an average distance between adjacent markers of 15.5 cM (Fig. 2). Linkage groups comprised 6–18 loci (81.2–326.5 cM); the remaining 9 linkage groups comprised 2–3 loci (6.3–42.4 cM). In linkage group 4, the order was changed to the new order proposed by OUTMAP (3-opt algorithm), since a single family supported it with a substantially higher LOD score than the original CRI-MAP order.

To test the sensitivity of the constructed map, 4 "additional" microsatellites (L012, L022, L52, and L63) were genotyped and inserted into the existing map. The marker L52 showed linkage to 2 framework markers in linkage group 5. The marker L63 showed linkage to group 9 and was added to this pre-ordered group. The marker L012 was a problematic one, as it showed linkage to groups 1, 2, 5, and 7 with LOD >2.2, and to group 12 with LOD 1.82. All 7 markers for which the pairwise linkages were found were the framework markers, and 4 of them also had segregation distortion. We decided not to combine several linkage groups (1, 2, 5, and 7) into 1 large group, as the linkages found may have represented artifacts due to segregation distortion. However, if marker L012 had been included into the earlier map construction process we might have treated it differently, leading to different conclusions. We believe that this kind of sensitivity analysis gives a reasonable view of the uncertainty involved of the constructed map.

Discussion

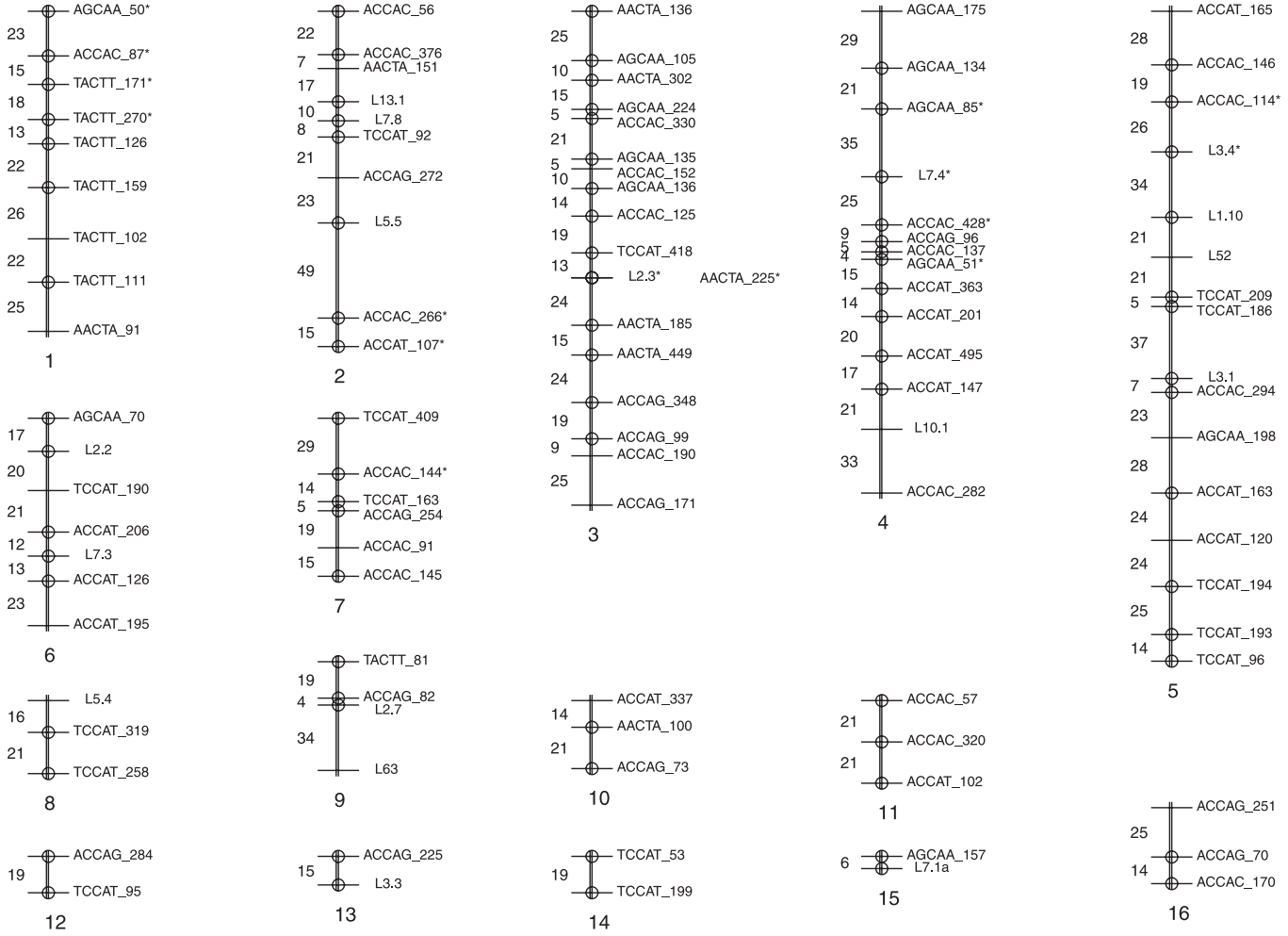
CRI-MAP (Lander and Green 1987; Goldgar et al. 1989; Green et al. 1990) was used to construct a consensus map by analyzing data over all families simultaneously. Note that most programs are applicable only for a single family at a time; they require known linkage phases for parents, and the family-specific maps are put together afterwards with some extra work (see Hu et al. 2004 for extensive comparison and benefits of simultaneous analysis over JoinMap).

The exhaustive enumerating algorithm of locus ordering used by CRI-MAP gives, in general, an optimal solution but cannot practically be applied for linkage groups that contain more than 8–10 loci because of too many possible orders. With CRI-MAP, large linkage groups must be ordered stepwise such that a small number of loci are ordered in each round with respect to the given fixed order constructed in the previous round. This, of course, does not lead to optimal ordering because maximization in each step is based only on partial data, and thus there are good grounds for using the 2-stage mapping strategy, which was applied here.

In OUTMAP (Ling 1999; Butcher et al. 2002), sophisticated optimization methods, like the 2-opt, the 3-opt (Johnson 1990), and the simulated annealing (Kirkpatrick et al. 1983; Falk 1992; Weeks and Lange 1987; Ling 1999; Jansen et al. 2001), are applied for simultaneous locus ordering. The optimality of the solution in n-optimization increases with number of edges (n), as does the computation time. In practice, 2-opt and 3-opt algorithms already give adequate answers. To attain a more global solution, Lin (1965) combined n-optimization with randomization. Lin's algorithm finds locally optimal solutions by n-optimization for a randomly chosen set of initial situations. Further modifications of the 2-opt and 3-opt algorithms are used in OUTMAP for locus ordering. In OUTMAP, the algorithms do not use randomly chosen initial locus orders but use, instead, the obtained locally optimized solution as a new initial order for the following optimization round. Genetic algorithms (Carlborg et al. 2000; these are so far not applied to locus ordering) and Markov Chain Monte Carlo sampling (Stephens and Smith 1993; George et al. 1999; Rosa et al. 2002) are alternative effective optimization methods.

The best order was obtained through ordering the 11 loci present in family K (Fig. 1) by OUTMAP followed by insertion of the 3 subsequent loci (segregating in other families) by CRI-MAP, and applying the flips of 4 (for each locus order obtained by permuting a 4-tuple of adjacent loci within an initial locus order) option of CRI-MAP. The fact that linkage group 4 was the only one having an increased LOD score after OUTMAP ordering indicates that the stepwise procedure applied with CRI-MAP was indeed successful in our data set. In many cases, however, the difference in scores was not great. There were unexpectedly large differences between the maps created by OUTMAP's different optimiza-

Fig. 2. Genetic linkage map of silver birch constructed by amplified fragment length polymorphism (AFLP) and microsatellite markers. Microsatellites are coded by L and followed by a number (according to their clones). AFLP markers are described in terms of the 2 or 3 selective nucleotides used; for example, in AACTA_100 the *Eco*RI primer is given to the left (AA), the *Mse*I primer to the right (CTA), followed by the number indicating the approximate size in basepairs of the scored fragment. Genetic distances are given in centiMorgans (cM). Kosambi units are on the left side of each linkage group, and marker names are given to the right of each linkage group. Linkage groups covering 1561 cM are indicated by numbers 1 through 16. * indicates significant ($P < 0.05$) deviation from the expected 1:1 segregation. The framework markers are shown with circles.



tion algorithms (results not shown). This is because the algorithms find only a local maximum of the LOD score, and the low information content within a single family seemed to increase this characteristic. None of the algorithms managed clearly better than the others. However, it has been shown that performance of OUTMAP is superior in large linkage groups (Butcher et al. 2002). The differences between our results and those of Butcher et al. indicate that our data set was extreme in many ways: (*i*) the information content of dominant AFLP markers was low, (*ii*) the material consisted of multiple families with small numbers of progenies, (*iii*) the linkage groups were small, and (*iv*) all our markers did not segregate in all families.

Markers seem to be well distributed, and no evidence of clustering was noticed. Most marker intervals are 15–25 cM, perhaps because of the small number of informative meioses and even fewer recombinants in the data to support linkages in shorter distances. The high proportion of unlinked loci (40%) is higher than in most of the plant maps (Akkaya et al. 1995; Byrne et al. 1995). The failure to obtain the basic chromosome number may be a consequence of the structure and small size of the mapping population. This has also been reported for other species and marker systems. Perhaps additional markers could have been ordered in the map and certain gaps filled in by increasing the population size.

The observed deviation from the Mendelian expectation of a marker locus might be due to selectional forces, sampling or scoring errors, or some noise, such as null alleles. In a case of an AFLP locus, however, a more essential question arises: the pseudo-testcross configuration of dominant AFLP markers is based on the assumption of heterozygosity of the parent having the dominant phenotype. However, again this assumption is based on the observed phenotypes of the progeny. So the decision to be made is whether the loci that departed from the Mendelian 1:1 ratio should be disregarded, or whether the fact that there is at least 1 descendant expressing the recessive phenotype should be taken as evidence for the heterozygosity of the dominant parent. We

Table 2. Genome length estimates based on the number of significant pairwise linkages (*n* links) between 84 framework markers exceeding a specified LOD score level.

LOD	*n* links	Estimated genome length* (cM)
≥1.6	145	1906.00
≥1.7	110	2512.46
≥1.8	107	2582.90
≥1.9	86	3213.61
≥2.0	85	3251.41
≥2.1	79	3498.36
≥2.2	77	3589.22

Note: In all cases, the maximum recombination fraction is 0.33, corresponding to a genetic distance of 39.64 cM (Kosambi map-function).

*The estimates were derived from the formula G = M (M–1) X / (*n* links) with values M = 84 and X = 39.64 cM.

were inclined to the latter. This was supported by the fact that there were no loci with only 1 descendant of either phenotype. Also, the clustered location of the highly distorted markers indicates that the distortion might be due to some biological cause (for instance, lethal genes or fragment complexes). Clustering behavior has also been observed in a study by Kuang et al. (1999) concerning inbreeding depression in *Pinus radiate*.

Genome length estimation

Betula pendula is a diploid species (2*n* = 28). No published estimate of the genome size exists, but the closely related tetraploid *B. pubescens* has a genome size of 2C DNA = 1.4 pg (Grime and Mowforth 1982).

The method-of-moments type estimator for genome size is obtained by equating the observed and expected proportions of locus pairs that exceed a specified LOD score value (Chakravarti et al. 1991). The number of significant locus pairs varies according to the given LOD level, implying dependence between estimated genome size and the LOD value (Table 2). Genome size estimates from pairwise linkages (only framework markers were used to avoid an overestimate of genome coverage) ranged from 1906 cM for LOD scores of 1.6–3589 cM for LOD scores of 2.2. Thus, the 101 markers in the current map coverage ranged from 44% to 82% of the estimated silver birch genome sizes. It is hard to deduce how large a fraction of the genome is thereby covered, notably because we do not know the total genetic length of the silver birch genome. The possibility of detecting linkage between markers residing on the same chromosomes will depend obviously on the number of informative meioses shared between the markers. This, in turn, will be a function of marker heterozygosity in the segregating generations and the number of offspring in which transmission can be followed. Our mapping population included 1 half-sib family with 60 offspring and 2 full-sib families with 30 offspring. In the half-sib family the number of potentially informative meioses will be only half that obtained in the analysis of the same number of offspring from full-sib families.

Acknowledgements

We thank Veijo Hakamäki, Erkki Kosonen, Osmo Luostarinen, and Elvi Pääkkönen for collecting the material from progeny tests and clone archives, Raili Tallqvist for sample preparation, and Sven Kevin for technical assistance. The work was supported by TEKES (FIBRE-project) and by a research grant (202324) from the Academy of Finland.

References

Akkaya, M.S., Shoemaker, R.C., Specht, J.E., Bhagwat, A.A., and Cregan, P.B. 1995. Integration of simple sequence repeat DNA markers into a soybean linkage map. Crop. Sci. **35**: 1439–1445.

Bucci, G., Kubisiak, T.L., Nance, W.L., and Menozzi, P. 1997. A population 'consensus', partial linkage map of *Picea abies Karst*. based on RAPD markers. Theor. Appl. Genet. **95**: 643–654.

Butcher, P.A., Williams, E.R., Whitaker, D., Ling, S., Speed, T.P., and Moran, G.F. 2002. Improving linkage analysis in outcrossed forest trees — an example from *Acacia mangium.* Theor. Appl. Genet. **104**: 1185–1191.

Byrne, M., Murrell, J.C., Allen, B., and Moran, G.F. 1995. An integrated genetic linkage map for eucalyptus using RFLP, RAPD and isozyme markers. Theor. Appl. Genet. **91**: 869–875.

Carlborg, Ö., Andersson, L., and Kinghorn, B. 2000. The use of genetic algorithm for simultaneous mapping of multiple interacting quantitative trait loci. Genetics, **155**: 2003–2010.

Chakravarti, A., Lasher, L.K., and Reefer, J.E. 1991. A maximum likelihood method for estimating genome length using genetic linkage data. Genetics, **128**: 175–182.

Echt, C.S., and Nelson, C.D. 1997. Linkage mapping and genome length in eastern white pine (*Pinus strobus L.*). Theor. Appl. Genet. **94**: 1031–1037.

Falk, C.T. 1992. Preliminary ordering of multiple linked loci using pairwise linkage data. Genet. Epidemiol. **5**: 75–80.

George, A.W., Mengersen, K.L., and Davis, G.P. 1999. A Bayesian approach to ordering gene markers. Biometrics, **55**: 419–429.

Goldgar, D.E., Green, P., Parry, D.M., and Mulvihill, J.J. 1989. Multipoint linkage analysis in neurofibromatosis type I: an international collaboration. Am. J. Hum. Genet. **44**: 6–12.

Grattapaglia, D., and Sederoff, R. 1994. Genetic linkage maps of *Eucalyptys grandis* and *Eucalyptys urophylla* using a pseudotestcross mapping strategy and RAPD markers. Genetics, **137**: 1121–1137.

Green, P., Falls, K., and Crooks, S. 1990. CRI-MAP. Version 2.4 [computer program]. Washington University School of Medicine, St. Louis, Mo., U.S.A.

Grime, J.P., and Mowforth, M.A. 1982. Variation in genome size — an ecological interpretation. Nature (London), **299**: 151–153.

Hu, X.-S., Goodwillie, C., and Ritland, K.M. 2004. Joining genetic linkage maps using a joint likelihood function. Theor. Appl. Genet. **109**: 996–1004.

Hulbert, S.H., Ilott, T.W., Legg, E.J., Lincoln, S.E., Lander, E.S., and Michelmore, R.W. 1988. Genetic analysis of the fungus, *Bremia lactucae*, using restriction fragment length polymorphisms. Genetics, **120**: 947–958.

Hurme, P., Sillanpää, M.J., Arjas, E., Repo, T., and Savolainen, O. 2000. Genetic basis of climatic adaptation in Scots Pine by Bayesian quantitative trait locus analysis. Genetics, **156**: 1309–1322.

Jansen, J., de Jong, A.G., and van Ooijen, J.W. 2001. Constructing dense genetic linkage maps. Theor. Appl. Genet. **102**: 1113–1122.

Johnson, D.S. 1990. Local optimization and the traveling salesman problem. *In* Automata, Languages and Programming, 17th International Colloquium, ICALP90, Proceedings. Warwick University, England, 16–20 July 1990. *Edited by* M.S. Paterson. Springer Verlag, Berlin, Germany. pp. 446–461.

Kirkpatrick, S., Gelatt, C.D., and Vecchi, M.P. 1983. Optimization by simulated annealing. Science (Washington, D.C.), **220**: 671–680.

Kuang, H., Richardson, T., Carson, S., Wilcox, P., and Bongarten, B. 1999. Genetic analysis of inbreeding depression in plus tree 850.55 of *Pinus radiate*. I. Genetic map with distorted markers. Theor. Appl. Genet. **98**: 697–703.

Kulju, K.K.M., Pekkinen, M., and Varvio, S. 2004. Twenty-three microsatellite primer pairs for *Betula pendula* (Betulaceae). Mol. Ecol. Notes, **4**: 471–473.

Lagercrantz, U., Ellegren, H., and Andersson, L. 1993. The abundance of various polymorphic microsatellite motifs differs between plants and vertebrates. Nucleic Acids Res. **21**: 1111–1115.

Lander, E., and Green, P. 1987. Construction of multilocus genetic linkage maps in humans. Proc. Natl. Acad. Sci. U.S.A. **84**: 2363–2367.

Lin, S. 1965. Computer solutions of the traveling salesman problem. Bell System Tech. J. **44**: 2245–2269.

Ling, S. 1999. Constructing genetic maps for outbred experimental crosses. Ph.D. thesis. University of California, Berkeley, Calif..

Morgante, M., and Olivieri, A.M. 1993. PCR-amplified microsatellites as markers in plant genetics. Plant J. **3**: 175–182.

Nelson, C.D., Kubisiak, T.L., Stine, M., and Nance, W.L. 1994. A genetic linkage map of longleaf pine *(Pinus palustris Mill.)* based on random amplified polymorhic DNAs. J. Hered. **85**: 433–439.

Rosa, G.J.M., Yandell, B.S., and Gianola, D. 2002. A Bayesian approach for constructing genetic maps when markers are miscoded. Genet. Sel. Evol. **34**: 353–369.

Sewell, M.M., Sherman, B.K., and Neale, D.B. 1999. A consensus map for loblolly pine (*Pinus taeda L.*). I. construction and integration of individual linkage maps from two outbred three-generation pedigrees. Genetics, **151**: 321–330.

Stephens, D.A., and Smith, A.F.M. 1993. Bayesian inference in multipoint gene mapping. Ann. Hum. Genet. **57**: 65–82.

Taramino, G., Tarchini, R., Ferrario, S., Lee, M., and Pe, M.E. 1997. Characterization and mapping of simple sequence repeats (SSRs) in *Sorghum bicolor.* Theor. Appl. Genet. **95**: 66–72.

Travis, S.E., Ritland, K., Whitman, T.G., and Keim, D. 1998. A genetic linkage map of pinyon pine (*Pinus edulis*) based on amplified fragment length polymorphisms. Theor. Appl. Genet. **97**: 871–880.

Vos, P., Hogers, R., Bleeker, M., Reijans, M., van de Lee, T., Hornes, M., Frijters, A. et al. 1995. AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res. **23**: 4407–4414.

Weeks, D.E., and Lange, K. 1987. Preliminary ranking procedures for multilocus ordering. Genomics, **1**: 236–242.