Genetic Basis of Climatic Adaptation in Scots Pine by Bayesian Quantitative Trait Locus Analysis

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ABSTRACT

We examined the genetic basis of large adaptive differences in timing of bud set and frost hardiness between natural populations of Scots pine. As a mapping population, we considered an "open-pollinated backcross" progeny by collecting seeds of a single F_1 tree (cross between trees from southern and northern Finland) growing in southern Finland. Due to the special features of the design (no marker information available on grandparents or the father), we applied a Bayesian quantitative trait locus (QTL) mapping method developed previously for outcrossed offspring. We found four potential QTL for timing of bud set and seven for frost hardiness. Bayesian analyses detected more QTL than ANOVA for frost hardiness, but the opposite was true for bud set. These QTL included alleles with rather large effects, and additionally smaller QTL were supported. The largest QTL for bud set date accounted for about a fourth of the mean difference between populations. Thus, natural selection during adaptation has resulted in selection of at least some alleles of rather large effect.

THE genetic basis of adaptive variation in natural populations is still largely unknown. Fisher (1930) suggested that natural selection would fix alleles conferring small effects at a large number of loci. Later work by Kimura (1983) and Orr (1998) has shown that in fact the mutations fixed during an adaptive process due to directional selection are not expected to be uniformly small, but larger effects are fixed first. Fisher ignored the fact that advantageous mutations of small effect are more susceptible to the effects of drift while they are still rare. Later work by Orr (2000) has completed the picture and further emphasized that the effects of mutations that are fixed are not likely to be very small.

Counter to the prevailing understanding, existing empirical studies provide evidence that many adaptations could be based on even single loci, as reviewed by Orr and Coyne (1992). Early studies relied on quantitative analysis of line crosses using variants of the Castle-Wright estimator of the number of segregating loci (Lynch and Walsh 1998). With the advent of molecular markers and dense maps (*e.g.*, Tanksley 1993), more powerful tools have become available. For inferring the genetic architecture underlying adaptation, variation within or between populations needs to be studied.

Few such data are available, as quantitative trait locus (QTL) mapping studies on adaptive traits have been mainly on domesticated species (see references in Tanksley 1993), but these studies do not relate the QTL effects to those segregating in natural populations. Moderately large QTL effects in natural populations have been found for frost tolerance in *Eucalyptus nitens* (Byrne *et al.* 1997), and large effects have been found for flowering time in *Arabidopsis thaliana* (Clarke *et al.* 1995; Mitchell-Olds 1996; Kuittinen *et al.* 1997) and for bud flush in a cross between Populus species (Bradshaw and Stettler 1995). These and other studies in trees have been recently reviewed by Sewell and Neale (1999).

We have attempted to examine the genetic basis of adaptation to climate in Scots pine (*Pinus sylvestris*), which ranges from Spain (38°N) in the south to northern Finland (68°N), and from western Scotland (6°W) to eastern Siberia (135°E; Mirov 1967). Scots pine is wind pollinated with very efficient pollen flow (Koski 1970). The whole range of Scots pine seems to be part of a panmictic population. Allelic frequencies are homogeneous at marker loci, with F_{ST} of 0.03 at isozyme loci between Sweden and eastern Siberia (Wang *et al.* 1991). Between northern and southern Finland, $F_{ST} = 0.02$ for allozymes and restriction fragment length polymorphisms (Karhu *et al.* 1996). Scots pine thus has an effectively infinite population size, and the population structure corresponds to the model assumed by Fisher (1930).

However, the environmental variation within this large range is enormous. Even within Finland, there is a steep climatic gradient. In southern Finland the length of the growing season (days with average temperature

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>5°) is ~170 days at latitude 60°N and in northern Finland <120 days at latitude 69°N. Scots pine colonized these areas <10,000 years ago, after glaciation (Hyvärinen 1987). The colonization has required a major adaptive shift in relation to the length of the growing season. This has led to population differentiation in many growth-related traits, such as timing of vegetative growth and frost tolerance (see references in Savolainen and Hurme 1997). Reciprocal transfer experiments indicate that such differences have a genetical basis (Eiche 1966; Eriksson *et al.* 1980; Beuker 1994). In common garden experiments, growth cessation, terminal bud set, and frost hardening of young seedlings (and of adults) take place earlier in northern than in southern populations (Mikola 1982; Toivonen *et al.* 1991; Aho 1994; Hurme *et al.* 1997). The genetic differentiation between populations is very high, as >80% of total genetic variation in bud set date is found between northern and southern populations (Hurme 1999). Differences in quantitative traits are likely due to natural selection. The homogeneity of neutral markers shows that population substructuring or drift cannot have a major role.

The outcrossing mating system of forest trees gives rise to problems in QTL mapping. Populations may not be fixed for different alleles, and many different alleles may segregate within and between populations. F_2 progeny would be the best mapping population for outcrossing species. In forest trees, however, the types of mapping populations used are largely determined by the pedigrees available, as obtaining multiple-generation pedigrees takes decades in many species. Methods for F_2 require either genotypic information from grandparents or known haplotypes from parents. If these are not available, it is possible to apply the methods developed by Jansen *et al.* (1998) and Sillanpää and Arjas (1999). Sillanpää and Arjas (1999) devised a Bayesian method that combines parameter estimation and model selection (corresponding to different numbers of QTL) in an efficient way.

We examine here the genetic basis of variation in timing of bud set and development of frost hardiness in Scots pine. The very high differentiation between northern and southern Finnish natural populations provides the starting point for QTL dissection. F_2 or backcross progenies are not available for Scots pine, but we were able to obtain an "open pollinated backcross" progeny (two-generation half-sib progeny) by collecting seeds from a single F_1 tree (a cross between trees from southern and northern Finland) growing in southern Finland. We have modified slightly the method of Sillanpää and Arjas (1999) to fit this design. We compare the size of the QTL effects we detected in this one pedigree to the variation found between and within natural populations. Studying only one cross of course limits the conclusions. Our other goal was to compare Bayesian methods of mapping and effect estimation to single-locus ANOVA-based methods.

MATERIALS AND METHODS

Pine material: The mapping population was derived as follows. The Finnish Forest Research Institute has its clonal archives (vegetatively reproduced trees) in Punkaharju [61°48′N, 29°19′E; 90 m above sea level (a.s.l.)]. A tree of northern origin (P315, Kemijärvi, 66°35′N) had been crossed with a southern tree (E1101, Punkaharju, 61°48′N), and the F_1 trees had been planted in Loppi (southern Finland, 60°37′N, 24°26′E; 100 m a.s.l.). We collected open-pollinated seeds from one of the F_1 progenies in the spring of 1994. Thus, QTL detection is based on observing marker genes in the maternal component only.

The experiment was conducted twice, first in 1994 and the second time in 1996. In both years, the backcross progeny and four population samples (Salla, Sotkamo, Kerimäki, and Bromarv) ranging in latitude from 60° to 67°N in Finland were included. By using population samples as controls, we were able to observe the phenotypic variation of the backcross progeny in relation to geographical variation in the same growing season. Seeds from the populations were bulk samples from the forests of the Finnish Forest Research Institute.

In both years, in the beginning of June, 450 seeds from the backcross and 450 seeds from each control population were sown in the greenhouse in Punkaharju Forest Research Station. The plants were arranged in 10 blocks, with each tray consisting of 45 seedlings from a backcross or one of the populations. Seedlings were grown under natural daylength and ambient temperature, except that the temperature was not allowed to decline below +5°.

Scoring bud set and frost damage: Timing of growth in first-year pine seedlings is a reflection of general climatic adaptation. The differences between the populations may be expected to be found also in older populations of Scots pine. At least in *Picea abies*, the bud set dates of populations of 1- to 6-year-old seedlings were correlated over years (Ununger *et al.* 1988; Ekberg *et al.* 1994), and in *Pinus contorta* and *Picea sitchensis*, evidence of similar correlations of growth cessation with latitude has been obtained in adult trees as well (Cannell and Willet 1975).

First-year pine seedlings, grown in the greenhouse in southern Finland, will set an easily visible terminal bud toward the end of the summer. Bud set was scored twice per week from the beginning of August to the end of October in 1994, and until October 10 in 1996. The date of bud set was scored as the number of days from sowing to the date when an unambiguous bud could be seen from above the seedling. Germination occurred within a week in all seed lots. There was no latitudinal variation, and variation in germination date did not contribute to variation in bud set date.

Frost hardiness was studied in 1996 by exposing all seedlings of the backcross progeny to a predetermined freezing temperature during the frost hardening period. The frost treatment took place on October 8–9 when most of the buds had been formed. Frost damage of seedlings was based on visual scoring of needles.

For the frost treatments, a treatment temperature causing intermediate frost damage (LT_{50} = 50% needle damage) for the backcross progeny was determined in advance. Eight backcross seedlings were tested at each of six temperatures (+5, −4, −12, −22, −34, and −46°), and level of frost damage for needles was determined with the electrolyte leakage method (Repo *et al.* 1994; Hurme *et al.* 1997). The inflection point of the temperature response curve was recorded as the LT_{50} temperature. LT_{50} in the backcross was −24°, but since freezing testing of all seedlings was done 1 wk after preassessments, additional hardening was assumed to have taken place. The freezing temperature was chosen to be −28°.

The preassessments and the frost treatment to all seedlings were carried out in air-cooled chambers (ARC 300/−55+20, Arctest, Finland). The freezing programs started at 10° and the temperature was gradually cooled to the target temperature at a rate of 5°/hr. The minimum temperature lasted 4 hr, after which the temperature was raised back to 10° at 5°/hr. After the frost treatments were applied to all plants, they were transferred to a greenhouse and kept at 22°/15° (day/night), with a long photoperiod of 18 hr/6 hr (day/night). Needle damage to the seedlings was scored 10 days after freezing, on a visual scale from 0 to 10, where 0 is no injury and 10 is complete injury (all needles brown).

Randomly amplified polymorphic DNA amplifications and map construction: A randomly amplified polymorphic DNA (RAPD) map was constructed as a basis for single-marker mapping and for choosing marker cofactors for Bayesian QTL analysis (see below). Conifer seeds have haploid megagametophyte storage tissue surrounding the developing embryo. The megagametophyte has the same genotype as the egg cell. As the seed germinates, the megagametophyte can be collected off the developing seedling, and it yields sufficient DNA for a large number of PCR reactions. In this way, we could assess the genotypes of the female gametes produced by the heterozygous F_1 maternal tree. A RAPD map (Williams *et al.* 1990) was constructed from a sample of 84 megagametophytes independent of the phenotype collected off the seedlings of the backcross progeny. Methods for RAPD reactions are described in Hurme and Savolainen (1999).

Those loci where the megagametophyte genotypes segregated 1:1 (χ^2 test) in the 84 backcross progeny were chosen for mapping. The RAPD map was constructed with MAPMAKER/Exp 3.0 (Lander *et al.* 1987) using the Haldane mapping function. Grouping was done with LOD 4.0 and maximum interval 50 cM ($\theta = 0.31$) as thresholds, after which multipoint analysis was performed with log-likelihood difference ≥3 for framework markers. Other markers were located in relation to the framework. All potential scoring errors given by MAPMAKER were verified, scoring uncertain double recombinants as missing data.

Mapping the QTL: The QTL analyses for bud set were made separately for years 1994 and 1996. For improved QTL detection efficiency, selective genotyping was used (Lander and Botstein 1989; Darvasi and Soller 1992; Tanksley 1993). On the basis of phenotypic information from the seedlings in the backcross progeny, seedlings from the tails of the phenotypic distribution of bud set dates (years 1994 and 1996) and frost damage scores (year 1996) were used, while seedlings in the middle of the distribution were not genotyped. All markers with minimum distance of 1 cM in the RAPD map were amplified for the extremes as well.

In 1994, we had phenotypic data on 353 seedlings. RAPD markers were amplified from megagametophytes of 96 seedlings from the extremes of bud set dates (48 early and 48 late). Altogether, the 1994 data contained genotypes on 171 seedlings (of which 16 had missing phenotypes), including the random individuals used for RAPD map construction in addition to the extremes of selective genotyping. The 1996 data consisted of 405 phenotyped individuals, of which 96 extreme individuals were genotyped. The data sets 1994 and 1996 were also analyzed together. The results on the combined analysis are reported later only in part. For frost hardiness in 1996, we had phenotypic data for 379 individuals, of which 92 (46 low and 46 high) from both tails of the distribution were genotyped.

Single-marker analysis: Single-marker analysis (ANOVA) was used for preliminary mapping. This analysis was done as a covariance analysis (GLM) using SAS/STAT computer software (SAS 1987), with block effect as a classification variable in the regression model. Four block levels (combination of 10 blocks into 4) were used to indicate the locations of the seedling trays in the greenhouse. One marker was considered at a time and individuals with missing genotypes or phenotypes were omitted. For bud set, the single-marker analysis was done separately for 1994 and 1996.

The results were also used for choosing marker covariates to control background variation in the Bayesian QTL analysis. A similar principle is also used in composite interval mapping (Jansen 1993; Zeng 1993, 1994; Jansen and Stam 1994). In each data set, one significant marker from each linkage group showing potential effects ($P \sim 0.05$) was selected as a background control. For bud set, seven and eight background controls were used in years 1994 and 1996, respectively. For frost hardiness data, four markers were used (see Figure 2).

Bayesian analysis: Sillanpää and Arjas (1999) presented a hierarchical Bayes model for the QTL mapping of outcrossing species, where the number of QTL in the analyzed linkage group as well as the unobserved (parental) linkage phases and missing incomplete marker genotypes were all treated as random variables with unknown values. In this model, some QTL effects from other linkage groups are controlled for by using marker covariates representing potential QTL. During the estimation procedure, the model parameters (such as the number of QTL, their locations, genotypes, and the corresponding phenotypic effects) are all updated according to a Markov chain Monte Carlo (MCMC) sampling scheme, eventually resulting in dependent samples from the posterior distributions of these parameters. The Bayesian inference is then based on conclusions drawn from these samples.

Bayesian QTL analyses were executed with a modified (see appendix) C program (Multimapper/OUTBRED; Sillanpää and Arjas 1999), assuming a known marker map (see map construction above) but unknown maternal linkage phases. The linkage phases estimated for the marker map were not utilized here, because a smaller offspring data set was used in estimating the marker map. For each linkage group of each data set, the method was run 2×10^6 cycles in the DEC ALPHA 21164/437MHz processor at the Center for Scientific Computing of Finland. No initial sample value was rejected because of starting-value dependency (burn-in), but the chain was thinned because of limited storage capacity so that only every 10th iteration was saved.

In the preprocessing, all haplotypes of the offspring and genotypes of the mother tree were already known based on the experimental design, but the possible alleles of the missing diploid offspring genotypes and their origins were completed by using genotyping rules of Wijsman (1987). Technically (see Appendix A in Sillanpää and Arjas 1999), the known homozygote genotype was artificially given to all marker loci in the "synthetic father" to ensure that its assumed (background) contribution to the analysis was a constant. With this assumption, the environmental contribution of the variance was inflated by the paternal additive genetic contribution, with a corresponding decrease in the additive genetic variance. This is a simplifying assumption, which does not quite hold (but note that 85% of the total variation was between the northern and southern grandparental populations, Salla and Kerimäki; see below). For the mother tree, a known heterozygote genotype was given to all loci with unknown linkage phases.

The same environmental covariate (block) as in the single-marker analysis was also used here. The initial value of the number of QTL was three, with the corresponding loci evenly placed along the linkage group to be mapped. Following Sillanpää and Arjas (1998, 1999), the Poisson mean was set to two and the maximum number of QTL in one linkage group to three. Uninformative priors (see below) were chosen for regression parameters because no prior knowledge was avail-

able on these. The residual standard deviation was chosen to be uniform over ranges [0, 13.37], [0, 15.91], and [0, 3.27] for bud set data from 1994 and 1996, and frost hardiness, respectively. The right endpoint of the interval was the phenotypic standard deviation of the particular data set. The prior of the intercept for bud set (frost hardiness) was chosen to be uniform on [−200.0, 200.0] ([−40.0, 40.0]) and those of the QTL genotypic effects were independent normal distributions with mean zero and variance 2000 (400). The priors of the background control effects were uniform on [−200.0, 200.0] ([−40.0, 40.0]). The prior of the QTL location was uniform between zero and the length of the analyzed linkage group. The following proposal distributions were chosen on the basis of several test runs. The random walk proposal ranges (symmetric uniform density around previous value; Chib and Greenberg 1995) in the MCMC analyses were chosen to be 2.0 (location), 5.0 (intercept), 1.0 (residual SD), 7.5 (QTL coefficients), and 10.0 (cofactor coefficients) for bud set. The corresponding values applied to the frost hardiness data were one-tenth of those for bud set, except for location, which was 2.0. The proposal distributions for the genotypic effects of bud set and frost hardiness were chosen to be $N(0, 10.0)$ and $N(0, 2.5)$ in cases where the addition of a new QTL to the model was proposed.

Estimating QTL effects: We estimated QTL effects using haplotype information (megagametophytes) from random sets of seedlings in each year to avoid overestimation of QTL effects due to selective genotyping and genotype × environment interactions (Lande and Thompson 1990; Melchinger *et al.* 1998).

In 1994, these seedlings included the random sample with respect to the quantitative traits used for RAPD map construction (84 seedlings) and an additional random set, giving a total of 113 seedlings. In 1996, a random set of 115 megagametophytes was genotyped. For frost hardiness, 113 random seedlings were included.

The additive effects of the putative QTL were first estimated from regression coefficients from ANOVA. The model in each data set included markers closest to the QTL (one marker per QTL) and a block effect. The regression coefficient of the marker genotype gives the difference between the two QTL genotype effects *Aa-aa*. The signs of the estimates were determined by their estimated marker phases in the mother tree, which had to correspond to the ones in the RAPD map. This is because the origins (northern or southern grandparent) of the RAPD alleles in the backcross progeny determine whether genotype class 0 (or 1) is homozygote (*aa*) or heterozygote (*Aa*) at the respective QTL locus. The genetic variance associated with each QTL was calculated as $[(Aa\text{-}aa)/2]^2$, and the relative QTL effect size was estimated by dividing the genetic variance estimate by the phenotypic variance estimate of the random sample.

A second way to estimate the QTL effects was to estimate a coefficient of determination (R^2) with ANOVA. The full model in each data set included markers closest to the QTL (one marker per QTL) and the block effect. The effect of one QTL at a time was studied by excluding the respective marker from the model. The R^2 of this reduced model was subtracted from the R^2 of the full model, giving an estimate of the effect of the respective marker. The effect of all markers was obtained by subtracting the R^2 of the model where only the block effect was included from the R^2 of the full model.

A third approach for estimating phenotypic effects was by restricting the Bayesian model to only those chromosomal regions that showed elevated posterior QTL intensity in the Bayesian analyses. Two flanking markers around each putative QTL were genotyped in each random sample and the linkage phase of the mother tree for one of these two markers was assumed known (same as those used in the ANOVA estimations). We applied either the single-QTL model or the two-QTL model enlarged with the background controls and an environmental block effect. Additive effect estimates (median and 2.5 and 97.5% quantiles) were determined from the posterior cumulative distribution function of the phenotypic effects. This was constructed as the expectation over the range of phenotypic effects associated with the different locations in the marker interval flanking the particular QTL. Genetic variance estimates (median and quantiles) were likewise determined as the expectations over the range of genetic variance associated with the different locations in the interval. QTL effect sizes were calculated from Bayesian estimation in two ways: directly from the genetic variances and indirectly from the additive effects. In the indirect estimation, the additive effects were used to estimate genetic variances and QTL effect sizes.

Epistasis and genotype × environment interactions: Interactions between marker loci closest to putative QTL were tested with ANOVA for epistasis. Markers and a block were the main effects, and an interaction term between one marker pair at a time was included.

Genotype × environment interactions were studied for bud set between years 1994 and 1996. All the markers closest to the putative QTL (one per QTL) were studied. The analysis was done with nested ANOVA with an interaction term between marker and year included. One QTL was tested at each time, and the whole model consisted of a marker, year, block (year), and the interaction term marker × year.

RESULTS

Bud set dates and frost damage scores: The control population samples (Salla, Sotkamo, Kerimäki, and Bromarv) showed regular clinal latitudinal variation in bud set in both years, and population differences were significant ($F = 84.4_{\text{d.f.}=3}$, $P < 0.001$ and $F = 629_{\text{d.f.}=3}$, $P < 0.001$, in 1994 and 1996, respectively; see Figure 1). The difference in the median date of bud set between the northernmost and the southernmost populations (Salla and Bromarv) was ~22 days in 1994 and 38 days in 1996. The difference between the two grandparental populations of the backcross (Salla and Kerimäki) was 15 days in 1994 and 28 days in 1996. The proportion of the total variance between the two grandparental populations was 48% in 1994 and 85% in 1996. In 1994, the bud set period was shorter, and the populations overlapped in bud set. In 1996, the bud set periods of the populations were not longer than in 1994 (similar variances), but the populations overlapped less in 1996 than in 1994.

In the backcross progeny, the median date of bud set was 103 (September 12) in 1994 with SD 10.3. In 1996, the median date of bud set was 94 (September 6) with SD 10.1. The bud set of the backcross was intermediate between the grandparental populations (Figure 1). The backcross progenies and the grandparental populations overlapped more in 1994 than in 1996, consistent with comparison of the population samples.

The frost damage scores of the backcross progeny after frost treatment in −28° in October 1996 were distributed close to normally, apart from a slight skewness

to the left (Figure 1). The average frost damage score was 6.9 with SD = 2.1.

Log-transformation did not make any of the distributions of bud set or frost damage more normal, so untransformed data were used in the QTL analysis.

RAPD map: Altogether 179 polymorphic loci were found with 74 RAPD primers (2.4 polymorphic loci/primer), but 12 (6.7%) of them showed segregation distortion and were excluded. Finally, the RAPD map contained 164 RAPD markers, distributed in 16 linkage groups (Figure 2), and 3 markers remained unlinked. There were 12 large groups (from 35 to 136 cM), corresponding to the haploid chromosome number of pines ($n = 12$), and 4 smaller ones (from 2 to 11 cM). The map spans 1000 cM, covering about half of the genome, estimated with the method of Chakravarti *et al.* (1991). Spacing between markers varied from 1 to 31 cM, with an average of 9.5 cM.

QTL mapping with Bayesian analysis: The posterior probability distributions for the number of QTL summarized potential QTL activity in each linkage group (Table 1). Here, the presence of one or more putative QTL in a linkage group was inferred, when the posterior probability for one or more QTL ($N \geq 1$) was at least two times the probability for the absence of a QTL ($N = 0$). In this way, the estimated number of QTL per linkage group [$E(N|\text{data})$] ranged between 0.76 and 2.40. The localization of the QTL was shown by the QTL intensity curves, where the relative frequency of QTL indications is visualized along each linkage group (Figure 3).

QTL activity for bud set in 1994 was found in linkage group (LG) 6 in the 1994 data, and in LGs 4 and 11 in 1996 (Table 1, Figure 3). In the combined data, QTL found in the separate data sets were also found, and additionally 1 putative QTL in LG 5 (Figure 3). The 1996 data indicated even the possibility of 2 QTL (posterior expectation 1.86 QTL) inside an area of 1.5 cM in LG 4, which may as well indicate 1 larger QTL.

QTL activity for frost hardiness was detected in linkage groups 1, 2, 5, 6, and 9, with two QTL in LGs 1, 2, and 5 (Table 1, Figure 3). The QTL in linkage groups 1 and 2 were clearly separated from each other, but close to each other in LG 5, possibly indicating a single QTL.

Linkage groups 5 and 6 showed QTL activity for both bud set and frost hardiness. In LG 5 the QTL were clearly separated, but were close to each other in LG 6. No other genetic association between the traits was found, corresponding to the low phenotypic correlation (0.24).

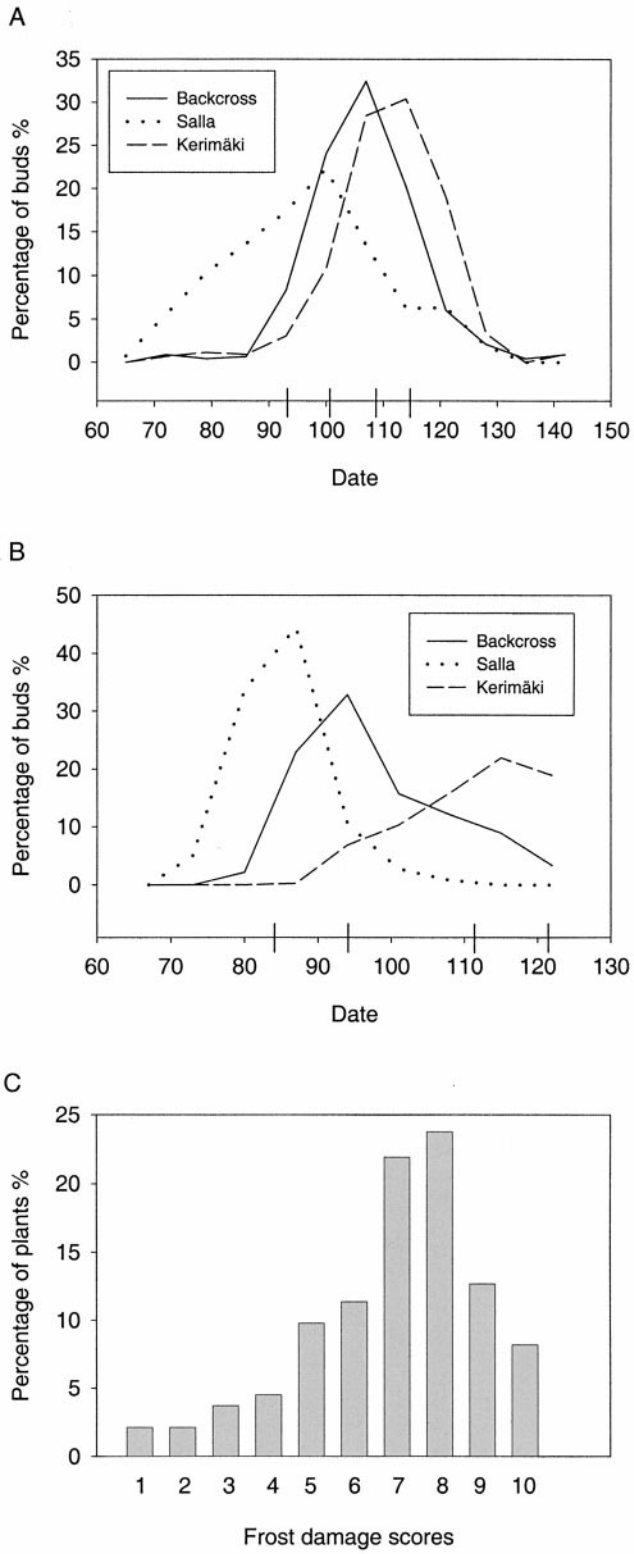


Figure 1.—(A) Frequency distributions of date of terminal bud set in 1994 in the backcross progeny and in the two populations (Salla, northern Finland; Kerimäki, southern Finland) representing latitudes of the grandparents of the backcross. Median bud set dates of the populations from north to south (Salla, Sotkamo, Kerimäki, and Bromarv) are marked on the axis. (B) Frequency distributions of date of terminal bud set in 1996 in the backcross progeny and in the two populations (Salla, northern Finland; Kerimäki, southern Finland) representing latitudes of the grandparents of the backcross. Median bud set dates of the populations from north to south (Salla, Sotkamo, Kerimäki, and Bromarv) are marked on the axis. (C) Frequency distribution of frost damage classes among the backcross progeny in 1996.

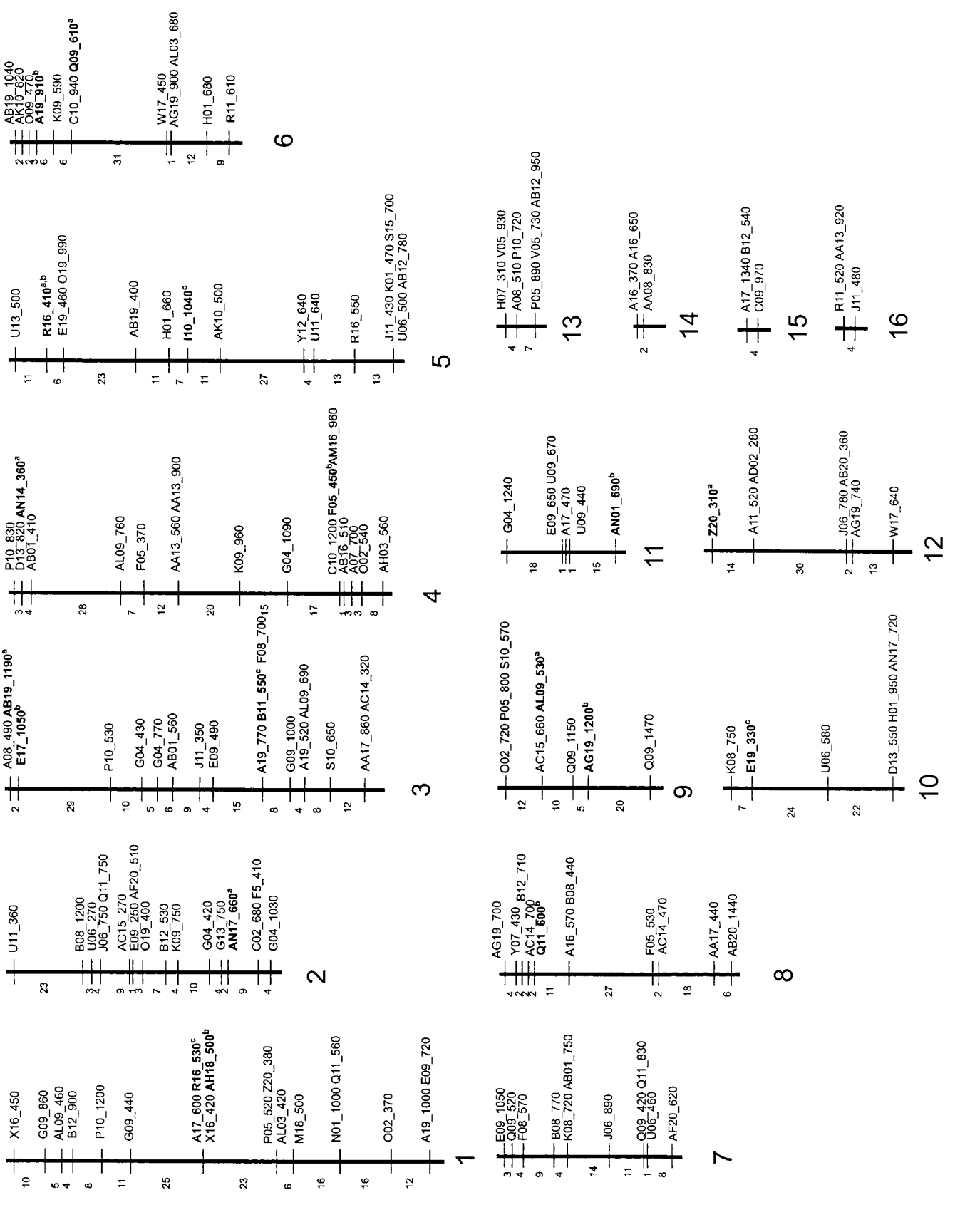

X16_450
G09_860
AL09_460
B12_900
P10_1200
G09_440
A17_600 R16_530c
X16_420 AH18_500b
P05_520 Z20_380
AL03_420
M18_500
N01_1000 Q11_560
O02_370
A19_1000 E09_720
1
U11_360
B08_1200
U06_270
J06_750 Q11_750
AC15_270
E09_250 AF20_510
O19_400
B12_530
K09_750
G04_420
G13_750
AN17_660a
C02_680 F5_410
G04_1030
2
A08_490 AB19_1190a
E17_1050b
P10_530
G04_430
G04_770
AB01_560
J11_350
E09_490
A19_770 B11_550c F08_700
G09_1000
A19_520 AL09_690
S10_650
AA17_860 AC14_320
3
P10_830
D13_820 AN14_360a
AB01_410
AL09_760
F05_370
AA13_560 AA13_900
K09_960
G04_1090
C10_1200 F05_450bAM16_960
AB16_510
A07_700
O02_540
AH03_560
4
U13_500
R16_410a,b
E19_460 O19_990
AB19_400
H01_660
I10_1040c
AK10_500
Y12_640
U11_640
R16_550
J11_430 K01_470 S15_700
U06_500 AB12_780
5
AB19_1040
AK10_820
O09_470
A19_910b
K09_590
C10_940 Q09_610a
W17_450
AG19_900 AL03_680
H01_680
R11_610
6
E09_1050
Q09_520
F08_570
B08_770
K08_720 AB01_750
J06_890
Q09_420 Q11_830
U06_460
AF20_620
7
AG19_700
Y07_430 B12_710
AC14_700
Q11_600b
A16_570 B08_440
F05_530
AC14_470
AA17_440
AB20_1440
8
O02_720 P05_800 S10_570
AC15_660 AL09_530a
Q09_1150
AG19_1200b
Q09_1470
9
K08_750
E19_330c
U06_580
D13_550 H01_950 AN17_720
10
G04_1240
E09_650 U09_670
A17_470
U09_440
AN01_690b
11
Z20_310a
A11_520 AD02_280
J06_780 AB20_360
AG19_740
W17_640
12
H07_310 V05_930
A08_510 P10_720
P05_890 V05_730 AB12_950
13
A16_370 A16_650
AA08_830
14
A17_1340 B12_540
C09_970
15
R11_520 AA13_920
J11_480
16

TABLE 1

Bayesian posterior probability distributions for the numbers of QTL

Data set	Group	$N = 0$	$N = 1$	$N = 2$	$N = 3$	$E(N\|\text{data})$
	Prior	0.16	0.32	0.32	0.21	
Bud set 1994	6	0.22	0.71	0.06	0.003	0.85
Bud set 1996	4	0.00	0.19	0.76	0.049	1.86
	11	0.19	0.69	0.11	0.007	0.94
Frost hardiness	1	0.00	0.19	0.79	0.020	1.83
	2	0.00	0.00	0.60	0.396	2.40
	5	0.00	0.05	0.72	0.227	2.17
	6	0.00	0.96	0.04	0.001	1.04
	9	0.30	0.65	0.05	0.002	0.76

Groups with QTL activity are shown. Group refers to the linkage group, N is the Bayesian posterior distribution of number of QTL, and $E(N|\text{data})$ the posterior expectation of number of QTL. Number of MCMC iterations was 2×10^6, and thinning of the chain was 10. The truncated Poisson prior distribution of number of QTL is also shown.

Single-marker analysis *vs.* Bayesian analysis: For bud set, all QTL supported by the Bayesian analysis were also significant in the single-marker analysis. However, some additional marker areas were statistically significant in the single-marker analysis. For instance, the QTL in LG 5 was found in single-locus analyses individually in both the 1994 and 1996 data sets, but the Bayesian analysis detected it only in the combined data set. Some other cases of discrepancies are likely to have been false positives.

For frost hardiness, the methods performed differently. Single-locus analysis revealed only one significant QTL in LG 1 (the one that proved to have the largest effect on frost hardiness) and two marginally significant QTL in LG 5 and LG 9. The Bayesian analyses found two loci in LG 1 and several other QTL in other linkage groups (see Table 1, Figure 3). In LG 1, the large amount of missing data in the flanking markers at the other QTL may have led to low power and lack of significance in the single-marker analysis. The QTL in LGs 5 and 9 with the Bayesian analysis were at different locations than indicated by the single-locus analysis.

QTL effects: The individual QTL effect estimates were similar to the two methods based on ANOVA (Table 2). The ANOVA estimates were also mostly similar to the Bayesian estimates based on using additive effects. A few larger differences occurred, however, in LG 6 in bud set data from 1994 and in frost hardiness.

Some individual QTL effects obtained from the genetic variances with the Bayesian method were very different from other estimates. The large credible regions (quantiles) of the genetic variances resulted in inaccurate genetic variance estimates, and thus the QTL effect sizes obtained from these estimates are not reported.

With ANOVA the QTL effects calculated from R^2 values ranged up to 12.7% for bud set and up to 11.1% for frost hardiness, which were close to the estimates obtained from the ANOVA additive effects (Table 2). The estimates from the additive effects with the Bayesian method ranged up to 13.0% for bud set and up to 11.7% for frost hardiness. The largest QTL for bud set (effect 12.7% from ANOVA R^2) was located in LG 4 in the 1996 data. For frost hardiness, the largest QTL (11.1% from ANOVA R^2) was located in LG 1 (Figure 3). The closely linked QTL for bud set and frost hardiness in LG 6 (distance of the peaks ~15 cM) had parallel effects (positive signs; the later the bud set, the more frost damage). There may be two closely linked QTL or one single pleiotropic QTL.

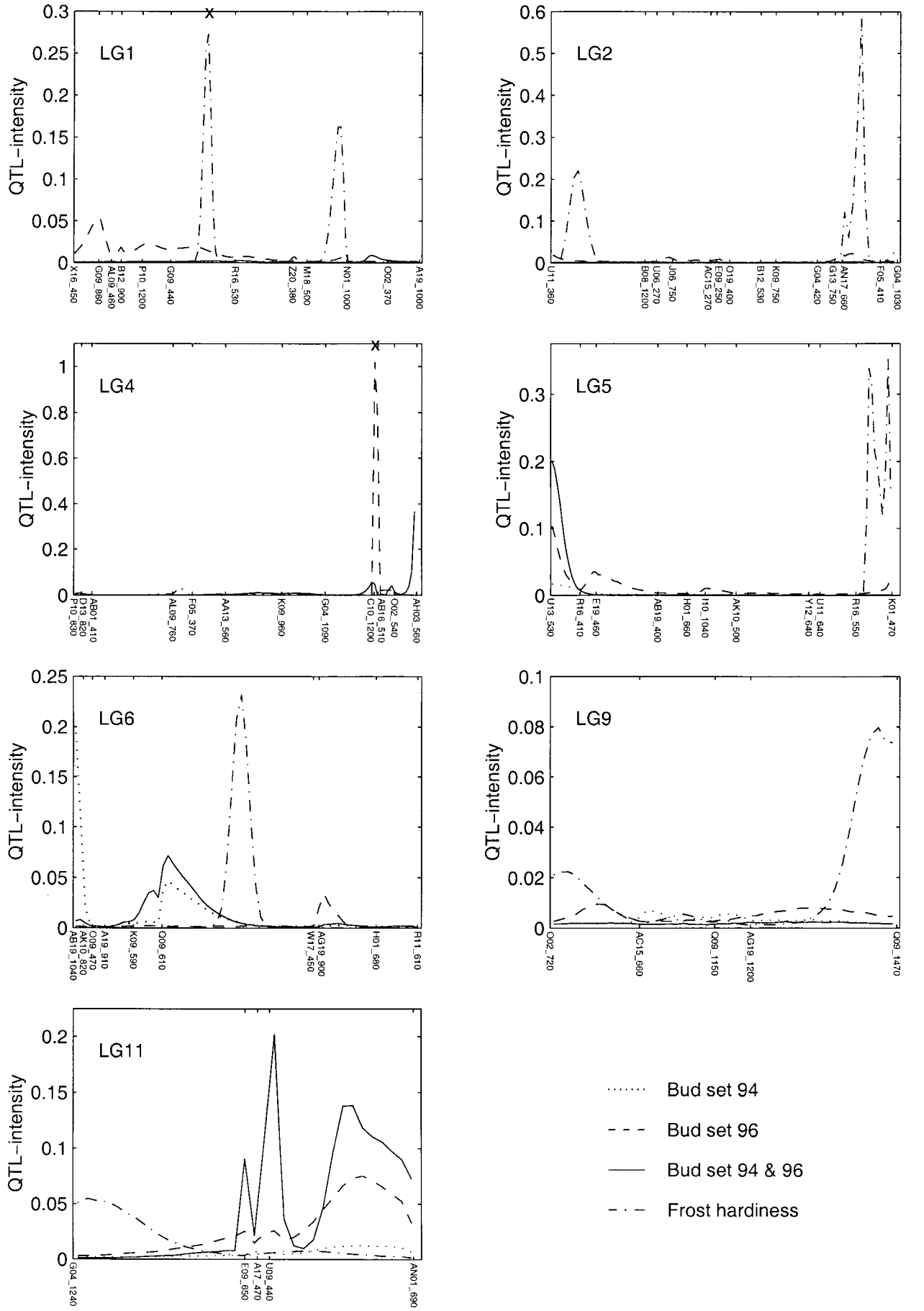
Altogether, based on the R^2 values, the markers explained 3.5 and 15.4% of the total phenotypic variation in the backcross bud set data from 1994 and 1996, respectively. For frost hardiness, 24.9% of the variation was explained by the markers.

Epistasis and genotype × environment interactions: There was no evidence for epistasis. None of the interactions between marker pairs were significant in ANOVA in any data set (data not shown). Nor did we find any genotype × environment interactions at the QTL, but the tests had low power. However, the control populations displayed significant population-by-year interaction ($F = 56.08_{\text{d.f.}=3}$, $P < 0.0001$; see Figure 1).

DISCUSSION

Size of factors underlying adaptive evolution in natural populations: As described in the Introduction, work by Kimura (1983) and Orr (1998, 2000) has suggested

Figure 2.—RAPD map of the F_1 tree (P315 × E1101). Markers used as background control markers in the Bayesian analysis chosen on the basis of single-marker regression are marked with boldface letters (**a**, 1994 bud set data; **b**, 1996 bud set data; **c**, frost hardiness data).


LG1
LG2
LG4
LG5
LG6
LG9
LG11
QTL-intensity
Bud set 94
Bud set 96
Bud set 94 & 96
Frost hardiness

that adaptation should be based on factors of larger size than proposed by Fisher (1930). Thus, during adaptation, alleles with intermediate effects should be fixed by natural selection. Fisher's theory concerns especially large continuous populations. On the basis of information from neutral markers, Scots pine adaptation fits this situation rather well.

We found two QTL in 1996 data, the effects of which on bud set date were estimated as 4.5 and 3.1 days. These are the differences between the heterozygote and the homozygote. As it is known from earlier studies that there is little dominance (Mikola 1982; Hurme *et al.* 1997 and references therein), the difference between the two homozygotes at these loci would be 9 and 6 days, respectively.

We try to relate the size of these QTL to variation in natural populations. Two of our four population samples correspond to the populations of the grandparental trees here, Salla and Kerimäki. The mean difference in bud set date between the two populations was 28 days. Another quantitative genetics study, conducted with similar methods, in the same greenhouse, in the same year, showed that the estimated additive genetic variation in a southern population was 23.6 days^2 (based on 19 families with 40 progeny in each; Hurme 1999). Thus, additive genetic standard deviation is 4.9. This is the background against which we compare the sizes of the effects we found.

Fixation for alternative alleles in the north and south populations at these two loci alone (9 and 6 days) could account for more than half the difference between the grandparental populations. Note that the largest effect, 4.5 days, is close to the additive genetic standard deviation of the southern population. Thus, effects at the two individual loci could possibly account for a significant part of the between-population variation. Further, the individual effects segregating between populations (4.5 and 3.1 days) are rather large relative to the additive genetic variance estimate within the southern population. However, it is likely that we have not detected all QTL even in this cross. In regions of the genome with low coverage there may be loci with large effects, and there will be smaller ones that we did not detect. Our conclusion is of course also limited because we only analyzed one cross. However, in our view the important point is that we have demonstrated the existence of alleles of relatively large effects at a few loci, even if we cannot estimate in what proportion of crosses such large effects would be found.

The proportion of variation in this backcross explained by the QTL: In all, the QTL accounted for a low proportion of the total phenotypic variation of the backcross in bud set date (15%) and frost hardiness (25%). However, our aim really was to compare the effects to genetic variation within and between populations, not to that of the backcross. The low proportion is accounted for by several reasons. First, there is much environmental variation in the backcross. Our earlier quantitative genetics study gave within-population heritabilities of between 0.3 and 0.6 for southern and northern populations (Hurme 1999). Further, we only knew the marker results for the maternal parent and did not have any control over the paternal parent. Any variation contributed by the paternal parents will be included in the environmental variance. Last, the map did not have full genome coverage. Thus, some QTL likely have gone undetected. These factors do not bias our estimates of QTL effects. In fact, the single-locus estimates of QTL effects are more likely to be underestimates, because recombination between the marker and the QTL will lower the effect estimates. Despite the limited power of the experiment, the finding of relatively large effects compared to between-population differences or within-population additive genetic variation will stand.

Comparison with QTL found in other organisms: In comparison to most QTL mapping studies, there are three relevant aspects to this data set. First, we studied natural populations, not influenced by human selection. Second, we studied QTL responsible for within-species differences. And most importantly, Scots pine is an outcrossing species. All of these factors could influence our expectations of sizes of effects, but all of the factors could also influence the statistical power to detect QTL. In outcrossers, background heterogeneity makes QTL detection more difficult, but if large differences exist between parents, QTL can still be detected.

There are only a few other studies on natural outcrossing populations, where the differentiation was generated by natural selection. In *E. nitens*, two QTL with effects of 8 and 11% of the total phenotypic variation were found for frost tolerance (Byrne *et al.* 1997). A direct comparison of the effects is not possible, as we do not know the heritabilities or differentiation between parental populations. In *Drosophila melanogaster*, variation in bristle number has been studied as a model trait of quantitative variation, and alleles with large effects at a neurogenic locus, *scabrous*, govern variation within natural populations (Mackay and Langley 1990; Mackay 1995). This locus explained 13 and 8% of the genetic variation in abdominal bristle number and sternopleural bristle number, respectively, demonstrating the segregation of alleles of large effect in natural

Figure 3.—The approximate posterior QTL intensities represented as frequency polygons for bud set timing and for frost hardiness in the backcross progeny. Posterior QTL intensities (*y*-axis) with bin length 1 cM are shown for only those linkage groups that showed elevated QTL activity at least in one trait. QTL with largest effects are marked with an x. LG number is in each top left corner; marker names are on the *x*-axis.

TABLE 2

QTL effects from ANOVA and Bayesian estimations

					ANOVA				Bayesian estimations		
	Marker	LG[a]	No. QTL[b]	Phenotypic variance[c]	*N* (obs.)[d]	Effect (R^2)[e]	Effect (addit.)[f]	Additive (SE)[g]	Effect[h]	Additive (quantiles)[i]	Genetic variance (quantiles)[j]
Bud set	AB19_1040	6	0.9	66.8	113 (81)	3.5	3.7	3.1 (1.9)	13.0	5.9 (2.2, 9.5)	8.1 (1.2, 20.9)
−94	Block					2.1					
	Markers					3.5					
	Model[k]					5.1					
−96	C10_1200	4	1.9	35.3	115 (92)	12.7	14.5	4.5 (1.2)	12.5	4.2 (1.9, 6.5)	4.5 (0.9, 10.5)
	AN01_690	11	0.9	35.3		5.6	6.6	3.1 (1.3)	4.8	2.6 (0.3, 4.9)	1.7 (0.04, 6.1)
	Block					2.6					
	Markers					15.4					
	Model					19.3					
Frost	G09_440	1	1.8	4.8	113 (53)	11.1	12.2	1.5 (0.6)	10.2	1.4 (−3.7, 5.2)	0.7 (0.0, 6.7)
hardiness	N01_1000			4.8		1.3	1.6	−0.6 (0.6)	1.9	−0.6 (−5.4, 5.1)	1.0 (0.0, 8.3)
	U06_270	2	2.4	4.8		0.1	0.1	0.2 (0.6)	0.2	−0.2 (−3.7, 3.7)	0.1 (0.0, 5.1)
	F05_410			4.8		2.2	3.2	0.8 (0.7)	1.3	0.5 (−4.3, 4.0)	0.3 (0.0, 5.8)
	R16_550	5	2.2	4.8		4.0	3.7	−0.8 (0.5)	0.8	−0.4 (−1.4, 0.5)	0.1 (0.0, 0.5)
	Q09_610	6	1.0	4.8		4.4	4.3	0.9 (0.6)	11.7	1.5 (0.3, 2.5)	0.5 (0.0, 1.6)
	Q09_1470	9	0.8	4.8		0.03	0.04	0.1 (0.6)	0.01	−0.03 (−1.1, 1.1)	0.03 (0.0, 0.4)
	Block					4.6					
	Markers					24.9					
	Model					28.4					

[a] Linkage group.
[b] Number of QTL supported by the Bayesian method.
[c] Phenotypic variances of the samples.
[d] Number of observables (number of observations when missing data omitted) in ANOVA.
[e] QTL effects calculated from the ANOVA R^2 values.
[f] QTL effects calculated from the ANOVA additive effects.
[g] Additive effects from ANOVA (standard errors).
[h] Bayesian QTL effects calculated from the additive effects.
[i] Additive effects from the Bayesian analysis (2.5, 97.5% quantiles).
[j] Genetic variances from the Bayesian analysis (2.5, 97.5% quantiles).
[k] Block and markers jointly.

populations (Lai *et al.* 1994; Long *et al.* 1995). Both Drosophila and Scots pine are effectively outcrossing, with very large effective population size, but comparisons are limited by different sampling (within *vs.* between population) and some differences in mating system.

A priori, one could expect that breeders would have efficiently selected for alleles with large effects and that domesticated populations would differ by alleles with larger effects than natural populations. Many QTL studies on crop plants do follow such a pattern (see references in Tanksley 1993; Lynch and Walsh 1998). For instance, in barley, frost tolerance loci accounted for 31 or 79% of the variation in different years (Pan *et al.* 1994). In the outcrossing *Brassica rapa*, smaller effects have been found (Teutonico *et al.* 1995). Arora *et al.* (1998) and Lim *et al.* (1998) suggested oligogenic inheritance for cold hardiness in cultivated blueberry and Rhododendron.

We could also expect to find larger effects in selfing species rather than in outcrossers. In selfing populations with smaller effective population sizes, alleles with larger effects (and more pleiotropic effects) could be fixed due to drift. Indeed, in selfing *A. thaliana*, flowering time differences between populations are largely due to one major gene and several minor ones (Clarke *et al.* 1995; Mitchell-Olds 1996; Kuittinen *et al.* 1997).

QTL fixed between species could be larger than within species due to longer separation and possible selection for reproductive isolation and QTL differentiation. At the interspecific level, a cross between Populus species revealed five QTL influencing bud flush, which explained 85% of the total variation (Bradshaw and Stettler 1995). For bud set, Frewen *et al.* (2000) found four QTL with phenotypic effects between 6 and 12%, confirming these expectations.

Specific features of the study: The large size of the backcross, ~400 segregating progeny per year, was a good starting point for an efficient study (see Melchinger *et al.* 1998). Considering the design, we had two grandparents from different populations in the cross. Although the populations were differentiated, they were each variable for bud set and frost hardiness loci. Only part of this variation was introduced into the cross, and only those loci heterozygous in the F_1 could be detected.

An important feature in the design was also the lack of information on the paternal component. A constant genetic effect from the father's side was assumed in the QTL analysis (fixed QTL in the pollen pool). We know, however, that the pollen pool is variable, and this may decrease the power of the analysis.

The different bud set QTL found between years 1994 and 1996 may have been due to environmental variance. The interactions between QTL and the environment may influence the number and the size of QTL contributions found between experiments (Melchinger *et al.* 1998). In the greenhouse, this would mean different conditions between years due to ambient weather fluctuations or due to microenvironmental differences. The environmental variations clearly differed between years, although there were no significant genotype × environment interactions. The control populations differed in their reactions between years ($P = 0.001$), even if the ranking order of bud set was maintained. Other QTL studies have provided evidence for G × E interactions (*e.g.*, Jansen *et al.* 1995).

Evaluation of the Bayesian method: Sometimes inbred line-cross (interval) methods are applied to outbred data, even though many outbred methods (Haley *et al.* 1994; Maliepaard and Van Ooijen 1994; Jansen 1996; Knott *et al.* 1997; Jansen *et al.* 1998; Sillanpää and Arjas 1999) are available. This may lead to unreliable results, since, in inbred methods, calculation of the QTL genotype probabilities is based on more restrictive conditions for parental haplotypes. Interval methods available for outbred half-sib designs (Georges *et al.* 1995; Knott *et al.* 1996) were not directly applicable to our design at the time we performed the analyses. Thus, we analyzed the Scots pine data with the Bayesian outbred method, which provided us a flexible multiple-QTL model with background controls, where multiple testing problems could be avoided (Shoemaker *et al.* 1999). Therefore, the Bayesian method was considered to be most suitable for our data.

The recently developed composite interval mapping method of Wu (1999) for megagametophytic information could be applicable to our design. In Wu's method the outcrossing rate and QTL genotype frequencies in the pollen pool are parameters to be estimated, whereas in our method these parameters were considered to be constants (homozygous pollen pool, outcrossing rate 100%). However, in Scots pine, empirical results in southern Finland suggest outcrossing rates ~95–100% (Muona and Harju 1989).

In our backcross, parental mating type (always *Aa* × *aa*) and therefore marker informativeness are the same for all markers, which is similar to the inbred line cross case. Due to the same information content, different chromosomal regions are treated equally in the analysis, and there is no tendency for QTL-intensity (or LOD-score) graphs to be biased toward highly informative areas. For the same reason, the absolute values of a test statistic from the single-marker analysis are also comparable between markers. However, incomplete marker coverage, unequal spacing, and missing genotypes may cause unbalance among chromosomal regions in the analysis.

QTL effects with ANOVA and the Bayesian method: The estimates of largest QTL effects from ANOVA were congruent with the QTL effect size estimates from the Bayesian method. In some other cases, the results obtained with the methods differed from each other (*e.g.*, bud set QTL in LG 6).

The two approaches, ANOVA and Bayesian estimations, are not directly comparable. ANOVA estimations were performed by considering only the observed data (omitting the missing data) and assuming that the QTL resides at a marker locus. Due to these conditions, the standard errors were relatively small. Further, ANOVA estimates of QTL effects may be underestimates, since the actual QTL location is usually at some distance from the marker. In the Bayesian estimation, on the other hand, uncertainty is incorporated by unknown positions and unknown genotypes of QTL within the given marker regions, as well as by partly unknown maternal linkage phases. As a result, credible regions and standard errors were different. In the Bayesian estimation, it could also be seen that the second moments (such as variances) are not as well estimated as the first moments (such as additive effects). The genetic variances from the Bayesian method did not fit well to the additive effects, but when these were estimated directly from the additive effect estimates, QTL-effect size estimates similar to ANOVA effects were obtained.

Some differences in the signs of the additive effects obtained from ANOVA and Bayesian method were found. In most cases this can be explained by the small effects (additive effects ~ 0). In bud set data from 1996, the estimate of the sign of the additive effect (2.6) in linkage group 11 with the Bayesian method depended on which one of the flanking markers was assumed to have a known linkage phase given by the RAPD marker map. Comparison with the single-locus method supported a positive effect.

The effects of some potential QTL found in the Bayesian mapping proved to be very small, $<1\%$. Yet these QTL were detected with Bayesian mapping. The main reason may be that different data sets (phenotypic extremes in QTL mapping and random data sets in effect estimations) were used. These may have weighed the effects differently, possibly due to the pattern of distribution of missing data.

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APPENDIX

Here we comment briefly on the order in which the updating of the MCMC algorithm takes place. There are two strong dependency relations in the offspring data: the vertical dependency between parents and their offspring, and the horizontal dependency between adja-

cent loci in each individual. If single-site updating is applied, the sampler can easily get stuck in some part of the sample space because of these dependencies (Sheehan and Thomas 1993; Janss *et al.* 1995; Jensen and Sheehan 1998). To facilitate movement in the sample space (mixing) of the MCMC sampler, especially in cases in which a large proportion of the data is missing and the markers are very close to each other, the following two-directional blocking scheme was implemented to the sampling algorithm of the Multimapper/OUTBRED program of Sillanpää and Arjas (1999). This modified program version is currently available on the web (http:/www.rni.helsinki.fi/∼mjs) and it was applied in all cases in this study.

Step 2 in the sampling scheme of Sillanpää and Arjas (1999; Appendix A) is modified in the following way: With equal probabilities, the sampler does either a (1) family block update or an (2) individual update.

1. The family block update is similar as before, except for step 2.5. In the new version, the grandparental origins are determined for offspring alleles having a heterozygous parent, but are proposed directly from the prior (Equation 4) for alleles inherited from homozygotes. The acceptance ratio is then modified accordingly.
2. Individual update: Proposals covering the entire chromosome (all markers jointly) of each offspring are constructed (conditional on parents) similarly as in step 2 of Sillanpää and Arjas (1999; Appendix A), but their acceptance is tested separately for each haplotype proposal of each individual. The acceptance ratio is again modified accordingly.