Evaluation of major genetic loci contributing to inbreeding depression for survival and early growth in a selfed family of *Pinus taeda*

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Abstract:

The magnitude of fitness effects at genetic loci causing inbreeding depression at various life stages has been an important question in plant evolution. We used genetic mapping in a selfed family of loblolly pine (*Pinus taeda* L.) to gain insights on inbreeding depression for early growth and viability. Two quantitative trait loci (QTLs) were identified that explain much of the phenotypic variation in height growth through age 3 and may account for more than 13% inbreeding depression in this family. One of these QTLs maps to the location of *cad-nl*, a lignin biosynthesis mutation. Both QTLs show evidence of overdominance, although evidence for true versus pseudo-overdominance is inconclusive. Evidence of directional dominance for height growth was noted throughout the genome, suggesting that additional loci may contribute to inbreeding depression. A chlorophyll-deficiency mutation, *spf*, did not appear to be associated with growth effects, but had significant effects on survival through age 3. Previously identified embryonic viability loci had little or no overall effect on germination, survival, or growth. Our results challenge, at least in part, the prevailing hypothesis that inbreeding depression for growth is due to alleles of small effect. However, our data support predictions that loci affecting inbreeding depression are largely stage specific.

Keywords: Conifers | fitness | genetic mapping | growth | inbreeding depression | loblolly pine | Pinus taeda | plants | stage specificity | survival

Article:

The extent to which mutations with major versus minor fitness effects are responsible for inbreeding depression at various life stages has been an important question in plant evolution. Highly deleterious alleles are easily purged in inbred populations, but alleles with only minor

effects on fitness will tend to persist and may become fixed in small populations (Lande and Schemske 1985; Byers and Waller 1999). Husband and Schemske (1996) used the relationship between allelic effects and purging to compare levels of inbreeding depression in predominantly selfing versus outcrossing taxa. They surmised that inbreeding depression for seed viability and germination is caused mostly by recessive lethals, whereas inbreeding depression for later-acting traits such as growth and fecundity is due primarily to slightly deleterious alleles. Byers and Waller (1999), however, have found inconsistent evidence for purging at any stage of development.

Genetic mapping can be a powerful tool for gaining further insights into the genetic architecture of inbreeding depression (Ritland 1996). Mapping adds the important dimensionality of linkage and locus order to the more traditional uses of genetic markers to sample genetic diversity in populations. Inferring the effects of segregating alleles at individual loci and their interactions has proven problematic with conventional quantitative methods, but these effects can be evaluated directly in many cases by mapping (Mitchell-Olds 1995b). Several recent studies have used segregation distortion of mapped markers to identify genetic loci affecting viability (Mitchell-Olds 1995a; Cheng et al. 1996; Kuang et al. 1998, 1999). Mapping of quantitative trait loci (QTLs) in selfed families could be similarly valuable for studying the effects of inbreeding depression on later traits such as plant growth. Not all QTLs identified in selfed families will necessarily be related to inbreeding depression. Loci with positive dominance coefficients for a trait, however, will cause inbreeding depression in proportion to the product of allele frequencies, the size of the dominance effects, and the inbreeding coefficient (Falconer and Mackay 1996). Loci identified in mapping studies will be specific to the particular families evaluated, and care must be taken in extrapolating findings to populations.

Conifers such as loblolly pine (*Pinus taeda* L.) are ideal organisms for the study of inbreeding depression in plants from several standpoints. Most conifers display exceptionally high levels of inbreeding depression, especially for viability during the embryonic stage (Lande et al. 1994; Williams and Savolainen 1996). The lack of a separately fertilized endosperm and absence of self-incompatibility mechanisms in conifers make estimation of early inbreeding depression relatively straightforward. These same features as well as conifers' longevity facilitate estimating the degree and specificity of inbreeding depression at different life stages (Charlesworth and Charlesworth 1987). The occurrence of multiple fertilized archegonia in a single ovule (polyembryony) in conifers, however, can confound estimates of early inbreeding depression from viability data (Sorensen 1969; Franklin 1972).

We recently reported a genetic-map-based study of the genetic architecture of embryonic inbreeding depression in a selfed family of loblolly pine (Remington and O'Malley 2000). Our approach used amplified fragment length polymorphism (AFLP) markers (Vos et al. 1995) from a previously constructed linkage map (Remington et al. 1999). In this paper, we extend those findings to include effects on germination and growth through the first three years of life. Objectives of this study were: (1) to evaluate the effects and modes of gene action at QTLs for seedling germination and early growth in the selfed family; (2) to map observed lignin biosynthesis and chlorophyll-deficiency mutations and evaluate their effects on survival and growth; and (3) to evaluate embryonic viability loci for effects on survival and growth. From these analyses, we were able to evaluate the contribution of major genes to inbreeding depression

for germination and growth in this family and the degree to which deleterious alleles are stage specific. Finally, we consider implications for the evolution of inbreeding depression and quantitative trait variation and for breeding strategies. The results from this study are specific to a single family and not to loblolly pine populations as a whole. Nevertheless, they provide a level of locus-specific detail that has not been obtainable to date against which population-level data obtained by traditional methods can be evaluated.

MATERIALS AND METHODS

Selfed Progeny

Selfed seed from loblolly pine clone 7–56, an elite first-generation breeding selection from the NCSU-Industry Cooperative Tree Improvement Program, were germinated in December 1995 under sterile conditions as described by Remington and O'Malley (2000). A total of 279 germinated seedlings were obtained from 373 filled seeds (74.8% germination). Germinated seedlings were grown in Ray Leach super cells with peat-vermiculite growing medium in a greenhouse under a high-nutrient regime, with supplemental lighting (approximately 16-h day length) until April 1996. On May 1, the seedlings were put under a nutrient stress regime (15–30–15 fertilizer at a rate of 5 ppm N) to provide growing conditions that have been reported to produce high juvenile-mature genetic correlations for shoot growth (Li et al. 1991). The seedlings were repotted in 10-L containers in a 4:1 mixture of bark:sand on July 3–6, and grown outdoors for the remainder of the summer and fall. Fertilizer concentrations were increased to 10 ppm N on September 26, and seedlings were moved into an unheated cold frame from November until March 1997. On June 27, 1997, the 258 surviving seedlings were transplanted to a field site near Tillman, South Carolina (provided by the South Carolina Forestry Commission) at a 2.4 × 3.0 m spacing. Most of the seedlings were in the second growing cycle of the season at the time of transplanting.

Measurements

New germinants were recorded at two- to three-day intervals during the germination period. Mortality dates were recorded for all germinated seedlings that subsequently died. A number of newly germinated seedlings with bright yellow chlorophyll-deficiency characteristics in the first primary needles were noted and recorded. The same seedlings showed similar phenotypes in the first flush of needles in the spring of the second growing season as well. Mutant *cad-n1* seedlings were also identified during the second growing season. The *cad-n1* mutants, originally characterized in this family, have a distinct dark brown wood color, associated with a nearly complete absence of cinnamyl alcohol dehydrogenase (CAD) activity in developing xylem. CAD catalyzes the final step in the synthesis of lignin precusors, and homozygous mutants display highly abnormal lignin composition (MacKay et al. 1997; Ralph et al. 1997). The total length of each successive cycle of shoot growth produced in the first growing season was measured for the main or tallest stem on each seedling. Total height was recorded for surviving trees at the end of the second and third growing seasons, and second-year and third-year growth were calculated from these measurements.

Marker Scoring

DNA preparations from seedling needle tissue and from ungerminated embryos, and AFLP template preparation, reactions, automated electrophoresis, and marker scoring have been described previously (Remington and O'Malley 2000). Almost all framework markers from a previously constructed AFLP linkage map (Remington et al. 1999) were scored, as well as some accessory markers that were included to provide full coverage with both linkage phases. Linkage group numbers and centiMorgan (cM) positions follow Remington et al. (1999). Accessory markers were assigned to the position of the nearest framework marker. The *cad-n1* and chlorophyll-deficiency mutations behaved as recessive traits, so they were assigned to map intervals by evaluating the number of recombinants to coupling phase markers using a spreadsheet program, and map positions were interpolated from the number of recombination events observed. These phenotypes were included as visible markers in subsequent analyses.

Half-Sib Family Evaluation

A large half-sib family of loblolly pine selection 7–1037 was planted in 1993 at a field site in Scotland County, North Carolina. The study contains 10 replications, two treatments (fertilized and control) per replicate, and 100 individuals per family per treatment per replicate. Selective genotyping was performed on megagametophyte DNA from seeds of the 25 largest and 25 smallest 7–1037 offspring per replicate in the fertilized plots based on year 4 stem cubic volume. Individuals were scored for a polymerase chain reaction (PCR) length polymorphism within the *Cad* promoter region that segregates in 7–1037 offspring (Wu et al. 1999) and for two AFLP markers on linkage group 1 (LG1) inherited from clone 7–56.

Data Analysis

QTL analysis for germination date, height at the end of each of the first three growing seasons, and annual growth for the second and third seasons was performed by interval mapping (IM) and composite interval mapping (CIM) with QTL Cartographer, Version 1.13 (Basten et al. 1994, 1998), models 3 and 6, respectively. QTL Cartographer estimates the conditional probabilities of homozygous and heterozygous genotypes for band-present dominant marker phenotypes, given the scores of neighboring markers, using a Markov-chain algorithm. This increases the power for QTL mapping with dominant markers in an F_2 population to a level approaching that with codominant markers, if markers in both linkage phases are well represented and the linkage map is already known (Jiang and Zeng 1997). Maximum-likelihood estimates for additive (*a*) and dominance (*d*) coefficients are obtained at 2-cM increments along each linkage group.

A likelihood-ratio test statistic threshold (*LR*) of 16.42, corresponding to a 0.05 experimentwise significance level, was derived empirically from 1000 permutations of year 2 height data in QTL Cartographer model 3. This corresponded closely to a 16.02 *LR* threshold estimated using procedures of Lander and Botstein (1989) and adjusted for an F_2 design in which *LR* has two degrees of freedom. Repulsion-phase pairs of markers assigned to the same map position were recoded as single codominant markers, primarily to reduce computing time. Additive and dominance effects and percentage of phenotypic variance explained were estimated for each identified QTL. Significance of apparent overdominant effects was evaluated by performing 100 bootstraps each on the data for individual linkage groups and evaluating the distribution of the

estimated additive and dominance coefficients $\hat{a}$ and $\hat{d}$, respectively) and of $\hat{d} - |\hat{a}|$. Marker-trait associations for germination were evaluated for individual markers using linear regression in JMP (SAS Institute 1996). A comparisonwise significance level of 0.00033 was used, which corresponds approximately to an experimentwise 0.05 level (Remington and O'Malley 2000). Germination effects were also evaluated as quantitative traits in QTL Cartographer. Linear regressions of year 3 stem diameter at breast height (DBH) and height and year 4 DBH, height, and stem cubic volume on *Cad* and putative QTL genotypes were performed in JMP.

The inbreeding depression explained by individual QTLs was estimated by subtracting the average genotypic value at each locus in the selfed family, relative to the population mean, from the average predicted breeding value for these loci in 7–56 selfed progeny. The mean genotypic value in the selfs, relative to the population mean, was estimated as

$$\sum_{\text{loci}} [\hat{a}(\tilde{P}_{QQ} - \tilde{P}_{qq}) + \hat{d}\tilde{P}_{Qq}] - \mu, \tag{1}$$

where $\tilde{P}_{ij}$ as the observed frequency of genotype ij among selfed progeny at a given locus, q is the frequency of the deleterious allele, and $\mu = (p - q)\hat{a} + 2pq\hat{d}$ ($\approx\hat{a}$ when $q \rightarrow 0$). The average breeding value of 7–56 selfed progeny associated with these loci was estimated as twice the value of

$$\sum_{\text{loci}} \left[\left(\tilde{P}_{QQ} + \frac{1}{2}\tilde{P}_{Qq} \right)(p\hat{a} + q\hat{d}) + \left(\tilde{P}_{qq} + \frac{1}{2}\tilde{P}_{Qq} \right)(p\hat{d} - q\hat{a}) \right] - \mu. \tag{2}$$

These estimates differ from standard formulas (e.g., Falconer and Mackay 1996, ch. 7), because genotype frequencies deviate from the expected 1:2:1 ratios due to linkage to embryonic viability loci. The average breeding value of selfed progeny is used in lieu of the breeding value of clone 7–56 itself to separate effects of the loci being tested from effects of allele frequency changes due to linked viability loci.

Directional dominance in year 3 height data was evaluated on linkage groups not associated with major dominant QTLs. The dominance coefficients under H_3, in which both a and d are estimated, for both models 3 and 6 in QTL Cartographer were used for this analysis. We divided each linkage group into segments in which all values of d were either positive or negative, and calculated the length (l) and average value of d for each segment. The distributions of $l \times |d|$ for segments with positive and negative values of d were compared using a two-sample t-test with the assumption of unequal variances.

The *cad-n1* and *spf* loci were evaluated for association with year 3 survival using contingency tests in JMP. Genotypes at each of the 21 markers most closely linked in coupling to identified and presumptive embryonic viability alleles were recorded for all germinated and nongerminating individuals. Band-absent individuals at one of these markers were assumed to be homozygous for the linked deleterious viability alleles. Germination, year 3 survival, and year 3 height were regressed on the number of assumed homozygous deleterious alleles (AHD alleles)

for embryonic viability, using linear regression in JMP. Numbers of samples for each number of AHD alleles were used as weights for germination and year 3 survival percentages.

RESULTS

Mapping of cad-n1 and spf Mutations

The *cad-n1* mutation mapped as a recessive Mendelian trait to the 88-cM position on linkage group 8 (LG8), approximately 20 cM from the terminal marker of the linkage map. An embryonic viability allele that caused a viability reduction of approximately 44% in homozygotes mapped to the same region, but was linked to the wild-type *Cad,* not the mutant. Homozygotes for *cad-n1* showed no effect on survival of germinating seedlings after three years (Table 1). Stems on the *cad-n1* homozygotes, however, were distinctly more supple than those of wild-type seedlings by the second year and tended to be more crooked.

Table 1. Map locations and effects of *cad-n1* and *spf* mutations. The *P*-values are two-tailed probabilities from Fisher's exact test.

			Year 3 survival/total scored		
Mutation	**Linkage group**	**Position (cM)**	**Wild type**	**Mutant**	***P***
cad-n1	8	89.0	160/178 (89.9%)	52/58 (89.7%)	1.0000
spf	1	92.0	194/222 (87.4%)	19/33 (57.6%)	0.0001

A transient chlorophyll-deficiency phenotype was observed in approximately 13% of seedlings. The abnormal bright yellow needles occurred on the first primary needles in new germinants and on needles produced on the first cycle of elongation in the spring of the second year in the same trees. Later needles produced each year were normal in appearance. The phenotype mapped as a recessive Mendelian trait to the 92-cM position on LG1. The mutant allele was linked in coupling with a recessive viability allele approximately 13 cM away, which explained the abnormal 6.7:1 segregation ratio. We concluded that the trait was controlled by a single recessive allele and named the mutation *springfever* (*spf*). Unlike *cad-n1,* the *spf* mutation showed a significant effect on survival, with only 57% of the homozygous mutant seedlings alive after three years, compared with 87% of wild-type seedlings (Table 1).

Germination Percentages

Germination percentages follow a binomial rather than normal distribution, so the statistical basis for both the least-squares regression used with single marker analysis and the maximum-likelihood estimation in QTL Cartographer are violated. Parameter estimates from both approaches seemed to correspond well with the observed discrepancies in marker frequencies between germinants and nongerminants, so both methods appeared robust in this situation. No loci with germination effects were identified that met the 0.00033 significance level with single marker analysis or the 16.42 empirical *LR* threshold with IM or CIM. Suggestive trait loci were noted on LGs 1, 6, 8, 9, 10, and 12, for which *P*-values less than 0.01 and/or *LR*-statistics greater than 10.00 were observed. One of these loci corresponded closely to the embryonic viability allele near the *Cad* locus on LG8. Both the embryonic viability and suggestive germination loci associated with *Cad* appeared to be asymmetrically overdominant (i.e., one homozygote showed lower fitness than the other). The less fit homozygote was linked to the wild-type *Cad* allele in

each case. The suggestive germination locus on LG6, with recessive effects on germination, also mapped in coupling to within 10 cM of a viability allele. Overall, 15 of 211 mapped markers showed germination effects significant at a comparisonwise 0.05 level, not greatly different from the 11 that would be expected by chance alone.

Growth Effects

Estimated QTLs for growth effects are summarized in Table 2. We evaluated QTL effects for each of the three years and cumulatively, even when effects were only significant in one year, to obtain less biased estimates of effects. Two QTLs showed consistent effects on growth that were significant or nearly so in each of the first three years. One of these QTLs mapped to the *Cad* locus on LG8, with large deleterious effects on growth associated with *cad-n1* homozygotes. The other, on LG1, had even greater effects, but was not associated with a discrete phenotype. Both loci showed asymmetrical overdominance for growth in both years 2 and 3 and for total height after year 2 (data not shown) and year 3. The $\hat{d} - |\hat{a}|$ values from bootstrap samples of data for growth in years 2 and 3 and for year 3 height were reasonably normal in distribution, except for the year 3 growth data for LG1 (Table 3). Using probabilities from the normal distribution, the values $\hat{d} - |\hat{a}|$ were significantly greater than zero only for the year 2 growth and year 3 height for the QTL on LG1. The LG1 and LG8 loci explained approximately 15% and 16%, respectively, of the within-family phenotypic variance in year 3 height. The size of effects at each locus as a percentage of the phenotypic mean was greater with each successive year, and the homozygotes for the deleterious alleles at the LG1 and LG8 loci were 42% and 29% shorter, respectively, than the homozygotes for the more favorable alleles after three years.

Table 2. Summary of quantitative trait loci for growth effects. Estimates, except for *R*2, are from QTL Cartographer model 6 (composite interval mapping), with a window size of 10 cM and five background parameters fitted. *R*2-values are based on within-family phenotypic variances. Peak *LR*-values and position of the *LR* peak are shown for each region. No *LR* peak was observed in the region of the linkage group 12 locus in year 1. Additive effects are for linkage phase A per Remington and O'Malley (2000).

				Effects: cM (% phenotypic SD)		
Linkage group	**Trait**	**Position (cM)**	***LR***	**Additive (*a*)**	**Dominance (*d*)**	**R^2**
1	Yr. 1 growth	8.0	20.11	–24.65 (49.0%)	13.65 (27.1%)	0.062
	Yr. 2 growth	23.8	17.42	–45.54 (33.1%)	95.80 (69.6%)	0.088
	Yr. 3 growth	21.8	24.43	–139.67 (46.1%)	194.99 (64.4%)	0.087
	Yr. 3 height	23.8	36.39	–225.30 (56.4%)	341.15 (85.4%)	0.145
3	Yr. 1 growth	9.1	9.34	15.11 (30.0%)	3.49 (6.9%)	0.038
	Yr. 2 growth	2.2	5.87	13.64 (9.9%)	33.80 (24.5%)	0.022
	Yr. 3 growth	8.2	16.64	149.04 (49.2%)	–28.70 (9.4%)	0.076
	Yr. 3 height	8.2	12.07	157.19 (39.4%)	21.15 (5.3%)	0.060
8	Yr. 1 growth	102.1	11.77	–16.32 (32.4%)	8.06 (16.0%)	0.052
	Yr. 2 growth	91.4	28.74	–44.58 (32.4%)	74.79 (54.3%)	0.133
	Yr. 3 growth	88.0	30.07	–100.26 (33.1%)	149.02 (49.2%)	0.119
	Yr. 3 height	87.4	39.07	–154.43 (38.7%)	226.54 (56.7%)	0.160
12	Yr. 1 growth	—	—	—	—	—
	Yr. 2 growth	103.1	18.19	–60.31 (43.8%)	7.50 (5.4%)	0.094
	Yr. 3 growth	117.6	10.25	–88.16 (29.1%)	13.67 (4.5%)	0.042
	Yr. 3 height	115.6	12.68	–134.51 (33.7%)	12.25 (3.1%)	0.057

Table 3. Test of overdominance for linkage group 1 (LG1) and LG8 quantitative trait loci (QTLs), based on 100 bootstrap replications for each linkage group and trait. *P*-values for $d - |a| \leq 0$ (i.e., absence of overdominance) are based on the cumulative normal distribution.

		$d - \|a\|$				
QTL	**Trait**	**Mean**	**SD**	**Shapiro-Wilk *W***	**Prob < *W***	***P*($d - \|a\| \leq 0$)**
LG1	Yr. 2 growth	53.4	25.2	0.9761	0.336	0.017
	Yr. 3 growth	60.6	66.3	0.9590	0.018	0.180
	Yr. 3 height	119.4	68.4	0.9791	0.482	0.040
LG8 (*Cad* locus)	Yr. 2 growth	27.4	30.8	0.9783	0.439	0.187
	Yr. 3 growth	71.3	82.7	0.9788	0.466	0.194
	Yr. 3 height	81.3	109.4	0.9752	0.302	0.229

We estimated the amount of inbreeding depression explained by each of these QTLs. This required an estimate of the population frequency (q) of the deleterious allele at each locus, which we assumed to be near zero for unconditionally deleterious alleles of large effect. Estimates of breeding value accounted for deviations in genotype frequencies from the expected 1:2:1 ratios at both QTLs. The *Cad* locus was associated with a viability allele, and the LG1 QTL mapped approximately 15 cM from a completely lethal viability allele, which substantially reduced the frequency of deleterious homozygotes. The predicted inbreeding depression for year 3 height in 7–56 selfs associated with the LG1 and LG8 QTLs was 13.1%. The predicted inbreeding depression would be 20.6% if the genotype frequencies in the selfs were in the expected 1:2:1 ratios. The amount of inbreeding depression would be less if values of q were substantially greater than zero, as would be expected if the alleles were overdominant for fitness.

Two other growth QTLs were significant in either year 2 or year 3, and showed similar trends in other years. In contrast with the LG1 and LG8 loci, the LG3 and LG12 QTLs appeared to be largely additive in effect, although the dominance estimates for the LG3 QTL varied considerably between years. No evidence was found for a growth QTL associated with the *spf* locus, which was located about 70 cM away from the LG1 QTL.

Table 4. Effects of *cad-n1* and deleterious linkage group 1 (LG1) allele in 7-1037 half-sib family at Scotland County site. Diameter breast height (DBH) values are in mm; height values are in cm; cubic stem volume values are in dm3. Year 3 values are calculated using replication effects as a covariate. Year 4 values are adjusted by overall replication means.

		cad-n1			**LG1 allele**		
Trait	**Mean**	**Estimated effect**	**SE**	***P***	**Estimated effect**	**SE**	***P***
Yr.3 DBH	26.94	0.19	1.06	0.85	0.32	1.33	0.81
Yr.3 height	250.19	1.39	4.51	0.76	0.07	5.65	0.99
Yr.4 DBH	49.76	–0.43	1.24	0.73	1.06	1.55	0.50
Yr.4 height	376.76	1.96	5.62	0.73	6.29	7.10	0.37
Yr.4 volume	3.97	< 0.01	0.17	0.99	0.16	0.21	0.45

To evaluate the population genetics of the LG1 and LG8 QTLs, we estimated effects at each locus in a large half-sib family of selection 7–1037 planted at a test site in Scotland County, North Carolina. Selection 7–1037 is an outbred daughter of 7–56 that inherited the *cad-n1* allele and the region containing the deleterious QTL allele for height growth on LG1. Neither locus had a significant effect on any of the evaluated size traits (Table 4). The deleterious LG1 allele, however, was associated with increases of 1.7% to 4.0% in the year 4 traits. This is the trend that

would be expected if the deleterious allele had a positive effect on growth as a heterozygote and were rare in the population.

Directional Dominance

We tested for evidence of additional inbreeding depression QTLs by evaluating directional dominance in the QTL Cartographer data. Inbreeding depression for a metric trait can only occur when the loci affecting the trait have dominance coefficients that are predominantly in a single direction, either positive or negative (Falconer and Mackay 1996). Reductions of height associated with inbreeding would require d to be generally positive. We hypothesized that linkage group segments in which $d > 0$ would be longer on average and would have greater absolute values of d than would segments with $d < 0$ if there were directional dominance. This would be expected to result in greater values for $l \times |d|$ for segments with positive values of d. We eliminated LG1 and LG8 from this analysis to exclude the dominant QTLs already identified. We found that segments with $d > 0$ were more than 50% longer on average and comprised 60–62% of the 10 linkage groups evaluated, depending on whether model 3 (IM) or model 6 (CIM) was used (Table 5). The tendency toward positive values of d was just barely significant at the 0.05 level under model 6 and was nearly significant ($P \approx 0.07$) with model 3, suggesting that additional loci contribute to inbreeding depression for growth.

Table 5. Evaluation of directional dominance effects for year 3 height, from models 3 and 6 in QTL Cartographer, Version 1.13d. Dominance coefficients are for H3 ($a \neq 0$, $d \neq 0$). Linkage groups 1 and 8 are excluded. Mean ($l \times |d|$) represents the mean value of the product of length (cM) and average absolute value of d over 2-cM intervals for each segment. The t-statistics are for a one-tailed, two-sample t-test assuming unequal variances.

	Model 6		**Model 3**	
	***d* < 0**	***d* > 0**	***d* < 0**	***d* > 0**
Number of segments	27	27	18	18
Total length (cM)	499.4	760.7	473.2	786.9
Mean ($l \times \|d\|$)	741	1465	1251	2497
SD	901	1942	2203	2796
t-statistic	–1.757		–1.501	
$P(T \leq t)$	0.0436		0.0715	

Relationships between Embryonic Viability and Later Inbreeding Depression

We used linear regression to evaluate whether alleles affecting embryonic viability also had pleiotropic effects on later traits. Germination percentage, cumulative survival of all embryos and of germinants after three years, and year 3 heights of individual trees were regressed on the number of AHD alleles for embryonic viability (Fig. 1). The regression coefficients of germination and year 3 survival on number of AHD alleles were slightly negative, but the effects were insignificant. Embryos with six AHD alleles (the highest number observed) did show reduced germination and survival, but this was based on a sample of only five embryos. Adding a quadratic term to the regressions did not improve the fit (data not shown). The number of AHD alleles showed no effect at all on year 3 height. The one surviving tree with six AHD alleles was in the 94th percentile for height after three years. Therefore, the data show little if any evidence for effects of embryonic viability alleles on later viability or growth.

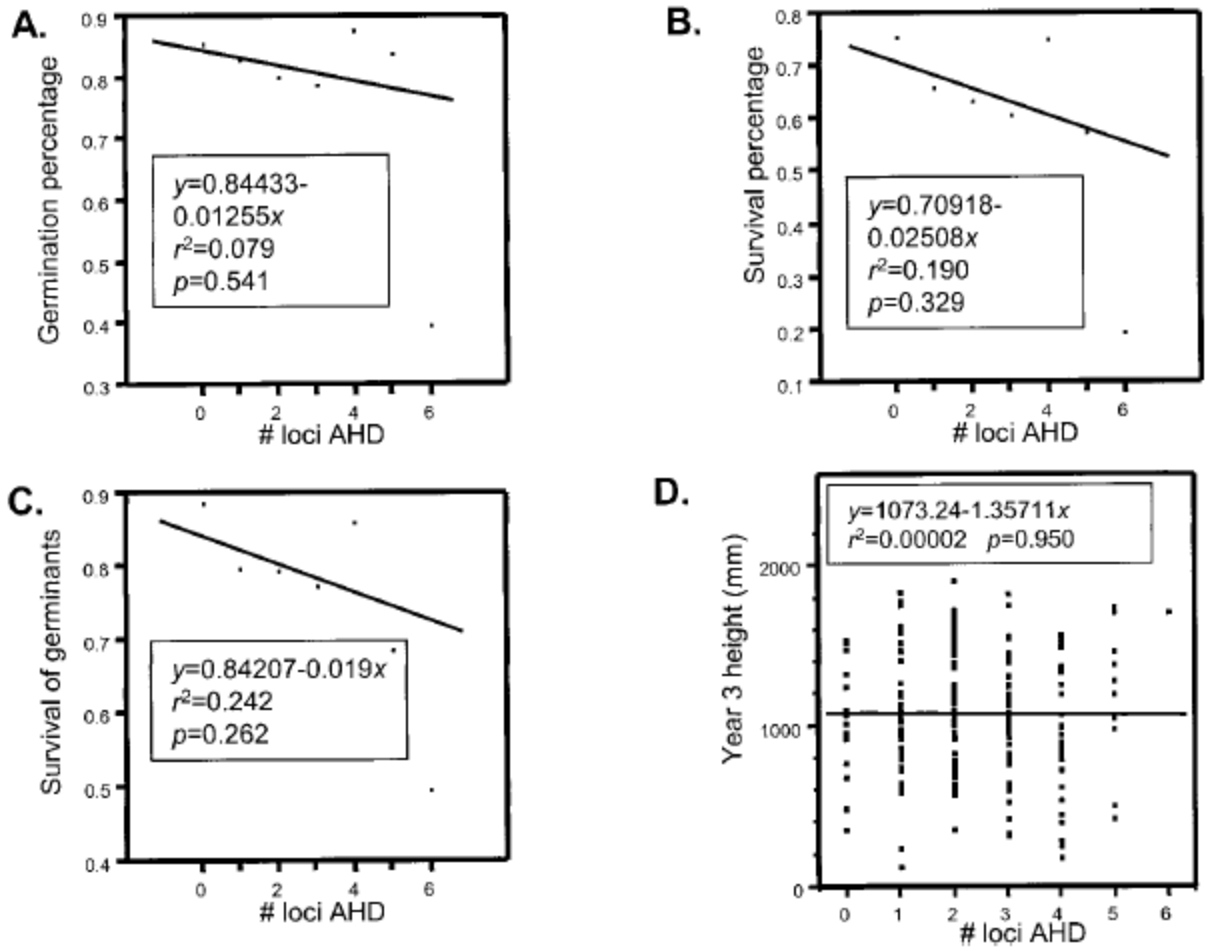

Figure 1. Regressions of (A) germination percentage; (B) survival percentage of all embryos; (C) survival percentage of germinating seedlings; and (D) height on the number of embryonic viability loci assumed homozygous for deleterious alleles (# loci AHD) at the end of the third growing season.

DISCUSSION

Role of Major Loci in Inbreeding Depression

It must be emphasized that the results obtained in this study are for a single selfed loblolly pine family. The number, positions, and effects of individual QTLs identified in this study have no particular relevance to the population as a whole. A number of additional families will have to be mapped before general conclusions can be drawn about the distribution and effects of major mutations in populations. These data are nevertheless pertinent to understanding the genetics and evolution of inbreeding depression for two important reasons. First, mapping provides detailed data on how individual mutations (or tightly linked clusters) affect multiple fitness components over time, precisely because individual families are mapped. Effects of individual mutations can only be inferred indirectly, and often inconclusively, from population-level data (Husband and Schemske 1996; Dudash and Carr 1998; Byers and Waller 1999). This is especially true if the individual alleles are rare in the population, as is expected with inbreeding depression. Although

this detail is initially obtained at the expense of generality until more families have been mapped, it is nevertheless valuable because it has been essentially unavailable until now.

Second, the very existence of mutations with large effects on inbreeding depression is of interest. Their presence is likely to produce high levels of variation in the nature and degree of inbreeding depression exhibited by individual families. The fate of small populations may be highly dependent on the nature of inbreeding depression in the founding parents. Consequently, the dynamics of conservation or breeding populations or of natural populations that become fragmented may be variable and difficult to predict in advance. Unpublished data from an ongoing study in loblolly pine do show a great deal of variation between families in inbreeding de pression for height growth, with some families showing nearly a 50% height reduction with selfing and others showing no inbreeding depression at all (S. E. McKeand, pers. comm.).

The major growth QTLs we found on LG1 and LG8 appear to be responsible for substantial amounts of inbreeding depression. The LG8 effect maps to the *cad-n1* mutation. The effects of *cad-n1* on stem rigidity and wood properties make it highly likely that the mutation itself, and not a linked locus, is responsible for the observed growth variation. Outcross families of parents known to carry *cad-n1* have not been reported to contain *cad-n1*-like mutants, which suggests the mutation is rare in the population. The deleterious LG1 allele likewise appears to be reasonably rare in the population, because it had no detectable deleterious effect as a heterozygote in the 7–1037 half-sib family. The average effect α of gene substitution is equal to $a + d(q - p)$, where q is the frequency of the less favorable allele (Falconer and Mackay 1996). Failure to detect an effect when a is so large suggests either that the deleterious allele is rare ($q \rightarrow 0$) and recessive ($d \approx a$) or overdominant and present at approximately equilibrium frequencies (but see discussion of growth and fitness below). Allelic effects at two other QTLs, on LG3 and LG12, appear to be largely additive and thus would not contribute significantly to inbreeding depression.

Our results from this family are at least partially inconsistent with the hypothesis that inbreeding depression for growth is due primarily to loci of small effect. The LG1 and LG8 loci explain a large percentage of the variance in year 3 height. The additive effects of these loci are 56% and 39%, respectively, of the phenotypic standard deviation for total height after three years, and the two loci may account for more than 13% inbreeding depression in year 3 height. The level of inbreeding depression explained by these loci would have been even greater if the LG1 locus were not linked to an embryonic lethal. This suggests that linkage among deleterious loci can obscure the true extent of inbreeding depression for quantitative traits. Moreover, the effects at these loci, expressed as a percentage of mean growth, appear to be increasing each year. The altered physical and chemical wood properties of *cad-n1* homozygotes seem likely to result in accelerated declines in growth and/or survival in subsequent years. Consequently, our results may underestimate the long-term effects attributable to these loci. Our study design does not allow us to estimate directly the total inbreeding depression for height growth in clone 7–56. These estimates would have required inclusion of outcross families of 7–56 in the study in a design that allows estimation of breeding value. Two separate field studies in loblolly pine each show an average height reduction of approximately 20% with selfing after 19 and 9 years, respectively (Bush and Smouse 1991; S. E. McKeand, pers. comm.). Our evaluation of directional dominance outside of LG1 and LG8 suggests that other loci with smaller effects are

also contributing to inbreeding depression. Whether these effects result from many slightly deleterious loci or lack of power to detect individual QTLs with smaller effects cannot be determined from this study.

Previous studies of growth QTLs in *Pinus* spp. have found year-to-year QTL effects to be inconsistent (Plomion et al. 1996; Emebiri et al. 1998; Kaya et al. 1999). Given these findings, the consistency of effects we observed at the LG1 and LG8 QTLs, and at the loci on LG3 and LG12 to a lesser extent, is noteworthy. Growing conditions were distinctly different during each of the three growing seasons. The seedlings experienced a combination of accelerated and stress growing conditions in year 1, were outplanted during the middle of the year 2 growing season, and experienced field conditions for all of year 3. Stability of growth QTLs across environments and developmental stages in pine may be greater than suggested by previous studies, and inconsistency may be primarily a function of statistical power to detect effects (Beavis et al. 1991, 1994). Continued measurements of this study over a longer period will be necessary to determine the true extent of QTL stability. Additional long-term studies with larger sample sizes are clearly needed if the genetic complexity of tree growth regulation is to be understood more completely (Wilcox et al. 1997).

We did not find clear support for individual loci affecting germination, although several chromosomal regions suggestive of germination effects were noted. The observed germination percentage of 75% was much lower than what we have normally observed for outcross seeds from clone 7–56 under laboratory conditions, which is suggestive of inbreeding depression. A larger study population would be necessary to determine whether the possible QTLs we observed for germination effects are real and whether inbreeding depression for germination in this family is primarily oligogenic or polygenic.

Apparent Overdominance at Cad and LG1 Loci

Overdominant effects have been reported frequently in QTL studies in both plants and animals (Stuber et al. 1992; Eshed and Zamir 1995; Li et al. 1995; Kirkpatrick et al. 1998). Distinguishing true overdominance from pseudo-overdominance caused by dominant genes linked in repulsion has proven difficult. In the present study, we found evidence of overdominance at the two loci with the largest effects on height growth. Overdominance at the LG1 locus was statistically significant for year 2 growth and year 3 height. Overdominant effects were not significant for the LG8 QTL associated with *cad-n1,* but the trend was apparent in growth for both year 2 and year 3, as well as for year 2 and year 3 total height. Neither locus, however, showed overdominance during the first growing season.

Our failure to detect a significant positive growth effect associated with the deleterious LG1 allele in the 7–1037 half-sib family, or any effect at all associated with *cad-n1,* may favor an explanation of pseudo-overdominance. Alleles with negative effects on height growth linked in repulsion to the major QTLs in 7–56 would not necessarily be present in 7–1037. We had already detected an allele deleterious to embryonic viability, and possibly to germination, linked to the wild-type *Cad* allele in 7–56. The coincidental presence of additional dominant QTLs linked in repulsion to both of the largest QTLs detected does still seem somewhat unlikely. The absence of heterozygote advantage for *cad-n1* in the half-sib family contrasts with the findings of

Wu et al. (1999), in which a significant growth advantage was associated with *cad-n1* in a 7–1037 half-sib family planted on a different site. Possible explanations for this discrepancy include limited statistical power to detect small effects, false positive findings in the previous study, environmental differences between the two planting sites, or different allele frequencies in the pool of male parents in the seed lots used to produce the two half-sib families.

Relationship between Tree Height Growth and Fitness

The potential rate of height growth in trees is expected to be under a form of stabilizing selection. Faster growth rates per se are strongly favorable to fitness, because taller trees compete more effectively for light in the ''stem exclusion'' stage of stand development and are much less subject to suppression-related mortality (Oliver and Larson 1990). Dominant trees also have larger crowns that provide more sites for seed production, so they may have higher fecundity as well. Bush and Smouse (1991) treated growth potential as a direct measure of fitness. However, increased annual growth tends to be correlated with increased susceptibility to cold and drought among provenances, which may reduce observed growth rates and lead to higher rates of mortality (Ma 1987; Rehfeldt 1992a,b; Schmidtling 1994). The expected net result of these antagonistic effects of growth potential on fitness would be to favor faster growth rates in provenances from less severe environments. Common-garden studies in a number of species, as summarized by Morgenstern (1996), indeed show clinal patterns of variation in growth potential, with decreased rates of growth in populations from successively higher latitudes or altitudes. Similar effects may also occur in increasingly droughty environments (Schmidtling 1994).

Given these considerations, loci showing overdominance for growth may not be overdominant for fitness and allele frequencies may be governed by mutation-selection balance rather than balancing selection. Traits under stabilizing selection typically have a concave-down fitness profile. Strength of selection against a phenotype is generally modeled with a quadratic function (Falconer and Mackay 1996). The degree of growth reduction associated with homozygotes for *cad-n1* and the deleterious LG1 allele (29% and 42%, respectively, relative to the wild-type homozygotes after three years) would almost certainly have strong deleterious effects on fitness. The relatively small heterozygote advantage observed for growth at these loci, however, is more likely to be in the nearly flat portion of the fitness profile, resulting in little if any heterozygote effect on fitness. Allele frequencies at overdominant loci with respect to fitness have an equilibrium ratio of s_2/s_1, where s_1 and s_2 are the respective selection coefficients against the homozygotes. The equilibrium frequencies of mutant alleles with recessive effects on fitness will be much lower, or approximately $\sqrt{u/s}$ under mutation-selection balance, where u is the per locus mutation rate (Falconer and Mackay 1996).

Stage Specificity of Inbreeding Depression

Husband and Schemske (1996) found evidence that genes responsible for inbreeding depression tend to be stage specific, but called for experimental verification from specific populations. Alleles affecting fitness at more than one stage of development would probably be eliminated more rapidly from populations than alleles whose effects are stage specific. In contrast, alleles with opposing effects on fitness at different life stages would be more likely to be maintained.

Evidence for alleles deleterious to early viability but with favorable heterozygous growth effects in *P. taeda* has been reported by Bush and Smouse (1991).

Our data favor stage specificity of inbreeding depression effects. We found no evidence that loci affecting embryonic viability have any overall effect on growth, and only weak support at most for effects on germination. Our data are for only three years of growth and are from a single family. Continued measurements and data from additional families will be necessary to verify these results. We did note several specific instances of linkage between loci affecting inbreeding depression at different stages. One or more alleles reducing embryonic viability and possibly germination are tightly linked to the wild-type *Cad* allele, but the effect is unlikely to be related to *Cad* itself. The LG1 growth QTL and *spf* each map in coupling to within 15 cM of embryonic viability alleles on LG1, but the respective loci appear to be in different marker intervals in both cases. Interestingly, the *cad-n1* and *spf* mutants show opposite effects on survival and growth so far. Homozygous *spf* individuals show significantly elevated mortality, but no obvious growth effects in the survivors. The *cad-n1* mutants have reduced growth rates, but no loss of survival so far. As the *cad-n1* mutants become larger, however, their less rigid stems are likely to cause increasingly severe form and growth defects, and accelerated mortality seems highly probable. The contrast in effects of different mutations on various fitness components further suggests that inbreeding effects are likely to vary greatly between families, depending on the nature of the mutations carried by the parents.

Our finding of stage specificity may not be favorable for selective breeding efforts in conifers. Deleterious embryonic viability loci that accumulate in breeding sublines might be eliminated in due course by selecting the fastest growing individuals in each generation, if these loci had pleiotropic effects on survival and growth. Our results, however, suggest that this is unlikely to be the case.

Application of Genetic Mapping in Evolutionary Studies

The use of mapped genetic markers in large families can provide much more powerful insights into the genetic architecture of trait variation and evolution than has been possible so far. Genetic markers, primarily allozymes, have been used for more than two decades to study the relationships between genetic variation, inbreeding, and fitness. Many basic questions addressed in these studies, including the existence of and basis for a relationship between heterozygosity and fitness and whether the actual marker alleles are neutral, remain unresolved (reviewed by David 1998). The added dimension of linkage and locus order information provided by genetic mapping allows resolution of marker-trait recombination, size of effects, additivity/dominance ratios, and linkage among multiple trait loci that was not previously possible. However, the effort required to obtain enough markers to move from marker sampling to mapping has been prohibitive for all but the most important crop or model organisms (Ritland 1996). A PCR-based marker technique such as AFLP, combined with the use of automated sequencers for resolving markers, allows rapid generation of large numbers of markers and may eliminate these limitations. AFLP markers present additional challenges because they are nearly always dominant and have a very low information content in F_2 mapping designs. This problem can also be largely overcome with appropriate linkage mapping strategies and statistical designs, as we and others have shown (Jiang and Zeng 1997; Remington et al. 1999; Remington and O'Malley

2000). Data from codominant multiallelic markers such as simple sequence repeats (SSRs) are much simpler to apply from a statistical standpoint, but the cost to develop enough SSRs to provide complete map coverage may be prohibitive for most plants.

We have successfully used an essentially complete genetic linkage map consisting entirely of dominant AFLP markers (Remington et al. 1999) to map and characterize the major loci affecting embryonic viability (Remington and O'Malley 2000) and, in the current study, early growth in a selfed *P. taeda* family. Some of our results confirm previous predictions, such as primarily stage-specific effects of genes causing inbreeding depression (Husband and Schemske 1996). However, finding such large amounts of inbreeding depression for growth associated with individual loci does not conform with previous theoretical and empirical expectations (Charlesworth 1991; Fu and Ritland 1994; Husband and Schemske 1996). Whether this discrepancy is due to differences between pines and other taxa, idiosyncrasies of the particular parent we studied, or the greater resolution provided by genetic mapping remains to be seen. We were less successful in distinguishing between true and pseudo-overdominance. Resolving this question may require selfing individuals from multiple crosses (Fu and Ritland 1994) or from multiple generations of a single pedigree. Variations of our methodology should be useful for studying the genetic architecture of inbreeding depression in any self-compatible plant species that can be crossed and cultivated.

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