Seedling Height and Diameter Variation of Various Degrees of Inbred and Outcross Progenies of Loblolly Pine[1])

By R. A. Sniezko[2]) and B. J. Zobel[3])

North Carolina State University,
Raleigh, North Carolina, 27695-8002, U.S.A.

(Received 26th June 1986)

Summary

From the ten loblolly pine families investigated in this study, it appears that some greenhouse traits such as seedling height and diameter (at 17 weeks) show inbreeding depression that is linearly related to the amount of inbreeding. However, there is also some indication of a non-linear response of seedling height to inbreeding.

The inbreeding depression present for height and diameter in the greenhouse was relatively small compared to that found in other studies with loblolly pine or other conifers. It is likely that the optimal growing conditions in this experiment may have led to a smaller response than expected.

The among-family variance for seedling diameter appears to increase with inbreeding, but large standard errors indicate that further investigation is necessary. The within-plot variance for seedling height and diameter is greater for inbred progeny than for noninbred progeny. The presence of a few very small seedlings, i.e. "runts", in some S_1 and S_2 progenies explains some of the increase in the within-plot variance. However, a portion of the increase in the within-plot variance may be due to an increase in the environmental component of this variance; if so, then inbred progeny, at least in the greenhouse, may have reduced homeostatic power.

Phenotypic correlations between seedling height and diameter indicate that the relationship of these two traits with inbreeding is fairly constant. The correlations among mating types were generally nonsignificant, indicating that for seedling height and diameter inbred performance may not be a good indicator of general combining ability.

Inbreeding has potential uses as a breeding tool, but practical difficulties in dealing with inbred trees, such as much lower yield of filled seed, make its use less desirable. Additional studies of inbreeding are necessary to provide knowledge of its effects in future advanced breeding populations.

Key words: loblolly pine, inbreeding, single crosses, directional dominance.

Zusammenfassung

Bei den in dieser Arbeit untersuchten 10 Familien von *Pinus taeda* zeigten sich für Höhe und Durchmesser der Sämlinge im Gewächshaus Inzuchtdepressionen, die anscheinend linear von dem Grad der Inzucht abhängen. Doch gibt es auch Anzeichen für einen nicht-linearen Einfluß der Inzucht auf die Höhe der Sämlinge.

Die im Gewächshaus für Höhe und Durchmesser festgestellte Inzuchtdepression ist im Vergleich zu anderen Ergebnissen mit *Pinus taeda* und anderen Koniferen relativ gering. Wahrscheinlich führten die in diesem Versuch optimalen Wachstumsbedingungen zu einer geringeren Reaktion als erwartet.

Die Varianz zwischen Familien für den Sämlingsdurchmesser schien mit der Inzucht zuzunehmen, aber große Standardabweichungen zeigen an, daß weitere Untersuchungen notwendig sind. Die Varianz innerhalb der Parzelle ist für die Sämlingshöhe und den Durchmesser bei ingezüchteten Nachkommenschaften größer als bei nicht ingezüchteten. Das Auftreten von einigen sehr kleinen Sämlingen, z. B. „runts" in einigen S_1- und S_2-Nachkommenschaften erklärt einen Teil des Anstieges der Varianz innerhalb der Parzellen. Jedenfalls dürfte ein Teil der Erhöhung der Varianz innerhalb der Parzellen einer Erhöhung der Umweltkomponente dieser Varianz zuzuschreiben sein. In diesem Fall könnten ingezüchtete Nachkommenschaften, zumindest im Gewächshaus, eine reduzierte homöostatische Fähigkeit haben.

Phänotypische Korrelation zwischen Sämlingshöhe und Durchmesser zeigen an, daß die Beziehung dieser zwei Merkmale mit der Inzucht nahezu konstant ist. Die Korrelation zwischen Kreuzungstypen waren generell nicht signifikant und machen deutlich, daß für Sämlingshöhe und Durchmesser das Auftreten von Inzucht kein guter Indikator für generelle Kombinationseignung ist.

Die Inzüchtung hat potentiellen Nutzen als Züchtungsinstrument, aber praktische Schwierigkeiten im Umgang mit ingezüchteten Bäumen, wie eine sehr viel geringere Vollkornausbeute bei Samen, machen ihre Verwendung weniger wünschenswert. Zusätzliche Inzuchtstudien sind nötig, um Kenntnisse ihrer Effekte in künftigen fortgeschrittenen Züchtungspopulationen zu liefern.

Introduction

Tree improvement programs in loblolly pine (*Pinus taeda* L.) and several other forest tree species are currently in the first or second generation and there has been an attempt to restrict inbreeding. However, in future generations, the breeding populations will become progressively more inbred. A knowledge of the effects of inbreeding is essential for formulating breeding strategies for the future.

Experiments in forest trees have shown that viable seeds can be produced on at least some individuals, through the first two generations of selfing. This result, bolstered by the success of the inbreeding-hybridization method in corn improvement, has led some forest geneticists to suggest the use of inbred material in forest tree improvement (Bingham, 1973; Andersson *et al.*, 1974; Lindgren, 1975; Rudolph, 1981). However, only a relatively few crosses have been made in forestry among unrelated first-generation selfs (S_1 trees). Further studies are needed to determine whether heterosis occurs following outcrossing of inbreds and the extent to

---

[1]) A portion of the research submitted as partial fulfilment of the requirements for the Degree of Doctor of Philisophy, Department of Forestry, North Carolina State University, by the first author.

[2]) Tree Breeder, Forest Research Centre (Box HG 595), Highlands, Harare, Zimbabwe.

[3]) Professor Emeritus, N.C. State University, Raleigh, N.C., U.S.A. Paper number 10552 of the Journal Series, North Carolina Agricultural Research Service, Raleigh, North Carolina 27695-7601.

which the original vigor of the population may be restored or improved upon. Low reproductive vigor of inbred trees and the long interval between generations have been the primary factors restricting investigations on inbreeding effects in forestry.

The effects of one generation of selfing which is the most severe type of inbreeding, has been investigated for some conifers. Compared to outcrossed material, selfing usually leads to inbreeding depression in many traits, including percent filled seed and height growth (BINGHAM and SQUILLACE, 1955; ORR-EWING, 1957; FRANKLIN, 1970; SORENSON and MILES, 1974; SORENSON *et al.*, 1976; WILCOX, 1983). In loblolly pine and in the other coniferous species studied, reduced yield of filled seed has been one of the most consistent and severe results of selfing (ORR-EWING, 1965; FRANKLIN, 1970; BRAMLETT and PEPPER, 1974). Investigations in several tree species indicate that additional inbreeding depression occurs for some traits in the second (S_2) and third (S_3) generations of selfing beyond that exhibited in the first generations of selfing (S_1) (BINGHAM, 1973; ANDERSSON *et al.*, 1974; ORR-EWING, 1976; RUDOLPH, 1976).

The presence of inbreeding depression for a trait indicates that directional dominance is present at the loci influencing the trait. The absence of any inbreeding depression is evidence that an additive model or a model that does not include directional dominance is operating. If inbreeding depression is present and if it is linearly related to the degree of inbreeding, then the intra-locus effects are additively accumulated over loci (FALCONER, 1981).

Another general consequence of inbreeding is the redistribution of the genetic variance. Inbreeding leads to genetic uniformity within families (or lines) and genetic differentiation among families. However, only under the very restricted conditions of continuous selfing and a genetic model that includes only additive effects can the genetic variance be easily partitioned (HALLAUER and MIRANDA, 1981). When dominance is included in the genetic model (as is generally the case when dealing with forest trees) the situation becomes more complicated and the changes in the variance cannot be determined without knowledge of the initial gene frequencies (FALCONER, 1981).

Inbred individuals often exhibit a greater environmental variance than noninbred individuals (FALCONER, 1981). The within-plot variance consists of both a genetic and an environmental component. At homozygosity ($F = 1$), the genetic component is assumed to be zero and in this case, an increase in the within-plot variance with inbreeding would be due to an increase in the environmental variance.

The primary objective of the study reported here included an examination of the effect of two generations of selfing in loblolly pine on the means and the within-plot and the among-family variances for seedling height and diameter. Also investigated were the change in the phenotypic correlation between seedling height and diameter over several levels of inbreeding, the correlation between inbred and noninbred progeny and the perfomance of S_1 outcosses relative to outcrosses of the noninbred parental generation (S_0).

The classes of inbreeding and outcrossing used in this study are: S_0 single cross ($F = 0$) S_0 open pollinated ($F = 0$), S_0 self ($F = 0.5$), S_1 single cross ($F = 0$), S_1 open pollinated ($F = 0$), S_1 crossed to another S_1 of the same line ($F = 0.5$), S_1 backcross ($F = 0.5$), and S_1 self ($F = 0.75$). The various types of matings and their inbreeding coefficients, F, are shown in *Figure 1*.

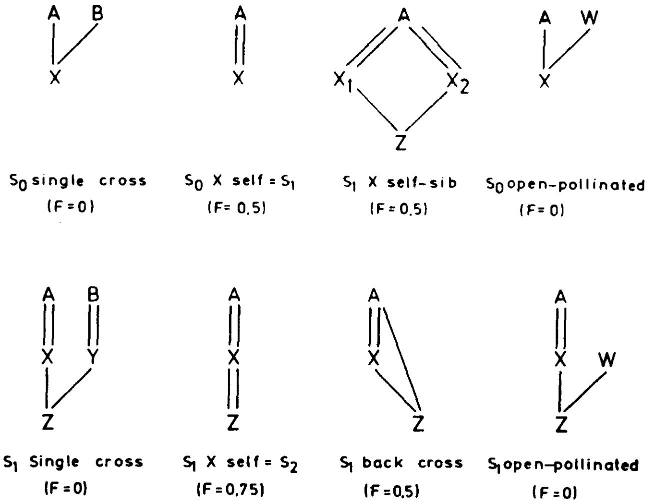

Figure 1. — Types of matings that were performed for this study and the inbreeding coefficients, F, associated with the offspring of these matings[1])

---

[1]) The presence of || indicates that the progeny were obtained by selfing.

Material and Methods

Field Procedures and Crossing Design

Ten unrelated and presumably noninbred loblolly pine clones from first-generation seed orchards of the North Carolina State University-Industry Tree Improvement Cooperative served as parents or grandparents of all the progeny used in this study. These clones were originally selected in natural stands in North Carolina and South Carolina. Most of these clones have been determined to be good general combiners and had previously produced moderate to large quantities of filled seed following self-fertilization. S_1 progeny from each of these ten clones had been planted in a special S_1 plantation of the lands of Champion International Corp. at Tillery, North Carolina in 1969 and many of them had just begun to flower when this study was initiated in 1978. Since some individuals within the S_1 families did not flower, it was necessary to select only those S_1 trees within each family that produced enough male or female flowers to make the necessary crosses.

Seeds for the inbred and outcross progenies used in this study were obtained from partial diallel crosses (*Figures* 2 and 3). From the first partial diallel, made from ten noninbred seed orchard clones (S_0 clones), seeds were obtained from crosses among S_0 clones (S_0 single cross seeds) and from self-fertilization of S_0 trees (S_1 seeds). Open-pollinated seeds (S_0 OP seeds) were also available for eight of the ten clones (*Figure 3*).

The second partial diallel included 40 parents, consisting of four individuals from each of ten S_1 families (*Figure 2*). The S_1 trees used were selfed progenies of the ten S_0 clones used. Crosses from this diallel included S_1 single crosses, S_2 (self-fertilization of S_1 trees), S_1 × self-sib (crosses among different S_1 trees of the same family), and S_1 backcrosses (crosses of S_1 trees with their S_0 parent). The S_1 backcross was performed only for family 6-20. Open-pollinated seeds (S_1 OP seeds) were available from 15 of the 40 S_1 trees (this represented eight of the ten families included in this study). Pollinations were made over a two-year period, in the springs of 1978 and 1979. Seeds from both years were sown in 1981 for a greenhouse study. In

	8-68.				6-20.				9-12.				6-22.				6-9.				3-13.				6-41.				8-1.				8-7.				8-127.			
Female	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Male																																								
8-68.1	S								R												R												R							
8-68.2	Y	S								R																														
8-68.3											R												R												R					
8-68.4			Y	S								R												R												R				
6-20.1					S								R																											
6-20.2						S								R												X														
6-20.3							S								R												X												X	
6-20.4							Y	S								R												X												X
9-12.1									S								X												X											
9-12.2										S								X												X										
9-12.3																			X																					
9-12.4												S								X																				
6-22.1													S								X																			
6-22.2																						X																		
6-22.3																																								
6-22.4																																								
6-9.1																	S																				X			
6-9.2																	Y	S								X														
6-9.3																			S								X													
6-9.4																			Y	S																				X
3-13.1																					S								X											
3-13.2																					Y	S																		
3-13.3																							S																	
3-13.4																																								
6-41.1																									S															
6-41.2																										S														
6-41.3							Z																																	
6-41.4								Z																				S												
8-1.1																													S								X			
8-1.2																													Y	S										
8-1.3																																								
8-1.4																																S								
8-7.1																																	S							
8-7.2																																		S						
8-7.3																																								
8-7.4																																								
8-127.1																																					S			
8-127.2																																					Y	S		
8-127.3							Z																																	
8-127.4								Z																															Y	S

The types of crosses yielding filled seed are indicated as follows:

X = S_1 single cross
R = reciprocal cross was made in place of the indicated S_0 single cross
Z = reciprocal cross also made
S = self-fertilization of S_1 tree (S_2)
Y = S_1 × self-sib

The numbers 8—68.1, 8—68.2, 8—68.3, 8—68.4 represent four S_1 trees of family 8—68. These trees are self-fertilized progeny of the clone 8—68. The S_1 trees from other families are represented in a similar manner.

Open-pollinated seed were collected from trees 8—68.1, 8—68.2, 8—68.4, 6—20.2, 6—20.3, 6—20.4, 9—12.1, 6—9.1, 6—9.4, 3—13.1, 6—41.2, 6—41.4, 8—7.3, 8—127.1 and 8—127.4.

Seed was also available for 6—41.3 × 6—41.5, 6—20.3 × 6—41.5, and from the backcrosses 6—20 × 6—20.1, 6—20 × 6—20.2 and 6—20 × 6—20.3.

Figure 2. — Partial diallel crosses made among S_1 trees.

Female	8-68	6-20	9-12	6-22	6-9	3-13	6-41	8-1	8-7	8-127
Male										
8-68[b)]	S		X			X			X	
6-20		S		X			R			R
9-12			S		X			R		
6-22				S		X			X	
6-9					S		R			R
3-13						S		R		
6-41							S		X	
8-1										X
8-7									S	
8-127										S
OP	W	W	W	W	W		W		W	W

---

a) The crosses from which seed were available are indicated as follows:
X = S_0 single cross seed.
R = reciprocal cross was made in place of the indicated S_0 single cross.
S = self-fertilization of S_0 trees (S_1)
W = open-pollinated seed

b) Tree numbers are clonal identity numbers.

Figure 3. — Partial diallel crosses made among S_0 clones.[a)]

some cases, seed was available from other sources and was used if the 1978 and 1979 pollinations produced insufficient seed.

Laboratory and Greenhouse Procedures

Filled seed were seperated from the empty seed by water flotation and soundness was verified by use of X-rays (radiographs). Two replications, each containing from four to 26 filled seed per cross (20 seed on the average) were sown in the greenhouse in a randomized complete block design for the 127 crosses in late January, 1981. When available, extra seed were sown to use as replacements for nongerminated seed.

Seven weeks after sowing, germination was completed and most of the early mortality had occurred. Obvious chlorophyll-deficient seedlings and dwarfs were of low frequency and were not included in the growth phase of the study due to their high mortality rate. As many as 11 seedlings from each cross were placed in each of four replications in a randomized complete block design. Within a replication, seedlings from all crosses were randomized, forming noncontiguous plots, ensuring exposure to the environmental variability within the replications (LIBBY and COCKERHAM, 1981; LAMBETH and GLADSTONE, 1983). Seedling height and root collar diameter were measured 17 weeks after sowing.

Measurements

Data collected during the course of this study included number of filled seed per cone, percent mortality, percent germination, rate of germination, hypocotyl length, number of cotyledons, seedling height and seedling diameter (root collar diameter). In general, only seedling height and diameter (at 17 weeks) are discussed in the present paper. Information on other measurements and general observations may be found elsewhere (SNIEZKO, 1984).

Data Analysis

Mean of all progeny types for each family and least square estimates of the means over all families were calculated for all traits in this study (BARR *et al.*, 1979). For the S_1 OP, S_1 single cross, S_2 and $S_1 \times$ self-sib crosses, means were calculated for each family on both a S_1 family basis (all lines of a S_1 family included) and on a S_1 line basis (separately for each S_1 parent tree).

Because not all progeny types were present for all families, separate analyses of variance were used to compare 1) S_0 OP and S_1 OP progenies, 2) S_0 single cross and S_1 single cross progenies and 3) S_1 and $S_1 \times$ self-sib progenies. In the analysis that included S_1 OP, S_1 single cross, S_2 or $S_1 \times$ self-sib crosses, all lines of a S_1 family were pooled. For example, the means of the three S_1 OP progenies (8-68.1 OP, 8-68.2 OP and 8-68.4 OP) derived from clone 8-68 were pooled to estimate the S_1 OP mean of family 8-68 in comparison of S_0 OP and S_1 OP families. For each analysis, only those families from which all progeny of the relevant progeny types were available were included (thus the different number of families or crosses in the different analyses). In addition, in a separate analysis using S_0 OP, S_1 and S_2 progenies, inbreeding effects were partitioned into that attributable to linear regression on the level of inbreeding (F) and residual. These analyses permitted tests of whether inbreeding depression in any trait was linearly related to the increase in F (and the associated increase in homozygosity that accompanies inbreeding). For all analyses, replications and families are considered random and progeny type (or inbreeding levels) are considered fixed effects. Due to the imbalance, all analyses of variance were performed using the General Linear Models Procedure (GLM) of the Statistical Analysis System (BARR *et al.*, 1979).

Variance components were estimated for seedling height and diameter for each progeny type. The Type I Method of the VARCOMP procedure was used in calculating the variance components (BARR *et al.*, 1979). Estimates obtained included family variance, plot error variance and within-plot variance.

Phenotypic correlations among family means between height and diameter were computed for each progeny type. Also computed were the phenotypic correlations among mating types for height and diameter.

Results

Regression of Traits on the Coefficient of Inbreeding (F)

The linear regression model was found to be highly significant (.01) for both seedling height and diameter (see F_{linear} in *Table 2*). For seedling height the residual mean square, $F_{residual}$, was significant (.05), indicating that a model involving a quadratic or higher order polynominal may give a better fit (*Table 2*). However, $F_{residual}$ accounted for less than 15% of the total variation due to inbreeding. The presence of one or a few "runts" in some of the S_1 and S_2 families (which differ greatly in size from the remaining S_1 and S_2 seedlings of those families) is responsible for some of the deviation from the linear model. Thus, the linear regression model may be a reasonable fit for seedling height and diameter. However, since there is an indication of a nonlinear response of seedling height to inbreeding, additional investigation will be needed to verify this and to specify the nature of the response. The overall means for both seedling height and diameter decreased with inbreeding (*Table 1*). This decrease was approximately 1.0% and 1.2%, respectively, for each 0.1 increase in F (SNIEZKO, 1984).

The family × inbreeding level interaction was highly significant for seedling diameter and nearly significant (.054) for height, indicating that not all families responded to inbreeding in the same manner. For seedling height, six of nine families had a significant or nearly significant in breeding depression that was linearly related to the coefficient of inbreeding. For diameter, F_{linear} and/or $F_{residual}$ were significant for seven of nine families. In general, family responses for diameter were more variable than for heights. In some families, diameter seemed to be affected very little even after two generations of selfing, while other families exhibited a very marked decrease, especially from the S_1 to the S_2 generation. The range in family means for seedling height and diameter is summarized in *Table 1*.

The S_1 progeny from family 8—68 had a much higher mean than the S_0 progeny of this family for both seedling height and diameter. One possible explanation is that, since the 8—68 S_0 OP seed came from storage, its performance was hindered by seed changes before planting. Another possible, but not probable explanation, is that the S_1 progeny of family 8—68 may be an accidental outcross. In any case, the performance of this S_1 progeny is superior to all families for at least some traits and will be closely monitored in the field study.

Table 1. — Mean and range of family means for seedling height and diameter for nine families for a noninbred generation and two generations of selfing.

		Seedling height (cm)			**Seedling diameter (mm)**		
Generation	**F**	**Mean**	**Minimum**	**Maximum**	**Mean**	**Minimum**	**Maximum**
S_0 OP	0	28.28	27.12	29.84	2.89	2.74	3.10
S_1	0.5	27.50	26.38	29.87[a)]	2.76	2.57	3.04
S_2	0.75	26.08	24.36	27.26	2.64	2.26	2.83

[a)] If family 8—68 which behaved differently from the others (see text) is excluded, the maximum height for S_1 progeny is 28.13 cm (and 2.92 for diameter).

Table 2. — Analyses of variance for seedling height and diameter for a noninbred generation and two generations of selfing (S_0 OP, S_1 and S_2 progenies) based upon nine families.

		Mean Squares	
Source	d.f.	Height	Diameter
Replications	3	542.47**	1.304**[a)]
Family	8	53.00**	1.673**
Inbreeding level (F)	2	626.15**	8.493**
F_{linear}	1	1085.3**	15.835**
$F_{residual}$	1	167.0*	1.151
Family X F	16	25.59+	1.128**
Plot error	78	15.76	0.218**
Within-plot	1238	15.84	0.137

[a)] +, *, ** indicate significance at the .10, .05 and .01 level of probability, respectively.

Comparisons of S_0 OP and S_1 OP Progenies

Progeny type was significant only for seedling height. S_0 OP progeny were significantly taller than the S_1 OP progeny (*Tables 3* and *4)* indicating some effect of parental inbreeding. Although not significantly different, the S_0 OP progeny had greater diameter than the S_1 OP progeny (*Table 3*).

The family × progeny type interaction was highly significant for diameter and significant for seedling height, indicating that the effect of parental inbreeding was inconsistent. Individual family comparisons of the S_0 OP and S_1 OP means showed that there were very few families that had significant differences between the progeny types; however, for the majority of families, the S_0 progeny were taller and greater in diameter than the S_1 OP progenies.

Comparison of S_1 and S_1 × Self-sib Progenies

No significant differences were found between S_1 and S_1 × self-sib progenies for either seedling height or diameter (Sniezko, 1984). Although the differences were not significant, S_1 progeny were slightly larger than those for the S_1 × self-sib progeny (*Table 3*).

The family × progeny type interaction was significant only for diameter. When families were examined separately, S_1 progenies generally performed better than S_1 × self-sib progenies (although the differences were often not significant).

Comparison of S_0 Single Cross Progeny and S_1 Single Cross Progeny

Seedling diameter showed significant differences between generations (S_0 single cross vs. S_1 single crosses) as can be seen from *Table 5*. For seedling diameter, the mean of S_0 single crosses was greater than that of S_1 single crosses (*Table 3*) again indicating the effect of parental inbreeding. The cross × generation interaction was highly significant for both seedling height and seedling diameter, indicating that differences among S_0 single crosses and S_1 single crosses depended upon the characteristics of the two families that were mated (*Table 5*).

In general, about half of the single cross progenies from S_0 parents were taller than the corresponding crosses from S_1 parents (only two were significantly taller). For most of the S_0 single cross progenies, seedling diameter was larger than for the corresponding S_1 single cross progenies (differences were often significant or highly significant).

Phenotypic Correlations

The phenotypic correlations (on family means) between height and diameter within a progeny type were positive in all cases and nearly significant (0.10) or significant (0.05) for the S_0 OP, S_1 OP, S_1 and S_2 progeny types (*Table 6*). The correlations ranged from 0.58 for S_1 × self-sib progenies to 0.91 for S_0 OP progenies and indicates that height and diameter appear to be affected similarly with inbreeding.

Diameter correlations between progeny types are generally small and nonsignificant, except for the correlation between S_1 OP progenies and S_2 progenies ($r = 0.70$, significant at .10), which indicates that S_2 performance may be an indicator of S_1 outcross performance (*Table 7*). Height correlations among progeny types are generally positive, but nonsignificant (SNIEZKO, 1984).

Variance Components

For both height and diameter, the within-plot variance component is smallest for S_0 OP progenies and largest for S_2 progenies (*Tables 8* and *9*). The within-plot variance component is similar for S_1 OP and S_0 OP progenies, and for S_1 and S_1 self-sib progenies. An examination of the variances by family for the three levels of inbreeding for height and diameter shows the general trend of increased variance in the S_1 and S_2 progenies over the noninbred progenies (*Figures 4* and *5*). In several families there is a large increase in variance for inbreds, while in several other families there is little or no increase in variance. An examination of variance of individual S_2 families relative to height or diameter growth showed no pattern for height, but possibly a slight increase in variance with declining mean for diameter. For some S_1 and S_2 progenies, part of the increased variance is due to the presence of one or few "runts". These "runts" differ significantly from the remaining progeny and thus both decrease the mean and increase the within-plot variance.

Table 3. — Mean performance for seedling height and diameter for 1) S_0 OP and S_1 OP progenies, 2) S_1 and S_1 × self-sib progenies and 3) S_0 single cross and S_1 single cross progenies [a]).

	Progeny type	Coefficient of inbreeding (F)	Height (cm)	Diameter (mm)
1)	S_0 OP	0	28.31	2.91
	S_1 OP	0	27.38	2.80
2)	S_1	0.5	27.61	2.77
	S_1 x self-sib	0.5	26.77	2.76
3)	S_0 single cross	0	28.05	2.91
	S_1 single cross	0	27.67	2.79

a) Means for height and diameter are based upon eight common families for 1), six common families for 2) and 13 common crosses for 3).

Table 4. — Analyses of variance of S_0 OP and S_1 OP progenies for seedling height and diameter.

		Mean Squares	
Source	d.f.	Height	Diameter
Replications	3	379.2** a)	1.598**
Family	7	48.1**	0.355**
Progeny type	1	158.9*	2.476+
Family x progeny type	7	27.8*	0.482**
Plot error	45	10.2	0.100
Within-plot error	856	10.1	0.101

a) +, *, ** indicate significance at the .10, .05, and .01 levels, respectively.

Table 5. — Analyses of variance of S_0 single cross and S_1 single cross progenies for seedling height and diameter.

Source	d.f.	Mean Squares Height	Mean Squares Diameter
Replication	3	$907.3^{**a)}$	3.405^{**}
Crosses	12	37.30^{**}	1.287^{**}
Generation (S_0 or S_1)	1	42.11	4.443^{*}
Crosses x Generation	12	36.12^{**}	0.933^{**}
Plot error	75	13.62	0.208
Within-plot error	1674	12.17	0.103

a) +, *, ** indicate significance at the .10, .05, and .01 levels, respectively.

Table 6. — Phenotypic correlations (on family means) between seedling height and root collar diameter within progeny types.

Progeny Types	Correlation	
S_0 OP	$0.907^{**a)}$	(8)
S_1 OP	0.669+	$(8)^{b)}$
S_1	0.665+	(9)
S_2	0.680^{*}	(10)
S_1 x self-sib	0.583	(7)

a) +, *, ** denote significance at the .10, .05, and .01 levels of probability respectively.

b) Numbers in parentheses are the number of families used for the correlation.

The plot error variance component was largest for S_2 progeny, but most estimates are small and nonsignificant.

The family variance components for diameter increased with inbreeding, which would indicate that the genetic variation among families increases with inbreeding. However, the standard errors of these estimates are relatively large and further investigation is needed. For height, there was no trend.

Discussion

In general, in loblolly pine, percent filled seed seems to decrease in at least the first two generations of selfing but is at least partially restored upon outcrossing (Sniezko, 1984). Some families and lines within families will no doubt be lost in the inbreeding process. However, this probably will not be too severe a limitation to further inbreeding studies unless a species is quite self-sterile, and its use for inbreeding and outcrossing as a means of population improvement is being contemplated. In this instance, selection intensity could be severely restricted.

Percent filled seed showed the greatest inbreeding depression of any trait in this study (Sniezko, 1984). This generally agress with the results found in other conifers (Franklin, 1970). It has been suggested that the genetic cause of the embryo collapse responsible for producing an empty seed is an inbreeding effect, caused by the action of homozygous lethal genes (Orr-Ewing, 1957; Sarvas, 1962). However, the presence of single recessive embryonic lethals does not explain why the second generation of selfing appears to yield a lower percentage of filled seed than the

Table 7. — Phenotypic correlations for seedling diameter among progeny types estimated from means of S_0 OP, S_1 OP, S_1, S_2 and S_1 × self-sib progenies.

Progeny type	S_0 OP	S_1 OP	S_1	S_2	S_1 x self-sib
S_0 OP	1.00	-.07(7)	.10(8)	.06(8)	.80 $(5)^{b)}$
S_1 OP		1.00	.16(8)	$.70^{+a)}$ (8)	-.001 (6)
S_1			1.00	.34 (9)	-.09 (6)
S_2				1.00	-.07 (7)
S_1 x self-sib					1.00

a) + indicates significance at the .10 probability level.

b) Numbers in parentheses are the numbers of families used in the correlations.

Table 8. — Estimates of variance components and their standard errors for height for five progeny types.

Variance component	Progeny type				
	S_0 OP	S_1 OP	S_1	S_1 x self-sib	S_2
Family	0.834 (0.497)[a)]	1.005 (0.460)	0.933 (0.562)	2.116 (1.077)	1.200 (0.657)
Plot error	-0.093 (0.262)	0.015 (0.253)	-0.462 (0.372)	-0.049 (0.437)	1.482 (0.586)
Within-plot error	8.805 (0.756)	10.157 (0.650)	15.171 (1.262)	15.660 (1.194)	18.656 (1.124)

a) Numbers in parentheses are standard errors,

$$S.E. = \left(\frac{2}{K^2}\sum_{g}\frac{MS_g^2}{f_g+2}\right)^{1/2}$$

K = coefficient of the variance component being estimated,

MS_g = the g^{th} mean square used to estimate the variance component,

f_g = degrees of freedom of the g^{th} mean square (Becker, 1975).

Table 9. — Estimates of variance components (and their standarr errors) for root collar diameter for five progeny types.

Variance component	Progeny type				
	S_0 OP	S_1 OP	S_1	S_1 x self-sib	S_2
Family	0.00954 (0.00590)[a)]	0.00809 (0.00503)	0.0208 (0.01323)	0.0175 (0.00974)	0.0425 (0.0143)
Plot error	0.00140 (0.00348	0.0123 (0.00492)	0.0197 (0.00927)	0.00799 (0.00547)	0.0200 (0.00723)
Within-plot error	0.09382 (0.00806)	0.09562 (0.00612)	0.1201 (0.00999)	0.1192 (0.00906)	0.1462 (0.00442)

a) See *Table 8* for calculation of standard errors.

first-generation of selfing (Andersson *et al.*, 1974). At least a partial explanation for the continued reduction in filled seed in the second generation of selfing is that the S_1 plants that are being pollinated are themselves inbred progeny and therefore exhibit reduced reproductive capacity as well as reduced vegetative vigor. This is further borne out by the poorer performance of outcrossed progeny from S_1 vs S_0 parents.

Most of the variation among inbreeding levels found in this study of loblolly pine (represented by S_0 OP, S_1 and S_2 progenies) for height and diameter was accounted for using a linear regression model. This is evidence that a model indicating a linear decrease in the mean of these two traits with inbreeding is a good fit. However, there is also evidence for seedling height that a nonlinear model may be a better fit. Results from other tree species indicate that a linear model is a good fit for at least some traits (Gansel, 1971; Anderson *et al.*, 1974). However, most of the evidence showing a linear decrease in the mean of a trait after inbreeding is from other plant or animal species (Hallauer and Sears, 1973; Cornelius and Dudley, 1974; Falconer, 1981). This relationship is consistent with a genetic model that includes dominance and additivity over loci (no net epistatic effects involving dominance). Evidence from the study reported here indicates that for loblolly pine, dominance is present and inbreeding depression is linearly related to F, the coefficient of inbreeding, for the early growth traits assessed. This indicates that as loblolly pine becomes more inbred, the height and diameter of the population will continue to decrease. Thus it is necessary in breeding populations to effectively use selection to overcome the deleterious effects of inbreeding.

The greenhouse conditions under which the progeny were grown were excellent for the growth of loblolly pine. This may have been responsible for the relatively small inbreeding depression for seedling height and diameter (which were approximately 1.0 and 1.2%, respectively, for each 0.1 increase in the inbreeding coefficient). This is less than those reported for some other studies (Franklin, 1968; Gansel, 1971; Anderson *et al.*, 1974; Rudolph, 1976; Wilcox, 1983). Even though deleterious recessive growth genes may accumulate in the homozygotes following inbreeding, those ge-

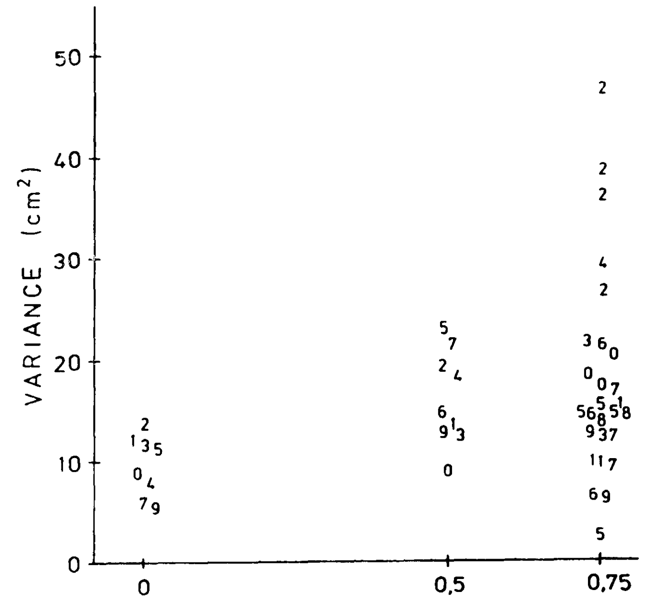

Figure 4. — Distribution of variance for height for three levels of inbreeding as represented by S_0 OP, S_1 and S_2 families (S_2 lines from a common family are shown separately) Numbers 0—9 represent families 8—127, 8—68, 6—20, 9—12, 6—22, 6—9, 3—13, 6—41, 8—1 and 8—7 respectively.

nes may have only limited or no deterimental effects under optimum growth conditions. In corn, heterozygous progenies have been shown in some cases to possess more phenotypic stability than inbreds (however selection for stable inbreds is no doubt possible). This is especially evident when grown in less favorable environmental conditions, where inbred line performance deteriorates much sooner than that of heterozygous progenies (McWilliams and Griffing, 1965). Some evidence for an increase in inbreeding depression under less favorable environments exists for Norway spruce (Anderson *et al.*, 1974).

From an operational point of view, some selfing or related mating will occur in most seed orchards. If inbreeding depression is minor or nonexistent in optimal environments, it will be difficult to rogue the inbred seedlings in the greenhouse or in the nursery before they are planted in the forest. Once in plantations, the inbred seedlings may grow more slowly or die, thereby reducing the yield of the plantation. This will be a problem only when the inbreeding occurring in the seed orchard is considerable. However, current estimates indicate that the proportion of selfed progeny from loblolly pine clonal seed orchards is less than two percent (Adams and Joly, 1980; Friedman and Adams, 1981). Additional testing of inbred seedlings over a range of environments is necessary.

The vigor of offspring from outcrosses of inbred progenies is expected to be restored to that of the original noninbred population (Falconer, 1981). Thus, the S_0 OP and S_1 OP progenies were expected to perform equally well, however, the S_0 OP progeny outperformed the S_1 OP progeny for several traits including seedling height. The S_1 single cross progeny performed very similar to the S_1 OP progeny, indicating that related matings among S_1 trees of the same family or pollen contamination from trees nearby in natural stands or in plantations were probably not responsible for the difference between the S_0 outcrosses and S_1 outcrosses. There are too few comparisons among S_1 outcrosses in forestry to determine whether our results are typical, or whether the differences between S_1 OP and S_0 OP will continue past the seedling stage in loblolly pine. In jack pine, S_1 outcrosses and S_0 outcrosses performed similarly for height growth (Rudolph, 1981).

In this experiment, for both height and diameter, the within-plot variance was greater for inbred than for noninbred progeny. However, the exact response appears to vary by family. The within-plot variance consists of both a genetic and an environmental component (and a G × E component). At homozygosity, the genetic component of this variance is assumed to be zero and the within-plot variance is made up of only an environmental component. Experiments in other organisms with highly inbred lines and the F_1 hybrids between them have also shown an increase in variance in the inbreds (Falconer, 1981). In these cases the increase in variance in the inbred lines has been attributed to an increase in the environment component.

However, in this experiment the inbreeding levels are between F = 0 and F = 1 and it is not possible to predict the behaviour of the genetic variance without making simplifying assumptions or having further information (on gene frequencies). In this case, the within-plot variance nearly doubles for height and increases by around 50 percent for diameter from the noninbred to the S_2 generation. Some families contribute more to this increase in variance than others, but the general trend is for the inbred progeny to demonstrate a greater variance than the noninbred progeny.

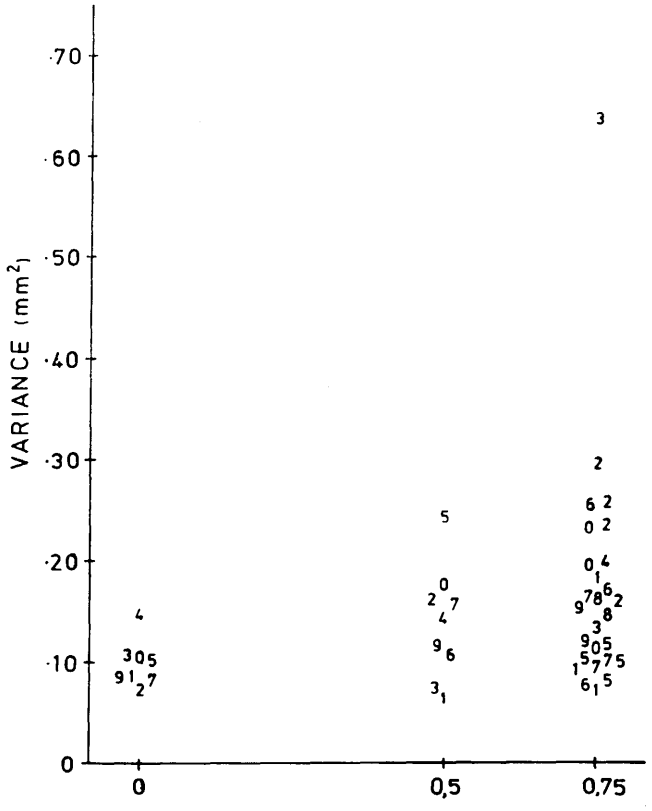

Figure 5. — Distribution of variance for diameter for three levels of inbreeding as represented by S_0 OP, S_1 and S_2 families (S_2 lines from a common family are shown separately). Numbers 0—9 represent families 8—127, 8—68, 6—20, 9—12, 6—22, 6—9, 3—13, 6—41, 8—1 and 8—7 respectively.

Although the within-plot variance consists of both a genetic and environmental (plus G × E) component, most of this variation for the S_0 OP progeny is nongenetic. If this is the case and assuming a constant environmental variance across generations, then a very large increase (2—5 fold) in the genetic component of the within-plot variance would be needed to account for the increase from the noninbred to the S_2 generation. When dominance is included in the genetic model it is difficult to specify the changes in genetic variance without further knowledge of the gene frequencies, but some increase might occur in the genetic component of the within-plot variance. The presence of a few "runts" in some of the S_1 and S_2 progenies is no doubt responsible for part of the increase in within-plot variance. However, an increase in the environmental component may also be partially responsible for this large increase. No definite cause for an increase in environmental variance has been demonstrated, but it has been suggested that homeostasis may be an important part of fitness and it would therefore be expected to decline with inbreeding if directional dominance was the underlying mechanism (Falconer, 1981). Also, the possession of different alleles at a locus may enhance the buffering capacity of an individual.

Inbreeding appears to affect the within-plot variance differently for different families in this greenhouse study. Further investigation in the field phase of this study and in other studies (and forest species) will be needed to determine whether the increase in the within-plot variance is a general result of inbreeding, and whether any increase is due to an increase in the genetic and/or environmental components of this variance.

In this study, the among-family variance component (and thus the genetic variance) for diameter appears to increase slightly with inbreeding, but the trend for height was less clear. Results from several other studies in forestry have shown an increase in family variance for at least some traits related to an increase in inbreeding (Anderson *et al.*, 1974; Rudolph, 1981; Wilcox, 1983).

The S_0 OP and S_1 OP progeny are the only progeny types for which the covariances between relatives can be easily related to genetic variances. In the case of inbred progeny, restrictions such as no dominance and no dominance types of epistasis, or gene frequency of one-half must be assumed to relate these genetic variances to those of the non-inbred generation (Cockerham, 1963). Therefore the only covariances among relatives that were related to genetic variances in our case were Cov (S_0oP) and Cov (S_1OP).

Assuming no epistasis (from Cockerham, 1961),

$$\mathrm{Cov}\,(S_0\,\mathrm{OP}) = 1/4\ (\text{additive variance}) \tag{1}$$

$$\mathrm{Cov}\,(S_1\,\mathrm{OP}) = 3/8\ (\text{additive variance}),\ \text{if there is one individual used per line} \tag{2}$$

$$\mathrm{Cov}\,(S_1\,\mathrm{OP}) = 1/4\ (\text{additive variance}),\ \text{if} > 5\ \text{individuals are used per line} \tag{3}$$

In our study, an average of two individuals were used to represent each S_1 line so the actual situation is somewhere between (2) and (3) above. In this experiment, the genetic variance among S_1 progenies was slightly greater for height and slightly less for diameter than of S_0 OP progenies, but neither of these differences is significant. A larger experiment will probably be needed to detect significant differences between the two progeny types.

Phenotypic correlations among progeny type means were generally small and nonsignificant, indicating that self-pollinated family performance (at 17 weeks) would not be a reliable indicator of general combining ability. In another study involving loblolly pine, the correlation between S_0 outcross and S_1 family means for height (at six months and at two years) were positive and moderately high (Franklin, 1969). In *Pinus radiata* the correlations between S_0 outcross and S_1 family means were moderately strong for several traits, including height, diameter and volume at age four (Wilcox, 1983). In corn, genetic and phenotypic correlations for most traits among selfed generations are often high, but correlations of inbred parents with their hybrids are usually fairly low (Falconer, 1981, Hallauer and Miranda, 1981). If this proves to be the case in loblolly pine or other tree species, then extensive testing of many single cross hybrids will be necessary to find the best lines to use in single crosses.

Acknowledgements

The support of this research by the N.C. State University — Industry Cooperative Tree Improvement Program and its staff is gratefully acknowledged. Special thanks go to Dr. G. Namkoong and Dr. F. Bridgwater for their comments on the manuscript.

Literature Cited

Adams, W. T. and Joly, R. J.: Allozyme studies in loblolly pine seed orchards: Clonal variation and frequency of progeny due to self-fertilization. Silvae Genetica **29** (1): 1—4, (1980). —— Andersson, E., Jansson, R., and Lindgren, D.: Some results from second generation crossings involving inbreeding in Norway spruce (*Picea abies*). Silvae Genetica **23**: 34—43, (1974). —— Barr, A. J., Goodnight, J. H., Sall, J. P., Blair, W. H. and Chilko, D. M.: SAS user's Guide. 1979 edition. SAS Institute, Inc., Cary, N.C. 494 pp, (1979). —— Becker, W. A.: Manual of Quantititive Genetics. Third ed. Washington State Univ. Press, Washington. 168 pp, (1975). —— Bingham, R. T.: Possibilities for improvement of western white pine by inbreeding. USDA Forest Service Research Paper INT-144, (1973). —— Bingham, R. T. and Squillace, R. E.: Self-compatibility and effects of self-fertilization in western white pine. Forest Science **1**: 121—129 (1955). —— Bramlett, D. L. and Pepper, W. L.: Seed yield from a diallel cross in Virginia pine. *In* J. Kraus (ed.), Seed yield from southern pine seed orchards. Georgia Forest Resource Council. pp. 48—54, (1974). —— Cockerham, C. C.: Implications of genetic variance in a hybrid breeding programme. Crop Science **1**: 47—52, (1961). —— Cockerham, C. C.: Estimation of genetic variances. *In:* W. D. Hanson and H. F. Robinson (eds.). Statistical Genetics and Plant Breeding. NAS-NRC 982, Washington, D.C. pp. 53—93, (1963). —— Cornelius, P. L. and Dudley, J. W.: Effects of inbreeding by selfing and full-sib mating in a maize population. Crop Science **14**: 815—819, (1974). —— Falconer, D. S.: Introduction to Quantitive Genetics. 2nd ed. Longman, New York. 340 pp, (1981). —— Franklin, E. C.: Artificial self-pollination and natural inbreeding in *Pinus taeda* L. Ph.D. thesis. Dept. of Forestry, North Carolina State University, Raleigh, N.C. 127 pp, (1968). —— Franklin, E. C.: Inbreeding as a means of genetic improvement of loblolly pine. Proceedings of the Tenth Southern Conference on Forest Tree Improvement. Houston, Texas. pp. 107—155, (1969). —— Franklin. E. C.: Survey of mutant forms and inbreeding depression in species of the family *Pinaceae*. USDA Forest Service Research Paper SE-61. 21 pp, (1970). —— Friedman, S. T. and Adams, W. T.: Genetic efficiency in loblolly pine seed orchards. Proceedings of the 16th Conference on Southern Forest Tree Improvement. Blacksburg, Virginia. pp. 213—220, (1981). —— Gansel, C. R.: Effects of several levels of inbreeding on growth and oleoresin yield in slash pine. Proceedings of the Eleventh Conference on Southern Forest Tree Improvement. Atlanta, Georgia. pp. 173—177, (1971). —— Hallauer, A. E. and Miranda, J. B.: Quantitative genetics in maize breeding. Iowa State University Press, Ames, Iowa. 468 pp, (1981). —— Hallauer, A. R. and Sears, J. H.: Changes in quantitive traits associated with inbreeding in a synthetic variety of maize. Crop Science **13**: 327—330, (1973). —— Lambeth, C. C. and Gladstone, W. T.: Statistical efficiency of row and non-contiguous family plots in genetic tests of loblolly pine. Silvae Genetica **32**: 24—28, (1983). —— Libby, W. J. and Cockerham, C. C.: Random noncontiguous plots in field layouts. Silvae Genetica **29**: 183—190, (1981). —— Lindgren, D.: Use of selfed material in forest tree improvement. Department of Forest Genetics, Royal College of Forestry, Research Notes No. **15**. Stockholm, Sweden. 76 pp, (1975). —— McWilliam, J. R. and Griffing, B.: Temperature-dependent heterosis in maize. Aust. J. Biol. Sci. 18: 569—583, (1965). —— ORR-EWING, A. L.: Further inbreeding studies with Douglas-fir. The Forestry Chronical 33: 318—332, (1957). —— ORR-EWING, A. L.: Inbreeding and single crossing in Douglas-fir. Forest Science 11: 279—290, (1965). —— ORR-EWING, A. L.: Inbreeding Douglas-fir to the S_3 generation. Silvae Genetica 25: 179—183, (1976). —— RUDOLPH, T. D.: Cone set, seed yield, seed quality, and early seedling development of S_2 generation jack pine. Proceedings of Tenth Central States Forest Tree Improvement Conference. Purdue University. pp. 42—60, (1976). —— RUDOLPH, T. D.: Four year height growth variation among and within S_0, $S_1 \times S_1$, S_1 open-pollinated and S_2 inbred jack pine families. Can. For. Research 11: 654—661, (1981). —— SARVAS, R.: Investigations on the flowering and seed crop of *Pinus sylvestris*. Comm. Inst. Forest Fenn. 53. 4. 198 pp, (1962). —— SORENSON, F. C., FRANKLIN, J. F. and WOODLARD, R.: Self-pollination effects on seed and seedling traits in noble fir. Forest Science 22: 155—159, (1976). —— SORENSON, F. C. and MILES, R. S.: Self-pollination effects on Douglas-fir and ponderosa pine seeds and seedlings. Silvae Genetica 23: 135—138, (1974). —— SNIEZKO, R. A.: Inbreeding and outcrossing in loblolly pine. Ph.D. thesis. Dept. of Forestry, North Carolina State Univ., Raleigh, N.C. 50 pp, (1984).. —— WILCOX, M. D.: Inbreeding depression and genetic variance estimated from self- and cross-pollinated families of *Pinus radiata*. Silvae Genetica 32: 89—96, (1983).

Linkage Relationships among Allozyme loci in Japanese Black Pine, Phinus thunbergii Parl.

By S. SHIRAISHI

Silviculture Division,
Forestry and Forest Products Research Institute,
P.O. Box 16, Tsukuba Norin Kenkyu Danchi-nai,
Ibaraki, 305 Japan

(Received 14th July 1986)

Summary

Linkage relationships among 32 polymorphic allozyme loci were investigated in Japanese black pine, *Pinus thunbergii* PARL. Haploid megagametophytes from 18 mother trees that possess doubly or more heterozygous loci were utilized for materials. Results of segregations in 221 instances among the possible 496 two-locus combinations of these loci were discussed by procedure of Akaike information criterion (AIC) in addition to conventional chi-square analysis. Segregation in most of the tested pairs revealed the independence in each locus. In fifteen pairwise combinations, however, linkage was recognized. *G2d* was always associated with *Pgm-1*. *Tzo-4* and *Lap-2* also showed almost complete linkage. *Me-1:Lap-2, Me-1:Tzo-4, Adh-3:Lap-1* and *Shd-1:Est-2* were linked closely with estimated recombination values of 0.13, 0.15, 0.26 and 0.27 respectively. *Mdh-1:Est-2, 6Pg-1:Est-4, Shd-2:6Pg-3, Got-2:Fm, Adh-3: Est-2* and *6Pg-3:Est-3* were linked with recombination values between 0.33 and 0.39. The remaining tested pairs were linked weakly with recombination values greater than 0.40. By AIC, linkages including nonrandom segregations in gametic genotype for specific loci were detected. Nineteen loci involved in these combinations were classified into six linkage groups, and a linkage map was made up on basis of the estimated recombination values and ordering of the loci in each group by three-point mapping.

Key words: Linkage, isozymes, allozymes, AIC, *Pinus thunbergii*, Japanese black pine.

Zusammenfassung

Die Kopplungsverhältnisse zwischen 32 polymorphen Allozym-Loci wurden bei *Pinus thunbergii* PARL. untersucht. Haploide Megagametophyten von 18 Sameneltern, die zwei oder mehrere heterozygote Loci aufwiesen, wurden verwendet. Die Segregation von 221 bei 496 möglichen Zwei-Locus Kombinationen wurden mittels des Akaike Informationskriteriums (AIC) und anhand der konventionellen χ^2-Analyse untersucht. Die Segregationsverhältnisse der meisten Paare zeigte die Unabhängigkeit der Loci an. Bei 15 paarweisen Kombinationen wurde jedoch Kopplung beobachtet. *G2d* war stets *Pgm-1* gekoppelt. *Tzo-4* und *Lap-2* zeigten meistens vollständige Kopplung. *Me-1:Lap-2, Me-1: Tzo-4, Adh-3:Lap-1* und *Shd-1: Est-2* waren mit einer geschätzten Rekombinationsrate von 0,13/0,15/0,26 bzw. 0,27 eng gekoppelt. *Mdh-1:Est-2, 6Pg-1:Est-4, Shd-2:6Pg-3, Got-2:Fm, Adh-3:Est-2* und *6Pg-3:Est-3* waren mit Rekombinationsraten zwischen 0,33 und 0,39 gekoppelt. Die übrigen der getesteten Paare waren mit Rekombinationsraten größer 0,40 nur schwach gekoppelt. Kopplung einschließlich nicht zufälliger Segregation der Parameter konnte bei einigen Loci beobachtet werden. Neunzehn Loci konnten in 6 Kopplungsgruppen eingeteilt werden. Die geschätzten Rekombinationsraten dienten zur Erstellung einer Kopplungskarte. Die Reihenfolge der Loci wurde anhand des "three point mapping" festgelegt.

Introduction

In breeding as well as genetic studies, it is very important to investigate the linkage relationship among loci and to make up the linkage map. It is necessary to clarify the relationship among loci as genetic markers when we study genetic structures and their dynamics in populations using isozymes and terpenes and so on. There are a few reports regarding linkage disequilibrium and epistatic selection in population (MITTON *et al.*, 1980; ROBERDS and BROTSCHOL, 1985). If the linkage map was made up on easily detectable marker genes such as allozymes, it would be easy to locate newly-detected genes on the map. When economically important genes are found and recombined by crossing in the future, the selection in the early stage is possible by using marker genes closely linked to them. Such a technique for early selection is useful, especially in forest trees which have very long vegetative period and it takes long time to manifest a lot of characteristics. The information obtained from linkage maps of various species also proved useful for characterizing phylogenetic relationships among them (CHELIAK and PITEL, 1985).

In coniferous species, linkage of genes which control isozymes can be revealed easily without making crosses by analysis of the independence of segregation in each locus