In this context then, flowering precocity acquires added importance in eucalypts.

Summary

Heterotic effect in regard to flowering precocity is reported in a controlled Eucalyptus tereticornis X E. *camaldulensis* cross. The implications of such precocious flowering in eucalypt improvement by selective breeding and hybridization is indicated.

Key *words:* Eucalyptus hybrids, Early flowering.

Zusammenfassung

Bei F_1 Hybriden aus der Kreuzung von Eucalyptus *tereticornis* Sm. mit E. camaldulensis Dehn. waren im Alter von 4 Jahren mehr blühende Individuen festzustellen als bei gleichaltrigen Individuen der reinen Elternarten, d. h *E.* camaldulensis blühte bis dahin überhaupt noch nicht. Die Eigenschaft des Frühblühens wird dem Hybridcharakter zugeschrieben.

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Inbreeding in Neighboring Trees in Two White Spruce Populations

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Introduction

White spruce [Picea glauca (Moench) Voss] has a transcontinental distribution, is one of the most important conifers in Canada, and is a genetically variable species (Nienstaedt and Teich, 1971). These factors combine to make it an excellent candidate for tree improvement work (Carlisle and Teich, 1970).

A temporal or spatial relationship among individuals in a population permits interbreeding and gene exchange between these individuals and therefore they may resemble each other more than they resemble members of different populations (Stebbins, 1949). Intuitively, there must be a tendency for close neighbors to mate, and proximity in time or space increases the probability that these neighbors will be related. If the trees growing near one another are related, then crossing these related individuals will result in inbreeding. In most naturally outbreeding plants, inbreeding reduces the mean phenotypic value shown by characters such as seed set, germination percent, and vigor. Franklin (1970) summarized much of the literature on inbreeding, particularly selfing, and the resulting reduction in seed yield, seed germination, survival, and early growth of seedlings for many Pinaceae species.

The amount of inbreeding in natural stands is determined largely by the distance and rate at which genes migrate within or between populations. As conifer seeds are seldom distributed more than 200 m (Fowells, 1965), pollen movement must account for most gene migration. If gene flow is extensive as a result of considerable long-distance pollen movement, natural inbreeding will be limited. If, however, effective pollen flow is limited, and gene exchange between neighboring trees is common, a significant amount of natural inbreeding may occur.

The genetic structure of natural populations has only recently received attention. The family group structure of trees has not been well documented although such relationships are generally accepted (Bannister, 1965; Koski, 1973; Sorensen, 1973; Ledig, 1974; Tigerstedt, 1974). Studies in Japan in natural forests of both *Cryptomeria japonica* and *Thujopsis dolabrata* have indicated relatedness of trees within groups (Sakai et al. 1970; Sakai and Miyazoki, 1972). Loblolly pines (Pinus taeda L.) growing within 100 m of each other in a small stand are probably related since the progenies from controlled crosses had depressed survival, green weight, and three-year height (Snyder, E. B., USDA Forest Service, Southern Forest Experiment Station, Gulfport, Miss., pers. comm. 1973). However, Tigerstedt (1973) found no gene clustering for trees in either of two stands of Norway spruce (Picea *abies* (L.) Karst.) in Finland. Spatial mapping of enzyme alleles in these populations indicated complete randomness of gene distribution.

The objective of this study was to determine the relationship among white spruce trees in groups within stands and to obtain estimates of the amount of inbreeding occurring under natural conditions.

Materials and Methods

Study Areas

Two central New Brunswick white spruce stands, separated by approximately 32 km, were chosen at study areas. One is a natural upland stand of mixed conifers at the Acadia Forest Experiment Station (AFES) and the other is a pure stand which has colonized abandoned farmland in the Tay River Valley (Tay). Based solely on an adequate number of male and female strobili, 17 trees at AFES and 9 at Tay were chosen for controlled pollination studies.

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Controlled Pollinations

Each of the 26 mother trees was selfed and crossed with a pollen mix composed of four pollens from the other stand (technique described by Nienstaedt and Teich, 1971, modified by Fowler, 1971). These two pollinations provided the extremes of distance: zero metres and 32,000 metres. To ensure an adequate number of short-distance crosses, ten pollinations were made between close neighbors; the remainder of the controlled pollinations were determined randomly. A range of distances between mother tree and pollen source tree was desired, however the availability of strobili largely determined how many and which crosses could be made with each tree.

Seed Set

Sound seeds were separated from empty seeds by flotation in 95% ethyl alcohol. The sound seeds were counted and the average weight of all seeds in each cross was determined. The large round empty seeds were counted and percent sound seed for each cross was calculated. This information was used to estimate genetic load or the number of lethal equivalents (Morton *et al.*, 1956) as presented by Sorensen (1969):

$$2B = -4 \log_n R$$

Where B is the average number of lethal equivalents per gamete, 2 B is the number per zygote that constitute the hidden, heterozygous, genetic load and R is the relative self-fertility, that is, percent sound seed from self-pollination divided by percent sound seed from a long-distance unrelated cross.

Wind-pollinated cones were severely damaged by spruce budworm *(Choristoneura fumiferana* [Clem.]) and as such, seed data were excluded from the calculations. The wind-pollinated seedling data were unaffected by budworm and were included.

Germination

The germination and growth studies were based on up to 50 sound seeds per cross. The seeds were germinated in a chamber maintained at 30° C during a daily 8-hour light period and 20° C during a daily 16-hour dark period (Heit, 1963). Seeds were considered germinated when the radicle was about 1 cm in length and percent germination was calculated for each cross. The rate of germination was determined as the number of days required for 50 percent of the total germination to occur.

Seedling Growth

Germinated seeds were planted in 2.5 inch (6.35 cm) peat pots in a random design on a greenhouse table at the Acadia Forest Experiment Station. Daylight was extended from 4 AM to 8 PM with incandescent lamps, and a commercial fertilizer (RX-30) was applied weekly.

Cotyledons were counted and averaged for each cross. Epicotyl length for each seedling was measured in millimetres on the 133rd day after the first seedlings were planted. Epicotyl lengths of each seedling were corrected for length of growing period, assuming a linear growth relationship, and were adjusted, by cross, for sound seed weight.

Coefficient of Relationship

The correlation of breeding values between members of families is a measure of the degree of relationship and is usually called the relationship coefficient (r). The correlation between the breeding values of relatives in a random-mating population is twice the inbreeding coefficient (F) of their progeny if the relatives were mated (Falconer, 1960):

$$r = 2F$$

The long-distance pollen mix was assumed to be unrelated and as such the progeny have a relationship coefficient of $r = 0$. The relationship coefficients were scaled from the data with progeny of self-pollinations having an $r = 1.0$ and progeny of long-distance pollinations having an $r = 0$.

Results

Seed Characteristics

The female parent provided the only significant effect on both sound seed weight and number of sound seeds per cone.

Table 1. — Means and F-values from analysis of variance of percent sound seed for each study area.

Location		Percent sound seed (standard deviation)	Source of variation	F-value
AFES	Mean of all crosses	35.8 (24.5)		
	Range of means of female parents	11.7 -61.5		
	Mean of self-pollinations	8.1 (7.7)		
	Mean of Tay mix-pollinations	50.7 (15.4)		
	Mean of AFES cross-pollinations	39.5 (23.2)		
			Females	2.11*
			Males	2.84**
Tay	Mean of all crosses	27.4 (18.4)		
	Range of means of female parents	20.6 - 36.5		
	Mean of self-pollinations	4.1 (3.8)		
	Mean of AFES mix-pollinations	35.8 (10.1)		
	Mean of Tay cross-pollinations	32.2 (17.4)		
			Females	0.47
			Males	4.14**

* Statistically significant at 0.05 level.

** Statistically significant at 0.01 level.

The variation in percent sound seed was caused by both male and female parent *(Table 1)*. However, analysis of variance showed that only the pollen parent had a significant effect at both stands and therefore percent sound seed was plotted over distance between the parents *(Fig. 1 and 2)*. The lower 95% confidence interval of the unrelated long-distance pollinations was placed on the graphs *(solid line Fig. 1 and 2)*. Data points falling below this line have only a 1 in 20 chance of resulting from outcrosses and may be considered a result of crosses between relatives. Points above the line result from either outcrosses or crosses between relatives but are impossible to distinguish. Within both study areas about 35% of the pollinations from within 100 metres are putative relatives. Beyond 100 metres crosses between relatives are much less common.

Genetic Load

Of the 26 trees in both stands on which controlled self-pollinations and long-distance pollinations were conducted, only 18 produced sound seed from both pollinations and could be used to calculate the genetic load. Estimates of the relative self-fertility (R) and the number of lethal equivalents per zygote (2B) are shown in *Table 2*. The number of embryonic lethal equivalents per zygote varied from 2.9 to 14.8 with a mean for both stands of 8.7.

Percentage and rate of germination

Germination of the sound seed was poor (54%) and slow, possibly due to lack of stratification. The seeds from self-pollination usually had a lower germination percent than those from the long-distance pollen mix. However, ana-

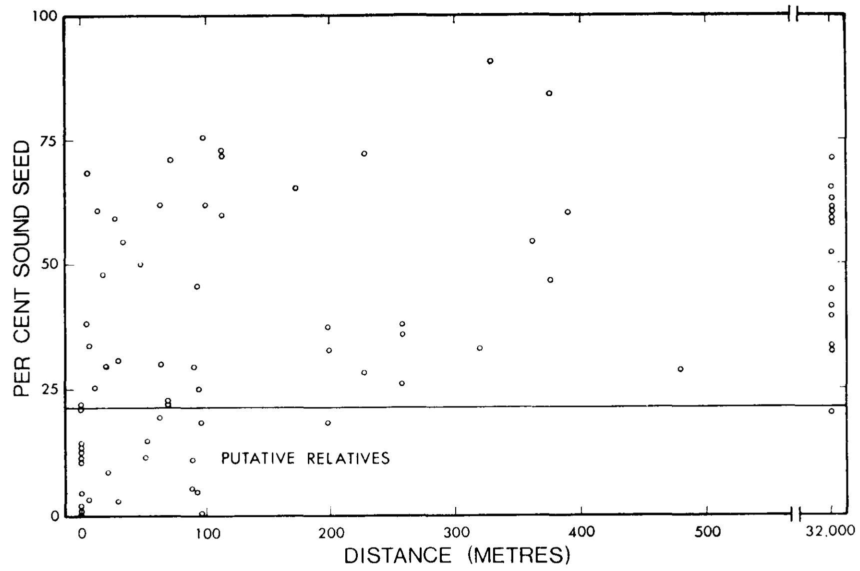

Figure 1. — **Percent sound seed over distance between mother tree and pollen parent at Acadia Forest Experiment Station.**

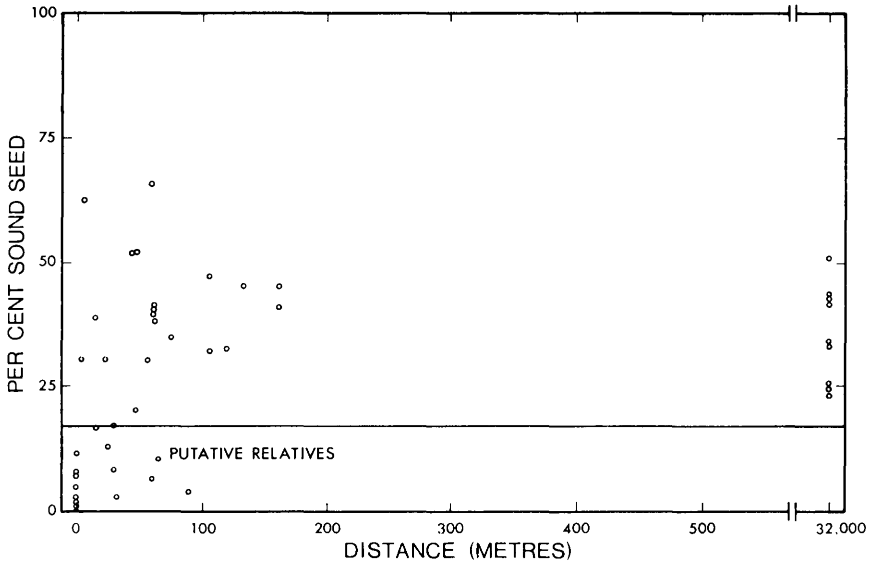

Figure 2. — **Percent sound seed over distance between mother tree and pollen parent at Tay River Valley.**

Table 2. — **Relative self-fertility and number of embryonic lethal equivalents per zygote for 18 white spruce trees in the study locations.**

Location	Tree Number	Relative self-fertility (R)	Number of embryonic lethal equivalents per zygote (2B)
AFES	552	.174	6.98
	553	.047	12.21
	554	.348	4.22
	561	.029	14.20
	795	.167	7.15
	607	.241	5.69
	791	.227	5.93
	792	.208	6.27
	732	.205	6.33
	735	.029	14.19
Tay	1	.220	6.06
	2	.260	5.39
	3	.061	11.20
	4	.485	2.89
	5	.111	8.79
	6	.035	13.38
	7	.056	11.56
	9	.025	14.75
	Mean	.157	8.73

lysis of variance showed that neither mother tree nor pollen parent had a significant influence on germination percent or rate of germination in either stand. The poor germination and the slow rate of germination may have masked any genetic differences.

Seedling Characteristics

Seedling mortality in the greenhouse was severe and made it impossible to detect any genetic effects of inbreeding on early survival.

The number of cotyledons varied little with pollen parent or between study areas. Analysis of variance showed that the female parent provided the only significant influence.

The means and standard deviations of epicotyl length after 133 days of growth, adjusted for sound seed weight, are shown in *Table 3*. Analysis of variance indicated that the mean epicotyl length of progeny by female parents differed significantly at both stands while the influence of the male parent was significant only at AFES. Epicotyl

Table 3. — Means and F-values from analysis of variance of epicotyl length of seedlings adjusted for sound seed weight by cross for each study area.

Location		Epicotyl length (mm) (standard deviation)	Source of variation	F-values
AFES	Mean of all crosses	75.2 (15.7)		
	Range of means of female parents	58.1 - 98.7		
	Mean of self-pollinations	57.3 (23.8)		
	Mean of Tay mix-pollinations	82.5 (10.3)		
	Mean of wind-pollinations	67.4 (4.5)		
	Mean of AFES cross-pollinations	76.9 (13.6)		
			Females	3.77**
			Males	1.94*
Tay	Mean of all crosses	60.0 (12.1)		
	Range of means of female parents	37.7 - 82.2		
	Mean of self-pollinations	45.7 (14.4)		
	Mean of AFES mix-pollinations	65.0 (7.9)		
	Mean of wind-pollinations	61.7 (7.4)		
	Mean of Tay cross-pollinations	62.0 (11.9)		
			Female	6.21**
			Male	1.06

*Statistically significant at 0.05 level.

**Statistically significant at 0.01 level.

lengths were plotted over distance between parents for both stands (*Fig. 3 and 4*). Epicotyl length observations below the solid line have only a 5% probability of resulting from outcrosses and may be considered the result of crosses between relatives. At AFES, the proportion of crosses between putative relatives to crosses between unrelated parents decreases beyond 200 metres. No crosses between putative relatives at Tay occur beyond 75 metres.

The mean epicotyl length of progeny from wind-pollination was about 12% shorter than that from the long-distance pollinations at both stands (*Table 3*).

Discussion

Genetic Load

Although empty seed in conifers result from several causes, genetic factors must be the most important because self-pollination consistantly produces large quantities of empty seeds. In this study the proportion of empty seeds

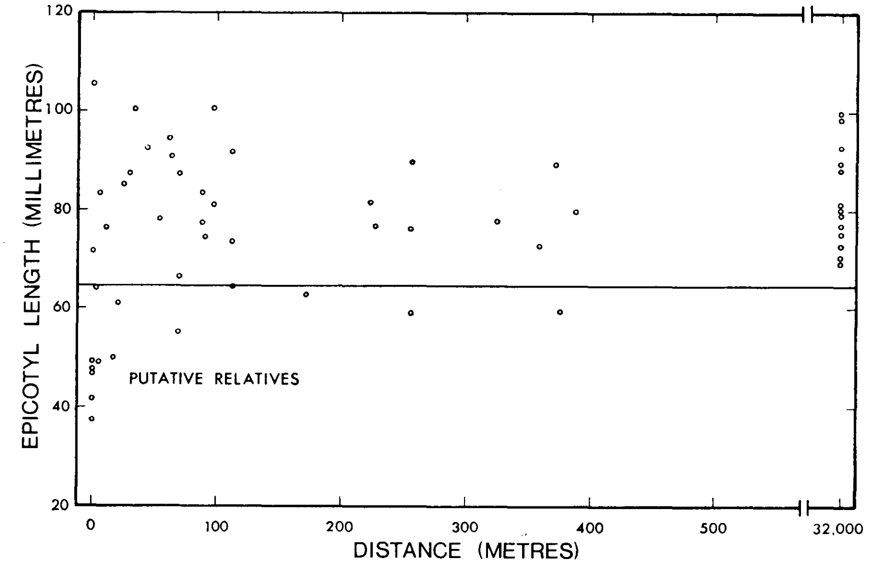

Figure 3. — Epicotyl length at 133 days adjusted for seed weight over distance between mother tree and pollen parent at Acadia Forest Experiment Station.

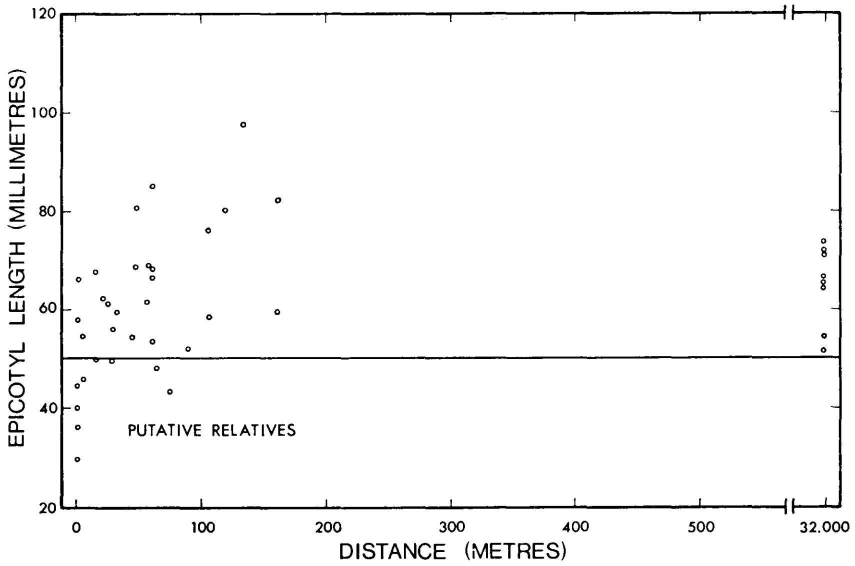

Figure 4. — Epicotyl length at 133 days adjusted for seed weight over distance between mother tree and pollen parent at Tay River Valley.

from controlled self-pollinations was 93.9% which was slightly higher than the 87% found by Mergen *et al.* (1965) for the same species.

The average genetic load of white spruce in this study is 8.7 embryonic lethal equivalents per zygote which is similar to the results reported by several authors for conifer species. For example, Franklin (1971) found 8.5 embryonic lethal equivalents per zygote in loblolly pine and Koski (1971) reported 8.9 embryonic lethals in Scotch pine *(Pinus sylvestris* L.) and 9.6 in Norway spruce.

Lethal genes are involved in such fundamental processes that their lethality takes effect early in the development of the zygote (Sarvas, 1968). In this study about 86% of the genetic load was expressed during embryo development. Koski (1973) stated that 87 and 89% of the genetic load was expressed before the seed matured for Scotch pine and Norway spruce respectively. As embryonic lethals eliminate between 85 and 90% of the inbred embryos, the effects of genetic load on germination capacity, seedling vigor, and survival can be expected to be reduced if the same genes contribute to load in both stages.

Epicotyl Length

The epicotyls of self-pollinated seedlings were 24% shorter than those of the long-distance pollen mix. This is similar to the findings of King *et al.* (1970). Mergen *et al.* (1965) also found that seedlings from self-pollinations were significantly shorter initially, but there was little difference in height between self- and wind-pollinated seedlings at the end of one growing season.

In both stands self-pollination also reduced seed set about 86% compared to the unrelated long-distance pollen mix. Obviously, in a white spruce improvement program there is much to be gained by minimizing the amount of selfing.

The epicotyls of progeny originating from wind-pollination at both stands were shorter than those from the long-distance pollen mix, indicating that some of the pollen reaching the wind-pollinated strobili was from self or related neighbors.

Coefficient of Relationship

Tigerstedt (1973) opposed the concept of gene clustering and inbreeding, and stated that mapping of Norway spruce populations indicated complete randomness of gene distribution. He concluded that population heterogeneity is maintained by strong stochastic gene flow as proposed by Koski (1970) and by extremely intense selection operating throughout the life cycle. Snyder (pers. comm. 1973), however, suggested that neighboring trees in wild stands of loblolly pine are related. He found that crossing neighboring trees within 300 feet (91 m) of each other resulted in average losses of 2 to 14% in seed and seedling characters when compared to the overall mean.

In this study, crosses between trees separated by 100 metres or less resulted in a 28% reduction in seed set compared to crosses between trees separated by more than 100 metres. This depression indicates that there is a detectable relationship among at least some of the trees within a radius of about 100 metres.

The relationship coefficients were scaled from the data in *Table 4*. The trees within a 100 metre radius of any mother tree at AFES have an average (of percent sound seed and epicotyl length) relationship coefficient of r = 0.30 while those at Tay have an average relationship coefficent of r = 0.23. These two relationship coefficients are high when compared to Morgenstern's (1972) estimates for black spruce *(Picea mariana* Mill. B.S.P.). He found a relationship coefficient of r = 0.16 for southern Ontario populations and r = 0.06 for northern populations.

Table 4. — Summary of percent sound seed and epicotyl length by distance classes for both study locations.

Location	Distance Class	Percent sound seed	Epicotyl length (mm)
AFES	Self-pollination (0 m)	8.1	57.3
	Wind-pollination		67.4
	1 to 100 m	32.7	78.1
	101 to 500 m	50.7	75.1
	32,000 m	50.7	82.5
Tay	Self-pollination (0 m)	4.1	45.7
	Wind-pollination		61.7
	1 to 100 m	29.8	59.8
	101 to 500 m	40.6	75.5
	32,000 m	35.9	65.0

White spruce does not grow in large contiguous stands in eastern Canada except when colonizing old fields. The white spruce in mixed stands, of which AFES is an example, may be so widely separated that gene flow between trees is reduced thus increasing the possibility of high relationship coefficients among the progeny. Gregorius (1973) stated that the coefficients of relationship increases steadily from generation to generation for plant populations of finite size whose individuals are stationary. Old field stands, of which Tay is an example, usually originate from mature trees along their perimeter. If these mature trees are few, as is often the case, the relationship coefficient within the old field stand could also be quite high.

Implications

Ledig (1974) analyzed two basic methods of selection of superior trees for breeding orchards: comparison tree selection and the individual tree or baseline selection. He suggested that the comparison tree method will be the most efficient only if family relationships are low. The relatively high relationship coefficients found in this study on white spruce indicate that the individual tree method of selection of superior trees will be the most efficient.

The increased growth of progenies from seed orchards over that of wind-pollinated controls is generally considered as a genetic improvement attributable to the genetic superiority of the parent types. Our data show that for white spruce a considerable part of this "improvement" may result merely from reduced inbreeding resulting from breaking up the family structure of stands of trees. In other species, the presence or absence of a relationship coefficient within stands will determine what portion, if any, of the improvement is the result of breaking up the family structure.

Abstract

The population structure of white spruce *(Picea glauca* [Moench] Voss) was studied in two stands in south central New Brunswick. Estimates of inbreeding were obtained by comparing the effects of self-pollination, cross-pollination between neighboring trees, and cross-pollination between widely separated trees.

Percent sound seed and seedling height varied significantly with pollen parent. Seed weight, cotyledon number, and germination percent were unaffected by pollen parent.

The relationship between inbreeding depression and nearness of pollen parent indicated that family relationships exist between near neighbors. Trees separated by greater than 100 metres were unrelated. Average relationship coefficients for trees within the two stands were found to be 0.30 and 0.23.

Key words: Population structure, Relationship, Inbreeding depression, *Picea glauca* (Moench) Voss.

Zusammenfassung

In zwei 32 km voneinander entfernten Beständen von *Picea glauca* (Moench) Voss, in New Brunswick, Kanada, wurde die Populationsstruktur untersucht, indem Selbstungen und Kreuzungen zwischen benachbarten Bäumen jedes Bestandes und Bäumen der beiden Bestände durchgeführt wurden. Hierbei variierte das Vollkornprozent der geernteten Samen und die Höhe der daraus angezogenen Pflanzen mit dem männlichen Elternteil im Gegensatz zur Anzahl der Kotyledonen und zum Keimprozent. Es konnte eine Beziehung zwischen Inzuchtdepression und naher Verwandtschaft zum männlichen Elternteil erkannt werden, woraus geschlossen wird, daß zwischen benachbarten Bäumen Familienverwandtschaft besteht. Bäume, die weiter als 100 m voneinander entfernt sind, waren nicht mehr als verwandt zu betrachten.

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