Population Variation in Sequoiadendron: Seed and Seedling Studies, Vegetative Propagation, and Isozyme Variation*)

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(Received 12th August 1980)

Summary

Seed samples were collected from 35 natural populations of giant sequoia and examined for seed weight, germination percent, cotyledon number, rootability of cuttings, and isozyme variation. Samples were significantly variable in percent seed germination, cotyledon number, isozyme allele frequencies, and observed heterozygosity. Seed germination varied among populations, but did not reveal any clear geographic patterns. Cotyledon numbers (of 871 seedlings) varied among populations and geographic areas. Cuttings (from 608 seedlings) rooted at 94 percent. Isozyme variation was found in every population sample at one or more loci. Little if any recent gene flow is likely to have occurred between the northern and southern populations. Relatively low heterozygosity among embryo samples suggests that inbreeding and/or population substructuring is likely in giant sequoia populations. Relatively higher levels of heterozygosity are found in the southern parts of the range, suggesting different local selective regimes. Early data suggest that the most northern native population (Placer Grove), may be substantially different from the other populations.

Key words: cotyledons, heterozygosity, inbreeding, mating system, rooted cuttings.

Zusammenfassung

Es wurden Samenproben aus 35 natürlichen Populationen von *Sequoiadendron giganteum* gesammelt und auf Samengewicht, Keimfähigkeit in Prozent, Anzahl der Kotyledonen, Bewurzelungsvermögen von Stecklingen und Isoenzymvariation untersucht. Die Proben zeigten signifikante Variabilität in Keimungsprozenten, Anzahl der Kotyledonen, Isoenzym-Allelhäufigkeiten und tatsächlich festgestellter Heterozygotie. Die Keimfähigkeitswerte verschiedener Populationen variierten, ergaben jedoch kein klares geographisches Muster. Die Anzahl der Kotyledonen je Keimling (aus 871 Keimpflanzen) schwankte zwischen den Populationen und geographischen Gebieten. Stecklinge (von 608 Keimlingen) bewurzelten sich zu 94%. Die Isoenzymvariation betraf innerhalb jeder Einzel-Populationsprobe mindestens einen Genlocus. Zwischen den nördlichen und südlichen Populationen hat in jüngerer Zeit wahrscheinlich höchstens geringer Genfluß stattgefunden. Relativ niedrige Werte für Heterozygotie in den Embryoproben machen das Vorkommen von Inzucht und/oder Untergruppen in Populationen von *Sequoiadendron giganteum* wahrscheinlich. Vergleichsweise höhere Werte für Heterozygotie finden sich in den südlichen Teilen des Verbreitungsgebietes, was auf unterschiedliche Verhältnisse bezüglich der lokal wirksamen Selektionsverfahren hinweist. Vorläufige Ergebnisse zeigen an, daß sich die nördlichste heimische Population (Placer Grove) von den übrigen möglicherweise wesentlich unterscheidet.

Résumé

La Variation dans la population de *Sequoiadendron:* Etudes sur les semences et les plants, reproduction végétative, et variation d'isozymes.

Les échantillons de semences provenant de 35 populations naturelles de séquoia géant ont été soumis aux tests de poids de la graine, du taux de germination, du nombre de cotylédons, de l'enracinement de boutures, et de la variation d'isozymes. On a observé une variation significative quant au taux de germination, nombre de cotylédons, fréquence de facteurs allélomorphes, et à l'hétérozygotisme. Entre les populations, la germination des graines a varié, mais sans révéler aucune tendance géographique. Le nombre de cotylédons (de 871 plants) a varié entre populations et aires géographiques. Le taux d'enracinement de boutres (prevenant de 608 plants) a atteint 94%. Dans chaque population, la variation d'isozymes a été observé sur une gene au moins. Tout transfer récent de genes entre les populations du nord et les populations du sud doit être trés faible. L'hétérozygotisme relativement faible entre les échantillons d'embryons suggère qu'un croisement consanguin et/ou une sous-structuration peut avoir lieu dans les populations de séquoia géant. Un degré d'hétérozygotisme qui est relativement plus élevé s'observe vers les limites australes de l'aire naturelle, ce qui suggère l'existence de différents régimes locaux de sélection. Les premières observations suggèrent que la population naturelle la plus méridionale (Placer Grove) pourrait être bien distincte des autres populations.

Mots clefs: cotylédons, hétérozygotisme, croisement consanguin, système d'accouplement, boutures avec racines.

Introduction

Giant sequoia (*Sequoiadendron giganteum* [Lindl.] Buch.) has been the subject of numerous popular articles since its "official discovery" in 1852. Yet, scientific studies of its ecological amplitude, wood quality, and physiological nature have appeared only in the last 20 years. We feel that giant sequoia is worthy of genetic study for at least four reasons: 1) some giant sequoias are, as far as we know, the most massive individual living organisms; 2) the species is second only to bristlecone pine in verified longevity (Hartesveldt et al. 1975); 3) giant sequoia has an unusual and interesting natural distribution (8 small, disjunct northern populations and about 65 larger, more continuous southern populations, all on the west slopes of the Sierra Nevada Mountains in California) *(Fig. 1);* and *4),* its fast growth rate and general resistance to insects and diseases make giant sequoia a potentially attractive addition to mixed conifer plantings in California and many other locations in the world. This paper is the first published study of patterns and amounts of genetic variation among samples from native populations of giant sequoia.

During 1974—76, we collected seed samples from 35 natural populations of giant sequoia, including all 8 northern ones. A long-term clonal study of the species, using open-pollinated families from 24 of the populations, was established in 1981. Observations, summarized below, on seed weights, germination percentages, cotyledon numbers and rooting percentages were made in the course of generating and expanding clones for that study. Seeds from these and additional families and populations were used in the isozyme analyses, and related seeds have been placed in

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long-term storage for gene conservation (Fins 1979). Seeds, seedlings, and rooted cuttings have also been distributed to North American, New Zealand, and European foresters and scientists interested in giant sequoia.

The Seed Sample

Our original research plan called for collections from equal numbers of trees from each sampled population. Since

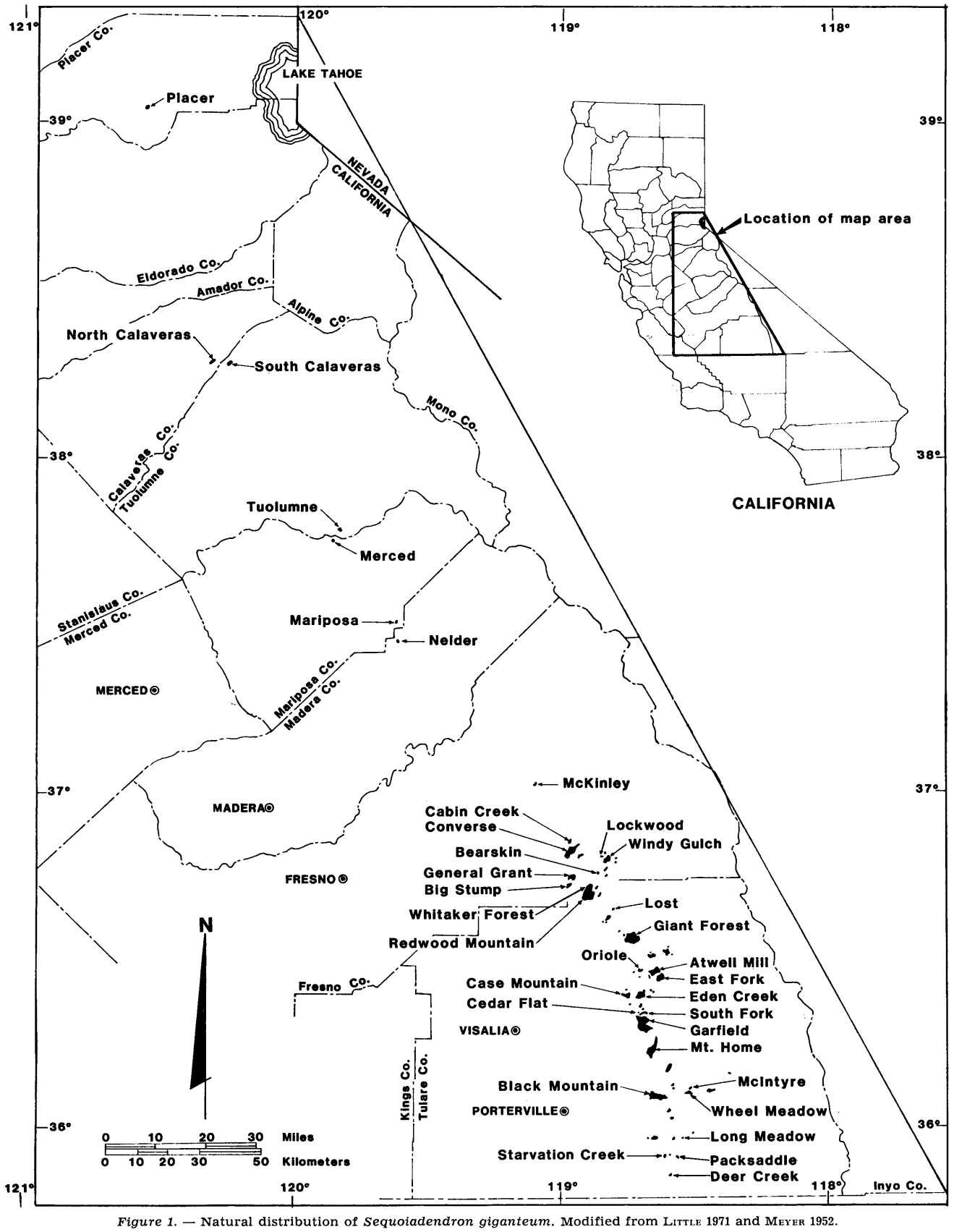


Figure 1. — Natural distribution of *Sequoiadendron giganteum*. Modified from Little 1971 and Meyer 1952.

giant sequoia cones remain green on the trees for several years, they were available during each of the collection years. However, we found that climbing mature giant sequoia trees for cone collecting was impractical, and shooting cones from the trees was an unacceptable means of collecting from many of the populations, particularly those in National and State Parks, and in National Forest groves accessible to the general public. We depended instead on chickaree squirrels, which, during the summers and falls of 1974, 1975, and 1976, cut cones in sufficient numbers for us to use for our research. Accordingly, we removed the restriction on equal numbers of trees from each of the sampled populations. Only in the 6-tree Placer (American River) Grove did we shoot cones from the trees to obtain our samples.

In general, the tree from which a cone had been cut was reasonably unambiguous, as giant sequoias are often widely spaced in natural stands. When two or more squirrel-cut cones lay beneath closely neighboring trees, and the identity of the mother tree was thus uncertain, we took only one cone for the genetic studies, making it unlikely that we collected two open-pollinated families from the same mother tree. Collections totaled 434 single-cone open-pollinated families from 35 populations. Details are given in FINS (1979).

Seed Weight, Germination, and Cotyledon Number

Materials and methods

Data for seed weight, seed germination, and cotyledon number were from collections from 26 populations. Twenty apparently-filled seeds were selected from each of 284 families; they were then weighed and stratified at 2.2° C for 7 days. Each sample was placed on a shaded germination plate (FINS 1979), and incubated indoors at ambient temperature. Germination was recorded at 2- to 3-day intervals. Seeds were considered germinated when the radicle extended at least 1 mm beyond the seedcoat. We counted cotyledon numbers on 871 seedlings, from 72 open-pollinated families in 26 populations.

Results

A one-way analysis of variance indicated no significant differences among population samples in seed weights of 20 apparently-filled seeds per family. The estimated population component of variance was positive, but accounted for only 3 percent of the observed variation *(Table 1)*. Average weight of the 20-seed samples was 0.116 gms.

Table 2. — Frequencies of cotyledon numbers by geographic area.

Geographic Area[1]	Cotyledon Numbers 3	4	5	6	Number of Observations	Average[2] Number of Cotyledons
North	.14	.59	.24	.03	281	4.16
Central	.21	.67	.12	.00	291	3.92
South	.16	.71	.13	.007	299	3.98
Average	.17	.66	.16	.01	871	4.02

[1] The 8 northernmost, 9 central, and 9 southernmost groves of the 26 groves sampled in 1974—75.

[2] χ^2 value for equal frequency distribution in each area = 33.8 ($P < .001$).

Overall germination was 23 percent, very close to the 22.5 percent figure reported by BEETHAM (1962) for seeds collected from the ground in 42 populations. Some of the open-pollinated families had no germinants. Analyses of germination percent, both including and excluding those families, show significant differences among population samples, accounting for 17 percent and 13 percent of the observed variation *(Table 1)*. Average germination in families with at least one germinant was 30 percent.

Mean numbers of cotyledons differed significantly between populations and between families within populations *(Table 1)*. The Placer Grove (the most northern population) had the highest mean cotyledon number (5.1) and the lowest seed weight (.095 gms) of the 26 populations analyzed. The sample from Deer Creek (the most southern population) had the next highest average cotyledon number (4.6) and the next lowest seed weight (.096 gms). The overall correlation between average seed weight and average cotyledon number (by population) was not statistically significant ($r = .29$, $P = .92$). Overall, 66 percent of the seedlings had 4 cotyledons. Variation in cotyledon numbers among geographic areas was statistically significant ($\chi^2 = 33.8$, $P < .001$). Samples from the 8 northern populations had high proportions of 5- and 6-cotyledon seedlings, most of the latter coming from the Placer Grove *(Table 2)*.

Rooting

Materials and methods

In May and June 1976, seeds were germinated from 155 open-pollinated families from 24 giant sequoia populations. We selected 608 of these seedlings, chosen randomly within

Table 1. — Summary of analyses of variance for seed weight, percent germination, cotyledon number, and rooting.[1])

Characteristic	Source	df	F Probability	Percent of Variance
Seed weight	Populations	25	.15	3%
	O-P families within pops.	258	-	97%
Percent Germination (includes nongerminating families)	Populations	25	<.001	17%
	O-P families within pops.	258	-	83%
Percent Germination (excludes nongerminating families)	Populations	25	.007	13%
	O-P families within pops.	163	-	87%
Cotyledon Numbers	Populations	25	<.001	12%
	O-P families within pops.	46	<.001	21%
	Within O-P families	799	-	67%
Rooting of Cuttings from Juvenile Donors	Populations	23	.12	2%
	O-P families within pops.	131	.06	6%
	Within O-P families	453	-	92%

[1]) Details in FINS (1979)

families, for use in a large clonal population experiment. Two cuttings (if available) were taken from each seedling in September 1976, and in December, 3 additional cuttings per seedling were taken, a total of 5 cuttings per clone for most of the 608 clones.

Cuttings were standardized to 6 cm in length and soaked in Benlate[1]) solution (1.13 gm/gal water) for one-half hour. The basal end of each cutting was freshly cut and dipped in an indole-butyric-acid solution of 4000 ppm in 95 percent ETOH. Cuttings were set in Leach supercells[2]) in a medium consisting of 1/3 Canadian sphagnum peat, 1/3 nitrogen-charged redwood sawdust and 1/3 commercially packaged oak-leaf-mold. The containers were randomized in racks. The racks were periodically moved about in the rooting beds of a greenhouse in Albany, California. They received 2 morning and 3 afternoon mist sprays, each of approximately one-minute duration, at one-hour intervals, and were fertilized weekly to saturation with Upstart[3]) at 4 ounces per gallon. We considered a cutting to be rooted when root tips extended through the bottoms of the containers.

These cuttings were intended for clonal expansion and were not intended to test rooting success by population. The donor seedlings had been grouped by family and population in the greenhouse, and cuttings were collected and set sequentially by family and population. These groupings may have introduced biases that could inflate both the population and family components of variation in the analysis of variance.

Results

Every clone rooted at least one cutting, and most rooted 4 or 5 of 5. Overall rooting was 94 percent after one year, with most rooting completed at 8 months. A nested analysis of variance (percentages transformed to arcsine) indicated no significant differences between open-pollinated families or populations in final rooting percent. F-probabilities of .12 and .06 suggest that the observed differences may not have been wholly due to chance *(Table 1)*, but the above-noted biases could have inflated these values.

Isozyme Variability

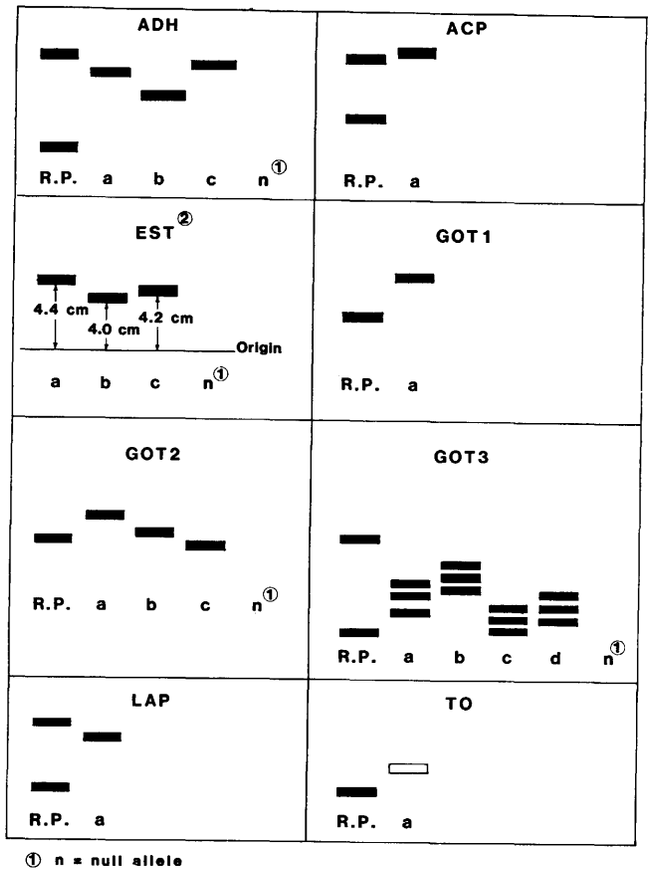
Materials and methods

We used starch-gel electrophoresis to analyze allelic variation in 357 open-pollinated families from 34 populations.

The biochemical methodologies we used were described by Fowler and Morris (1977) and Scandalios (1969), with some minor modifications in technique, such as restricting the gel fronts to 6 cm (as compared with 8 cm) and not removing wicks during the entire run (Fins 1979).

We used monomorphic red pine gametophyte tissue as standards in three locations on each gel (Fowler and Morris 1977). Of the eight loci scored, four were polymorphic: an alcohol dehydrogenase (ADH), an esterase (EST), and two glutamic-oxaloacetic transaminases (GOT); four were monomorphic: an acid phosphatase (ACP), a tetrazolium oxidase (TO), a leucine amino-peptidase[4]), and a GOT. Mobility relationships among alleles of these eight loci, as compared with red pine standards, are shown in *Figure 2.*

We tested allelism by scoring a minimum of 15 and a maximum of 50 gametophytes from individuals segregating apparently allelic bands. Where large numbers of viable seeds were not available from a single probable heterozygote, we pooled data from all families segregating for the same band mobilities. In all 13 chi-square tests, segregation



① n = null allele

② Red pine did not band for EST. Scoring was done on the basis of migration distance from the origin.

Figure 2. — Relative mobilities of 8 isozyme loci of giant sequoia and red pine (R. P.)

ratios were consistent with 1:1 expectations (Fins 1979). We used one-way analyses of variance to test differences in average level of heterozygosity among populations, G-tests to test heterogeneity of allele frequencies among population samples (Sokal and Rohlf 1969), and paired t-tests to test mean differences in heterozygosity between observed and expected levels of heterozygosity of embryo and mature population samples.

Morris and Spieth (1978) showed that, in the case of limited laboratory facilities and time, analysis of 3 haploid gametophytes per tree most efficiently gives both population and genotype information, with a .25 probability of misclassifying a heterozygous locus. We analyzed 3 gametophytes and their associated diploid embryos for each tree, and corrected for misclassification of heterozygotes among the maternal trees using the following formula:

$$H_{oc(m)} = 4/3\ H_{o(m)}$$

where $H_{o(m)}$ is the observed level of heterozygosity of the maternal trees and $H_{oc(m)}$ is their level of heterozygosity corrected for misclassification.

[1]) Fungicide manufactured by Dupont Company. Active ingredient Benomyl.

[2]) Tubular 140-cc plastic containers usually used for growing seedlings in greenhouses.

[3]) Ortho Upstart: 3N-10P-3K plus micronutrients.

[4]) LAP may actually be a variable locus. Differences in band thickness were observed and, from time to time, secondary bands appeared. However, there was no obvious genetic interpretation to these differences. With better separation techniques, genetic differences may be found at this locus.

Table 3. — Allele frequencies of gametophyte samples of mature giant sequoia, and numbers of embryos analyzed, (Populations listed from north to south.)

Population Name	Ave. Elev. (m)	ADH a	ADH b	ADH n+c	EST a	EST b	EST n+c	GOT 2 a	GOT 2 b	GOT 2 c	GOT 2 n	GOT 3 a	GOT 3 b	GOT 3 n+c+d	N maternal trees	N embryos
Placer	1580	1.00	-	-	.57	.43	-	.07	.93	-	-	1.00	-	-	3-6	30
North Calaveras	1400	.91	-	.09	.19	.78	.03	.03	.97	-	-	.875	.125	-	16	50
South Calaveras	1520	.96	-	.04	.18	.75	.07	.07	.89	-	.04	1.00	-	-	14	42
Tuolumne	1710	1.00	-	-	.60	.40	-	.15	.85	-	-	.60	-	.40	10	34
Merced	1680	1.00	-	-	-	.50	.50	-	1.00	-	-	.75	.25	-	6	18
Mariposa	1890	.89	.11	-	.33	.67	-	-	1.00	-	-	.83	.17	-	9	27
Nelder	1830	.96	-	.04	.625	.375	-	-	1.00	-	-	.71	.25	.04	12	38
McKinley	1950	1.00	-	-	.71	.21	.07	-	1.00	-	-	.79	.21	-	7	21
Cabin Creek	1750	.67	.33	-	.61	.39	-	-	.83	.06	.111	1.00	-	-	9	21
Converse Basin	1750	.73	.27	-	.50	.35	.15	.08	.77	-	.15	.85	.08	.08	13	37
Lockwood	1680	1.00	-	-	.60	.40	-	.20	.60	.20	-	.90	.10	-	5	12
Windy Gulch	1980	.86	.14	-	.79	.14	.07	-	1.00	-	-	1.00	-	-	7	21
Bearskin	1830	.83	.17	-	.67	.25	.08	-	1.00	-	-	1.00	-	-	6	12
Grant	1520	1.00	-	-	.545	.23	.23	.09	.82	.045	.045	.73	.27	-	11	33
Big Stump	1980	.50	.50	-	.75	.25	-	-	1.00	-	-	1.00	-	-	2	6
Redwood Mountain	1900	.76	.12	.12	.46	.52	.02	.10	.88	.02	-	.96	.04	-	25	77
Whitakers Forest	1900	.90	.03	.07	.77	.13	.10	.07	.87	.07	-	.70	.27	.03	15	50
Lost	2057	1.00	-	-	.83	.17	-	-	1.00	-	-	.83	.17	-	6	18
Giant Forest	1900	1.00	-	-	.60	.40	-	-	1.00	-	-	.80	.20	-	5	9
Oriole	1830	1.00	-	-	.25	.75	-	.17	.83	-	-	.67	.33	-	6	18
Atwell Mill	2130	1.00	-	-	.61	.36	.04	.04	.96	-	-	.64	.25	.11	14	33
East Fork	1680	1.00	-	-	.50	.375	.125	.125	.875	-	-	.75	.125	.125	4	12
Case Mountain	1680	.90	.10	-	.50	.40	.10	.20	.80	-	-	1.00	-	-	5	18
Eden Creek	1750	1.00	-	-	.50	.50	-	-	1.00	-	-	.75	-	.25	2	6
Cedar Flat	1580	.75	-	.25	.25	.67	.08	-	1.00	-	-	1.00	-	-	6	-
South Fork	1980	1.00	-	-	.83	.17	-	.17	.83	-	-	.67	.33	-	3	9
Garfield	2060	.92	.08	-	.44	.52	.03	-	.97	.03	-	.92	.08	-	18	54
Mountain Home	1890	.89	.07	.04	.52	.37	.11	.21	.76	-	.03	.85	.14	.01	50	150
McIntyre	1680	.83	.17	-	.50	.17	.33	-	1.00	-	-	.50	.33	.17	3	9
Wheel Meadow	2200	.95	.05	-	.59	.27	.14	-	.91	-	.09	.55	.18	.27	11	35
Black Mountain	1950	.84	.16	-	.54	.45	.01	-	.99	-	.01	.78	.18	.04	37	116
Starvation Creek	1830	1.00	-	-	1.00	-	-	-	1.00	-	-	.50	.50	-	2	6
Packsaddle	1950	.88	.08	.04	.42	.23	.35	-	.92	-	.08	.88	-	.12	13	37
Deer Creek	1830	1.00	-	-	.30	.70	-	-	.80	-	.20	1.00	-	-	5	16

Figure 2 and *Table 3* show that a 'null' allele was detected for each of the four variable loci. These nulls create difficulty, in that they could not be detected in heterozygous embryos in which the null was contributed by the pollen parent. (Nulls contributed by the female parent are detected in the gametophyte tissue.) However, giant sequoia pollen, unlike pine, spruce and fir pollen, has no air bladders. It seems reasonable to assume, then, that giant sequoia pollen does not travel great distances, that pollination is usually by local males, and that male and female allele frequencies in a sample are similar. Using this assumption, we estimated the proportion of misclassified heterozygote embryos in which the pollen parent contributed a null allele. The corrected observed frequency of heterozygosity among embryo samples ($H_{oc(e)}$) was calculated as follows:

$$H_{oc(e)} = H_{o(e)} + \frac{\sum_{i=1}^{k} \sum_{a=1}^{n \neq o} p_{io}\, p_{ia}}{k}$$

where P_{io} is the frequency of the null allele at the i^{th} locus, and p_{ia} is the frequency of all other alleles at the i^{th} locus of the maternal samples.

We applied an additional correction factor for small sample sizes (Kirby 1975) to all estimates of heterozygosity of both the maternal trees and the embryo samples as follows:

$$H_{occ} = (H_{oc})\,(1 - \frac{1}{2N - 1})$$

where N is the sample size for each population. Thus H_{occ} is the observed heterozygosity corrected for errors due to sample size and for misclassified heterozygotes.

Results

OVERALL VARIABILITY. Despite some small sizes, each of the 34 sampled population was variable at one or more loci *(Table 3).* The EST locus is the only one that was variable in all 34 populations samples. The middle range of frequencies found in most of the samples for the EST 'a' and 'b' alleles suggests that this polymorphism may be maintained through selection.

Allele frequencies were heterogeneous among population samples for all four variable loci, and suggest genetic differences between the northern and southern populations. For example, the 'c' allele of GOT 2 was present in 35 percent of the southern population samples (in 6 of 26 gametophyte population samples, and as part of the male contribution in 3 additional embryo population samples), and it was completely absent from samples of all eight northern populations. The 'b' allele of ADH was present in 25 percent (2/8) of the northern population samples and 65 percent (17/26) of the southern population samples.

POLYMORPHIC INDEX (PI) AND GENE DIVERSITY. The polymorphic index (PI) (HAMRICK and ALLARD 1972), and NEI's (1973, 1977) gene diversity ($\bar{H}_S$), are both measures of genetic variability whose magnitudes depend only on allele frequencies. They are independent of mating system and population structure, and are equivalent to the level of heterozygosity in populations whose genotypic arrays are in Hardy-Weinberg proportions.

For populations in which at least 3 standing trees were sampled, the average PI[5]) value for giant sequoias (weighted for sample size) was .140. PI values were not significantly correlated with latitude of the population samples.

Gene diversity (NEI 1973, 1977) is calculated as follows:

$$\bar{H}_T = \bar{H}_S + \bar{D}_{ST}$$

where $\bar{H}_T$ is equivalent to expected heterozygosity averaged over loci of a population whose genotypes are in Hardy-Weinberg (H-W) proportions, without reference to subpopulations; $\bar{H}_S$ is the weighted average over loci of expected heterozygosity of the subpopulations in H-W proportions; and D_{ST} is a measure of interpopulational gene diversity. As a measure of the relative magnitude of genetic differentiation between subpopulations, Nei used the following:

$$\bar{G}_{ST} = \bar{D}_{ST}/\bar{H}_T$$

These statistics are closely related to WRIGHT's (1943, 1951, 1965) F-statistics, which estimate inbreeding. Although Nei's gene diversity analysis was primarily designed to be applied to the average gene diversity among a finite number of subpopulations, it has also been used to estimate the proportions of the total genetic variability associated with differences within and between distinct populations of a species (O'MALLEY *et al.* 1979).

Giant sequoia's average genetic diversity ($\bar{H}_S$) was estimated to be .140. Total genetic diversity ($\bar{H}_T$) of the 30 populations sampled was .155. $\bar{D}_{ST}$ was .015, and $\bar{G}_{ST}$ was .097. Thus, approximately 10 percent of giant sequoia's genetic variation (as measured by our electrophoretic evidence) was between population samples, while 90 percent was within population samples.

GENETIC IDENTITY. We found little evidence of genetic divergence among most giant sequoia population samples. The average genetic identity value (NEI 1972) was .973 ± .01. The lowest identity values involved 2-tree samples, which can be explained as sampling error, and the 6-tree sample from the Merced grove, a population consisting of only about 20 large trees. A comparison of the Merced sample with the nearby Tuolumne sample shows the two to differ substantially from each other and from most other northern groves in their frequencies of the EST 'a' allele (completely absent from the Merced sample), the EST (n + c) alleles, the GOT2 'a' allele, and the GOT3 (n + c + d) alleles. Thus, whereas most giant sequoia groves appear to be very similar to each other genetically, there is some evidence of significant differentiation in these two small, adjacent northern populations.

OBSERVED HETEROZYGOSITY. Observed heterozygosity (including monomorphic loci, and weighted for sample size) of 351 maternal trees averaged 14.3 percent, and 1027 sampled embryos averaged 13.4 percent *(Table 4).* Paired t-tests, which we restricted to populations from which 18 or more maternal trees were sampled (Cabin + Converse Basin, Whitakers + Redwood Mountain, Garfield, Mountain Home, and Black Mountain)[6]), showed that the observed embryo heterozygosity was significantly less than expected heterozygosity (based on maternal allele frequencies) ($P = .02$). Using all populations, observed maternal heterozygosity levels were higher but not significantly different from H-W expected levels ($P = .09$). Observed embryo heterozygosity was lower and almost significantly different from observed maternal heterozygosity ($P = .06$).

Observed heterozygosity levels were significantly and negatively correlated with latitude: $r = -.44$ ($P = .01$) for maternal trees, and $r = -.41$ ($P = .02$) for embryos. Even without the eight northern populations, this trend was apparent for maternal trees ($r = -.37$, $P = .1$).

To test whether these heterozygosity levels are a function of population size and proximity, we compared 7 northern populations (Placer Grove excluded) with the 3 small southern populations that are the most disjunct of the sampled southern populations (Wheel Meadow, Packsaddle, and Deer Creek). A one-way analysis of variance showed these 3 small southern disjunct populations to be significantly more heterozygous than the 7 northern disjunct populations ($P < .05$).

Discussion

Genetic interpretations of family or population differences in seed weight and germination percent cannot clearly be made, since the seeds used were not from plants grown in common-garden conditions. For most species, seed weight appears to have a strong genetic component, even when seeds are developed in field populations (Y. B. Linhart, University of Colorado, personal communication). Thus, the statistically non-significant differences in seed weight among our giant sequoia population samples suggest that most of the genetic variation in this characteristic, if such exists, is between trees within populations.

Our isozyme data show that levels of heterozygosity are generally lower among embryo samples than among ma-

[5]) $PI = \frac{\sum_i \sum_j p_{ij}(1-p_{ij})}{m}$ where p_{ij} is the frequency of the j^{th} allele at the i^{th} locus, and 'm' is the total number of loci (including the monomorphic ones).

[6]) Only these populations were used because we felt that the expected (Hardy-Weinberg) values (based on the allele frequencies of the maternal population samples) would be better estimates than those from the smaller samples. Other comparisons a based on the data from the 29 or 30 populations in which 3 or more trees were sampled.

Table 4. — Comparison of observed and expected levels of heterozygosity among maternal trees and embryos of samples from three or more trees.

Population Name	Population Samples H_e[1]	Maternal Trees N	Maternal Trees $H_{occ(m)}$[2]	Embryos N	Embryos $H_{occ(e)}$[2]	Sign of Difference $H_{occ(m)}-H_e$	Sign of Difference $H_{occ(m)}-H_{occ(e)}$
North Calaveras	.100	16	.091	50	.101	-	-
South Calaveras	.084	14	.080	42	.083	-	-
Tuolumne	.162	10	.158	34	.143	-	+
Merced	.110	6	.126	18	.111	+	+
Mariposa	.115	9	.089	27	.059	-	+
Nelder	.123	12	.106	38	.153	-	-
McKinley	.097	7	.132	21	.083	+	+
Cabin Creek	.151 } .185	9	.139	21	.140 } .164	-	-
Converse Basin	.208 }	13	.209	37	.178 }	+	+
Lockwood	.153	5	.029	12	.129	-	-
Windy	.076	7	.110	21	.089	+	+
Bearskin	.096	6	.075	12	.130	-	-
Grant Grove	.165	11	.202	33	.179	+	+
Redwood Mountain	.151 } .153	25	.164	77	.147 } .147	+	+
Whitakers	.156 }	15	.193	50	.147 }	+	+
Lost	.070	6	.051	18	.061	-	-
Giant Forest	.100	5	.118	9	.157	+	-
Oriole	.137	6	.227	18	.143	+	+
Atwell Mill	.136	14	.149	33	.112	+	+
East Fork	.153	4	.178	12	.094	+	+
Case Mountain	.135	5	.148	18	.110	+	+
Cedar Flat	.108	6	.152	XX		+	X
South Fork	.125	3	.178	9	.104	+	+
Garfield	.111	18	.099	54	.106	-	-
Mountain Home	.177	50	.165	150	.169	-	-
McIntyre	.188	3	.222	9	.209	+	+
Wheel Meadow	.177	11	.230	35	.189	+	+
Black Mountain	.145	37	.111	116	.114	-	-
Packsaddle	.157	13	.173	37	.154	+	+
Deer Creek	.093	5	.148	16	.156	+	-
TOTAL	.140[3]	351	.143[3]	1027	.134[3]	18+ 12-	17+ 12-

[1] H_e is the level of heterozygosity expected in a population in Hardy-Weinberg proportions. It is equivalent in value to PI (Hamrick and Allard 1972), and to $\overline{H}_S$ (Nei 1977).
[2] H_{occ} is the observed heterozygosity corrected for misclassified heterozygotes and sample size.
[3] These figures are weighted averages.

ture-tree samples. This suggests that inbreeding may occur in giant sequoia populations, and that the statistically significant differences between population samples in germination rates may reflect different levels of inbreeding. However, variable germination rates can also be explained by differences in timing of pollination, in the cross- compatibility of parents, and/or in the spatial distribution of pollen-producing trees. Since each of these factors can (or does) vary among populations, it seems likely that various combinations of these causes resulted in the low and variable germination rates that we observed.

The biological significance of differences in cotyledon number is uncertain. However, cotyledons are easily observed, and such differences, likely to be genetically controlled, can contribute to our knowledge of variability between populations (Franklin and Greathouse 1968, Knauf and Bilan 1977, Sorenson and Franklin 1977). The significant differences we found in average cotyledon numbers among giant sequoia populations are consistent with our isozyme data, in that there is a substantial amount of variability among populations, and the northern populations appear to be genetically different from the southern ones. The most northern population (Placer Grove) is unusual in having a relatively high proportion of 5- and 6-cotyledon seedlings.

Cuttings from juvenile giant sequoia root easily, and differences in rooting between populations or families appear to be small. Clonal variation in rooting success was not reported here, but it similarly appears to be small (Fins 1979). Thus, rooted cuttings of giant sequoia may be particularly well-suited for research, or for mass propagation in plantation forestry.

Isozyme analyses

Gymnosperms usually have higher levels of isozyme variability than dicots and monocots (PI = .270, .113, and .165, respectively) (Hamrick *et al.* 1979). Our estimate of isozyme variability in giant sequoia is lower (PI = .140) than reported for most other gymnosperms. We offer three possible explanations:

1) If giant sequoia was once as variable as most other gymnosperms, severe or repeated bottlenecking during its southward and westward migration from Idaho and Nevada to California could have reduced its variability (see Axelrod 1959 for paleobotanic history). Too few generations may have passed since bottlenecking for selection, migration, and mutation to have returned within-population variability to previous levels.

2) Selection may also operate to reduce variation. However, for those of our samples in which $N_{(m)}$ was sufficiently large ($\geq$ 18), the data show a significant difference in average heterozygosity levels between embryo samples and Hardy-Weinberg expected heterozygosity, and suggest a real difference in average heterozygosity between embryos and maternal trees. The data, then, indicate a shift from relatively low embryo heterozygosity to higher levels among maternal trees. Thus, there is no indication, in this data set, of current selection for low levels of heterozygosity. Rather, it appears that there is selection for relatively higher levels of heterozygosity.

3) Biases in the early estimates of variability in other gymnosperms, and/or the small number of loci we investigated for giant sequoia, may have exaggerated the magnitude of the difference. Further studies of giant sequoia

and other gymnosperm species, including many more isozyme (and other) loci, will perhaps revise these early estimates of PI levels and differences.

Natural populations of some plant species have been found to be genetically subdivided into smaller subpopulations (SCHAAL 1975, LEVIN 1977). Studies using isozyme techniques have shown that populations of some tree species are similarly genetically substructured (FERET 1974, GRANT and MITTON 1977). Unrecognized substructing may lead to inaccurate expectations of heterozygosity, based on pooled population allele frequencies (WAHLUND 1928), and our data may suffer from this problem.

KAFTON (1977), in a study of natural stands of Monterey cypress, and BROWN *et al.* (1975), analyzing *Eucalyptus obliqua,* found lower than expected levels of heterozygosity among embryos and suggested inbreeding to be the likely cause. Since giant sequoia often comprises only about 5 percent of the stems in a forest stand, and its pollen has no air bladders to aid in dispersal, it is possible (and perhaps likely) that inbreeding through selfing, or pollination by near neighbors that are relatives, occurs with some regularity. Unfortunately, it is not possible to determine what proportion of the observed excess homozygosity among our embryo samples might have been caused by selfing, inbreeding other than selfing, or undetected population substructure. It seems likely that the embryo and adult populations are similarly subdivided, and if so, the differences in heterozygosity between them indicate that other causes must be affecting the distribution of genotypes. Inbreeding followed by selection for heterozygosity seems a plausible explanation.

Little, if any, gene flow appears to occur between northern and southern populations of giant sequoia, as evidenced by the presence of the GOT 'c' allele in southern populations, and its complete absence from embryo and gametophyte samples from northern populations, and a similar pattern in the occurrence of ADH 'b'. Comparatively high levels of heterozygosity were found in the southern part of the range in both embryos and mature populations. The theory of genetic drift could explain the relatively low heterozygosity found in the isolated northern populations, but the relatively high heterozygosity of three small isolated southern populations (.190 compared to .122 for the other 20 southern samples) suggests that genetic drift alone does not account for the observed differences. The paleontological data are currently insufficient to date the isolation of the populations, or to substantiate suggested migration routes, so no firm conclusions regarding the duration of genetic isolation or the effects of genetic drift can be drawn. We suggest that the observed progressive increase in heterozygosity from north to south might be explained by selective forces. The trend is apparent even without considering the eight northern populations.

Conclusions

The data from these and other studies on wild tree populations suggest that a substantial portion of seeds collected in natural forest stands may be inbred. The inbred seedlings from such collections would probably not survive as natural regeneration in the forest, but in nursery situations they may be kept alive because of reduced competition. Thus, by using wild seed, it is possible that we are planting a substantial number of seedlings destined to die shortly after planting, or that will produce trees with low growth potential and poor form. This argues for collecting seed from seed-orchards in which clearly unrelated individuals are brought into close proximity for the purpose of seed production. The result should be a higher proportion of outcrossed, viable, vigorous seedlings, capable of producing more valuable timber and a healthier planted forest.

In most forest species for which genetic tests have been established, important differences have been found among trees from the same populations and among trees from different geographic areas. The differences we found between giant sequoia populations show that substantial genetic variation is present in this species and that the most northern population is unusual, compared to the other 34 populations sampled. We expect that similar patterns of variation will also be found in morphological and physiological characteristics in the planned common-garden experiments. Careful attention to these patterns will allow us to match populations, families, or clones to appropriate growing sites, and will accelerate our progress toward the domestication and proliferation of this potentially useful California endemic.

Acknowledgements

We gratefully acknowledge our colleagues: M. T. CONKLE, W. B. CRITCHFIELD, R. P. GURIES, D. E. HARRY, J. V. HOOD, F. T. LEDIG, Y. B. LINHART, C. MILLAR, and J. B. MITTON for their valuable critiques and contributions to our manuscript.

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Nachbarschaftswirkungen in jungen Fichten-Provenienzflächen

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(Eingegangen 13. Juli 1981)

Zusammenfassung

Am Material des breit angelegten Hessischen Fichten-Provenienzversuches wird deutlich, daß die Vielfalt der Provenienzunterschiede auch solche der Reaktion auf Nachbarschaftswirkungen einschließt. Diese treten sowohl bei Konkurrenzwirkungen innerhalb von Flächen der Provenienzen als auch bei der Reaktion auf veränderte Wuchsbedingungen an Parzellenrändern auf.

Im Jungwuchs- und Dickungsalter sind sowohl für die Konkurrenzwirkungen innerhalb einer Provenienzfläche als auch für die Randwirkungen keine Provenienzunterschiede festzustellen. Der Anbau in Tief- oder Hochlagen führt ebenfalls zu keiner nachweisbaren Differenzierung zwischen den Provenienzen.

Wenn Fichtenbestände den Bestandesschluß erreicht haben und damit eine Beeinträchtigung des Wuchsraumes der Einzelbäume beginnt, zeichnet sich für die Hochlagen-Provenienzen eine starke Zunahme des Konkurrenzeinflusses innerhalb der Provenienzflächen ab. Dieser ist besonders ausgeprägt, wenn diese Provenienzen in Tieflagen angebaut werden. Bei Tieflagen-Provenienzen treten auch im beginnenden Stangenholzalter derartige Konkurrenzeinflüsse noch nicht klar hervor.

Die Reaktion auf Nachbarschaftswirkungen am Rande von Provenienzflächen zeigt die gleiche Tendenz wie die der Reaktion auf Nachbarschaftswirkungen innerhalb von Provenienzflächen. Im Jungwuchs- und Dickungsalter sind die Mittelhöhen von Parzellenrändern und Parzellenkernen gleich. Mit dem beginnenden Stangenholzalter ist ein gesicherter Einfluß der Randwirkungen festzustellen. Bei getrennter Betrachtung von Hochlagen-Provenienzen und Tieflagen-Provenienzen sind die Parzellenränder signifikant gesichert um etwa 2% höher als die Parzellenkerne. Erfolgt eine Aufgliederung nach kombinierten Provenienzgruppen und Versuchsorten dann ist dieser Unterschied nur für Hochlagen-Provenienzen auf Tieflagen-Versuchsorten gesichert nachweisbar. Damit wird die bei den Konkurrenzwirkungen innerhalb der Parzellen bei Hochlagen-Provenienzen festgestellte früher einsetzende Differenzierung bestätigt.

Bei der Auswertung von Fichten-Versuchen ist im Jungwuchs- und Dickungsalter ein Ausscheiden von Randreihen nicht erforderlich. Mit Beginn des Stangenholzalters tritt eine Zunahme der Durchschnittshöhe der Randreihen gegenüber derjenigen der Parzellenkerne ein, die jedoch für alle Provenienzen einheitlich und zunächst gering ist. Eine Beschränkung der Auswertung auf die Parzellenkerne erscheint daher bei Fichtenversuchen mit größeren Parzellen erst mit Beginn des starken Stangenholzalters notwendig. Bei kleineren Parzellengrößen (unter 50 Bäumen) üben die Randwirkungen jedoch einen so großen Einfluß aus, daß bereits im Stangenholzalter eine Ausscheidung der Randbäume erforderlich wird.

Schlagworte: Fichtenprovenienzen, Nachbarschaftswirkungen innerhalb und zwischen Parzellen, Parzellengröße

Summary

Evaluation of the comprehensive Hessian Norway Spruce Provenance Trial shows that differences between provenances exist also with respect to reaction against effects of neighbourhood. These become apparent when investigating competition within plots as well as modification of growth conditions at plot borders.

During the sapling stage no differences related to provenances can be observed with respect to competition within plots and effect of border conditions. Neither causes cultivation at low or high elevation a measurable differentiation between provenances.

When Norway Spruce stands reach the age of crown closure and consequently competition between individual trees starts, a considerable increase of the effects of competition within the plots can be observed for high-elevation-provenances. This becomes specifically apparent when theses provenances are cultivated at low elevation. For low-elevation-provenances such influences are not apparent even at the small pole stage.

The reaction on influences of neighbourhood at the plot borders shows the same trends as that on competition within provenance plots. During the sapling stage average heights of centre plot and border areas are equal. At the beginning of the pole stage a significant border effect can be observed. Assessing high-elevation-provenances and low-elevation-provenances separately the average height of border trees exceeds significantly that of the plot centre heights by 2%. If the material is classified according to the combination of provenance and cultivation groups this difference is significant only for high-elevation-provenances cultivated at low elevation. This confirms that fact of early differentiation of high-elevation-provenances already observed with respect to competition within plots.

Deletion of border tree rows is not necessary for the evaluation of Norway Spruce trials during the sapling stage. With the beginning of the small pole stage the average height of the border tree rows exceeds that of the plot centres, but this superiority is equal for all provenances and small at the beginning. Deletion of the border tree rows seems, therefore, to be necessary for Norway Spruce trials with larger plots only after beginning of the big pole stage. If smaller plots are used (less than 50 trees) the influence of border effects is such that already at the small pole stage deletion of border tree rows is required.

Key words: Norway Spruce provenances, effects of neighbourhood within and between plots, size of research plots.

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