Levels of Outcrossing in Two Loblolly Pine Seed Orchards[1])

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Summary

Outcrossing was estimated using allozymes in two seed orchards of loblolly pine *(Pinus taeda* L.). Unique markers, a single-locus mixed mating model, and a multilocus model were used. All the methods used agreed that outcrossing was very close to 1; the unique marker estimate was .982 (± .006), the single locus estimate .992 (± .007) and the multilocus model .994 (± .007). Based on the multilocus model, there was significant ($p < .05$) heterogeneity among clones in outcrossing rate in only one of the three years. Also based on the multilocus model, there were no significant differences among the rates of outcrossing in the three years sampled, or between the two orchards in any year.

Key words: Pinus taeda L., seed orchards, allozymes, inbreeding.

Zusammenfassung

In zwei *Pinus taeda* L.-Samenplantagen wurde anhand von Allozymen die Fremdungsrate bestimmt. Markergene, ein "single-locus mixed mating" Modell und ein Mehrfachlocus-Modell wurden zur Schätzung der Fremdungsrate benutzt. Alle angewandten Methoden lassen erwarten, daß die Fremdungsrate nahe bei 1 liegt. Markergene ergaben einen Schätzwert von 0,982 (± 0,006). Schätzungen anhand eines Genlocus betragen 0,992 (± 0,007) und anhand mehrerer Genloci 0,994 (± 0,007). Zwischen den Klonen bestand nur in einem der drei Saatguterntejahre signifikante Heterogenität (p 0,05) bezüglich der Fremdungsrate basierend auf dem Mehrfachlocus-Modell. Weder zwischen den drei Saatguterntejahren, noch zwischen den beiden Samenplantagen bestanden signifikante Unterschiede in den Fremdungsraten. Dieses Ergebnis wurde ebenfalls anhand des Mehrfachlocus-Modells abgeleitet.

Introduction

The genetic efficiency of seed orchards has been defined as the degree to which orchard seed crops reflect the genetic superiority and variability present among orchard clones. One of the potential problems in open-pollinated seed orchards is self pollination. In the pines, depression in a wide variety of seedling traits occurs under inbreeding, and self-fertilization is the most severe form of inbreeding. In loblolly pine, the effect of selfing on seed yield is a five-fold increase in empty seed after self-fertilization relative to that found after cross-fertilization (FRANKLIN 1968), and even filled seed often fail to germinate. Therefore, selfing can be a problem in maximizing seed production efficiency in seed orchards even if no self-fertilized progeny survive to the seedling stage. Self-fertilized progeny which do germinate successfully usually exhibit inbreeding depression, which may be carried to field plantings since nursery practices may allow more selfed seedlings to survive than under natural conditions (ERIKSSON and LINDGREN 1975).

Because most self-fertilized seeds are empty, the proportion of selfed progeny which survive to germination is usually much lower than the proportion of self-fertilizations which occur. For example, in an old-field stand of loblolly pine, the frequency of natural self-fertilization in the upper crowns was estimated to be 7 percent, while the frequency of selfed progeny was 1.75 percent (FRANKLIN 1968).

Several estimates of the outcrossing rate (t) in seed orchards have been obtained using allozymes. In three studies of different Scots pine *(Pinus sylvestris* L.) seed orchards, the proportion of outcrossed progeny was estimated to range from 0.874 to 0.977 (RUDIN and LINDGREN 1977, MÜLLER-STARCK 1979, SHEN *et al.* 1979). In these studies, outcrossing estimates were based on single clones which carried unique allozyme markers. A maximum likelihood estimator of t, which does not require the presence of unique markers, was used to estimate a mean t value of 0.90 in three seed crops in a Monterey pine *(Pinus radiata* D. Don) seed orchard (MORAN *et al.* 1980). A similar maximum likelihood method was also used to estimate a mean t of 0.91 in a seed orchard of Douglas-fir *(Pseudotsuga menziesii* [MIRB.] FRANCO) (SHAW and ALLARD 1982). Based on previous studies, then, from two to ten percent of seed orchard crops may result from self-fertilization.

Work done by SORENSON (1970) and SHAW and ALLARD (1982) indicates that clones may vary in their outcrossing rates. Based on a seed sample of five clones in two adjacent loblolly pine orchards, each with unique allozyme markers, ADAMS and JOLY (1980 a) found an average of 98.8 percent outcrossed progeny, and no evidence that outcrossing rates varied among clones, although sample sizes were small (range 60—80).

The purpose of this investigation was to increase the number of clones and seed sampled by ADAMS and JOLY (1980 a), to determine whether outcrossing varied by orchard or by year, and to compare different methods for estimating outcrossing.

Materials and Methods

Two loblolly pine seed orchards owned by Champion International near Newberry, S. C. were chosen for this study. Clones were selected from natural stands and placed in either the high specific gravity (HSG) or low specific gravity (LSG) orchard based on the wood specific gravity of the ortet. Most of these selected ortets were located within 50 km of the seed orchard site, and the most distant ortet was approximately 110 km from the orchards. Each orchard is approximately two hectares in size, and separated from the other by a 100 m wide strip which includes a virginia pine *(Pinus virginiana* MILL) seed orchard. The grafts were an average of 15 years old at the time of sampling and had been in full pollen and seed production for several years.

The seed used in this study was the result of wind-pollination during the springs of 1974, 1975 and 1977. There were 301 ramets of 23 clones in the LSG orchard, and 300 ramets of 27 clones in the HSG orchard in spring 1974. These were reduced to 202 and 183 ramets, respectively, for the 1975 and 1977 pollinations. After roguing, 1 to 39 ramets of each clone remained in 1974 and 1 to 25 in 1975 and 1977. Cone samples were collected in the fall of 1975,

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1976 and 1978. Cones were picked from the entire tree and placed at the base of the tree. Sampled cones were chosen at random from these. Seedlots from each ramet were kept separate. Ramets were chosen to sample ramets of different clones located throughout the orchard, and to sample some ramets in each of two years. Approximately 36 ramets of 25 clones in both orchards were sampled in each of 1975 and 1976, while 7 ramets of 7 clones were sampled in 1978. Trees in the surrounding natural stands outside the orchard were also sampled to obtain a population estimate of outcrossing under natural stand conditions.

By electrophoresis of both megagametophyte and embryo tissue, the genotype of the male gamete which fertilized an ovule can be inferred. Thus, gene frequencies in the pollen pool of a ramet can be obtained; i.e., the frequency of alleles carried by male gametes effective in fertilizing the ovules of a given ramet. Electrophoresis was accomplished according to the procedures described by ADAMS and JOLY (1980 b) on approximately 100 seed (megagametophyte and embryo) per ramet. Seven loci were chosen for use because they could be clearly distinguished in both megagametophyte and embryo tissue: GDH (glutamate dehydrogenase, E.C. 1.4.1.2), one locus of LAP (leucine aminopeptidase E.C.3.4.11.1), one locus of PGI (phophoglucose isomerase, E.C.5.3.1.9), one locus of GOT (glutamate oxaloacetate transaminase E.C.2.6.1.1), 6-PGD (6-phosphogluconate dehydrogenase, E.C.1.1.144), and two loci of PGM (phosphoglucomutase E.C.2.7.5.1).

Estimation Procedures

Three procedures for estimating the proportion of outcrossed progeny were used in this study. They include unique markers, a single locus mixed mating model, and a multilocus model. Both the unique marker method and the multilocus model allow estimates of outcrossed progeny in individual ramets and clones, while only population estimates can be obtained using the single locus mixed mating model.

Unique markers

The first and simplest procedure for estimating the proportion of outcrossed progeny is the use of allozyme markers unique to specific clones. These markers can be single alleles or combinations of alleles. If p equals the proportion of progeny in which the marker can be detected (for example, p = .5 for a clone heterozygous for a marker allele) then $\hat{t} = 1 - (x/pn)$, where t is the proportion of outcrossed progeny, and x is the number of observed markers out of n progeny sampled. The variance of t is then:

$$\text{var}(\hat{t}) = \frac{(1-\hat{t})\ [1-P(1-\hat{t})]}{pN}$$

Single locus mixed mating model.

The single locus mixed mating model was developed by BROWN and ALLARD (1970) to obtain an average estimate of the proportion of outcrossed progeny in a population. When this model is used, mating events are assumed to be either the result of outcrossing and random mating with probability t, or self-fertilization with probability s. The assumptions of this model are that an excess of self-fertilization of this only factor which may cause the system to deviate from random mating, that t is uniform for all maternal genotypes and pollen alleles, and that pollen gene frequencies are uniform over all maternal genotypes. If these assumptions are accepted, the probability of a certain genotype occurring in the progeny of a female of a certain genotype can be described using only two variables, t (s = 1 − t) and p (the pollen pool frequency of a certain allele). For example, if the maternal genotype at a locus is A_1A_1, the probability of finding an A_2A_2 progeny is zero, the probability of finding an A_1A_2 progeny is tq (the outcrossing rate times the frequency of allele A_2 in the pollen pool), and the probability of finding an A_1A_1 progeny is s + tp (the rate of selfing (s) plus the rate of outcrossing times the frequency of allele A_1 in the pollen pool). For the joint estimation of two parameters (t and p), data from at least two different maternal genoypes are required.

In his study, maximum likelihood estimates of p and t were found using methods similar to those described in BROWN and ALLARD (1970), except that female genotypes were known without error from ADAMS and JOLY (1980 a).

The single locus model, then, provides an overall estimate of outcrossing in a population for each locus separately, based on maternal genotypes and progeny arrays at one locus. If the above assumptions are violated by, for example, differential outcrossing rates by genotype, by differential selection between fertilization and measurement, or by heterogeneity of pollen pools, the estimates of t are expected to vary over loci.

Multilocus model

While single locus estimates of outcrossing require the assumptions mentioned above, the use of multiple loci to estimate t should provide a method less susceptible to single-locus confounding effects (SHAW *et al.* 1981). This method involves dividing the progeny of a parent into two classes, those which are identifiable outcrosses (i.e., those which possess genotypes which could not have resulted from selfing) and those which are non-discernible from selfs, a class composed of both true selfs and multilocus genotypes which cannot be distinguished from selfs. The proportion of discernible outcrosses is therefore a lower limit to the estimate of t. To get a better estimate of t, the proportion of non-discernible outcrosses is corrected (divided) by the probability of an ovule being fertilized by a pollen grain carrying a non-discernible genotype. This probability would be based on the frequency of such multilocus genotypes in the pollen pool. A multilocus estimator of t is then:

$$\hat{t} = x/n(1-a)$$

where x is the number of observed outcross progeny, n is the total number of progeny sampled, and a is the probability of a non-discernible outcross in the pollen pool (SHAW *et al.* 1981). Although this estimator was originally developed for estimating an overall average outcrossing rate from populations, we were interested in obtaining estimates for each individual clone (t_i) so that:

$$\hat{t}_i = x_i/n_i(1-a_i). \qquad (1)$$

In this case, x_i is the number of observed outcross progeny, and n_i is the number of progeny in the sample from clone i. The probability of a male gamete in the outcross pollen pool of clone i possessing a genotype which cannot be identified as different from the genotype of clone i is a_i. If the loci are independent, then a_i is simply the product of the frequencies in the overall pollen pool of the allele or alleles possessed by clone i over all loci. The loci used in this study are not linked, and disequilibrium is probably not

important (Shaw *et al.* 1981). The frequencies of the alleles in the pollen pool were estimated by summing the alleles in the pollen pools of all sampled ramets. This was done separately for each orchard each year.

In Shaw *et al.* (1981) a is treated as a constant, and the variance of t is given as:

$$\operatorname{var}(\hat{t}) = \frac{\hat{t}\,(1-t(1-a))}{n\,(1-a)} \qquad (2)$$

This variance on t can be considered a lower bound estimate on the variance of t. Since a is actually a random variable and has an associated variance, it is more correct to calculate the variance on t taking the variance on a into account. If $x_i/n_i = q_i$, then, from equation (1):

$$\hat{t}_i = q_i/(1-a_i)$$

$$\text{and } \operatorname{var}(\hat{t}_i) \simeq \frac{dt_i}{dq_i}^2 \operatorname{var}(q_i) + \frac{dt_i}{da_i}^2 \operatorname{var}(a_i) + 2\operatorname{cov}(q_i,a_i)\,\frac{dt_i}{dq_i}\,\frac{dt_i}{da_i}$$

$$\text{where } \frac{dt_i}{dq_i} = \frac{1}{1-a_i}$$

$$\text{and } \frac{dt_i}{da_i} = \frac{q_i}{(1-a_i)^2}$$

$$\text{so that } \operatorname{var}\hat{t}_i = \frac{1}{(1-a)^2}\operatorname{var}(q_i) + \frac{q_i^2}{(1-a_i)^4}\operatorname{var}(a_i) + \frac{+2\,q_i\operatorname{cov}(q_i,a_i)}{(1-a_i)^3}$$

where the variances of q_i and a_i are sampling variances.

In this study the pollen pool frequencies of all ramets were combined into an orchard pollen pool from which the a_i for each clone was estimated. Thus, the pollen pool samples used to estimate q_i (the pollen pool of a clone) and a_i (the pollen pool of all clones in the orchard) were not independent, and a covariance between q_i and a_i can be expected to exist.

For a similar situation, Omi (1982) compared variances based on gene frequencies independent of the clonal sample to those based on the total sample gene frequencies. The independent gene frequencies were obtained by using only the combined sample of all other clones in the orchard to estimate a_i's, while the total sample used all clones including the clone for which t was being estimated. Omi concluded that the covariance term of q_i and a_i was very small; therefore, this covariance term was dropped from the formula used to calculate a variance on the estimate of t. Hence, the following terms were added to equation (2) by including a variance on a_i: var (a_i) $(q_i^2)/(1-a_i)^4$.

Results

Unique marker estimates

Unique markers were available to estimate the proportion of outcrossed progeny in six clones, three from the LSG and three from the HSG orchard *(Table 1)*. Estimated t values were not significantly different ($p > .05$) based on a chi-square heterogeneity test weighted by variances, and were very close to 1. A weighted mean of .982 ± .006 was obtained by pooling estimates on five clones and inversely weighting by the variances. An unweighted mean of .972 was obtained based on all six clones. The weighted and unweighted estimates were therefore, very similar. In addition because the actual counts of selfed progeny were low (most were less than 5) Poisson confidence intervals were calculated to obtain estimates of the range over which t might occur *(Table 1)*.

The numbers of markers observed for each clone were obtained by combining counts of markers over all sampled ramets of each clone in each of two years. There were three clones for which it was possible to obtain t estimates in both the 1975 and 1976 seed years. The chisquare test was used on actual counts of markers to detect differences between years for the same clone. Of the three clones, only one clone exhibited significant heterogeneity ($p < .05$, X^2

Table 1. — Estimates of the frequency of outcrosses (t) in the progeny of loblolly pine seed orchard clones based on unique allozyme markers.

		Marker			1975 and 1976 Combined			
Orchard	Clone	Genotype	Expected* Frequency	Observed Selfs	N	t	s.e.(t)	Poisson 95% Conf. Limits
LSG	10	PGM2-1	0.50	6	430	0.972	0.028	0.939-0.990
	17	6PGD-3 and GOT2-1	0.25	2	339	0.976	0.024	0.915-0.997
	20	6PGD-1 and GDH-2	0.25	3	146	0.918	0.082	0.760-0.983
HSG	33	GDH-4	0.50	1	80	0.975	0.025	0.861-0.999
	36	LAP2-3	0.50	1	237	0.992	0.008	0.953-1.00
	46	6PGD-6	0.50	0	71	1.00	---	0.896-1.00
				Weighted mean		0.981[1]	0.006	
				Unweighted mean		0.972		

* Expected frequency of the marker in the male gametes ofselfed pollen.

[1] This estimate does not include clone 46.

heterogeneity test) in the number of markers observed from one year to the next. This is partially due to the fact that different ramets were sampled in each year's sample. Of the two ramets sampled in both years, there were significant differences between ramets ($x^2 = 6.11$ (1 d.f.) $p < .05$). 10C was observed to have significantly more observed selfed progeny than ramet 10A over years. This observation suggests that proximity to other ramets of the same clone may increase the production of self-fertilized progeny. In this case, ramet 10C is located within 15 meters of two other ramets of clone 10 while neither ramet 10A nor 10B (with no observed selfed progeny) have ramets of clone 10 within 15 meters.

Single locus estimates

Estimates of t were possible using from 3 to 7 loci in each of two years *(Table 2)*. Estimates at all loci were not possible because the necessary maternal genotypes were not sampled or did not exist in each orchard, or each year. Estimates of t ranged from .92 to 1.05, but only in one of the four orchard-year combinations (HSG 1976) were estimates heterogeneous over loci within a given orchard and year. Outcrossing estimates greater than 1 may be due to sampling error, or to some type of assortative mating. In 1975, the mean t estimate in each orchard differed significantly from 1. If the heterogeneity of the HSG 1976 estimates were ignored and the estimates pooled, neither the LSG nor the HSG 1976 differed significantly from 1. The difference between years for t estimates pooled over orchards was statistically significant (.97 vs. 1.02). A pooled overall estimate of t over orchards and years was .992 ± .007. This was in very close agreement with the estimate based on unique markers (.982 ± .006).

While three of the four orchard-year combinations did not exhibit significant heterogeneity among loci, the estimates for each locus ranged from .92 to 1.04 in the LSG in 1975, from .92 to 1.00 in the HSG in 1975, from .95 to 1.05 in the LSG in 1976, and from .94 to 1.12 in the HSG in 1976. These differences suggest that some of the assumptions of the single locus model are violated in these seed orchards.

Multilocus estimates

An estimate of the proportion of outcrossed progeny for each clone sampled in 1975 and 1976 was obtained. Of the 13 clones sampled in the LSG in 1975 and 1976, only clone 16 exhibited an estimated t significantly different from 1 and then only in 1975 (.94 in 1975, and 1.06 in 1976). Clone 26 was the only clone of the 11 in the HSG in 1975, and the 12 in 1976, to have an estimated t (.89) significantly less than 1. Clone 26 was not sampled in 1975, so that year-to-year variation could not be sampled for this clone. Estimates of t exhibited significant heterogeneity among clones, based on Fisher's weighted variance heterogeneity test, only within the LSG orchard in 1975. Therefore, estimates were pooled over clones. Pooled estimates of the proportion of outcrossed progeny for both orchards for 1975, 1976, and 1978 were once again very high *(Table 3)*. There were no significant differences between years, as in the estimates from the single locus model; nevertheless, the pooled t estimate was again lower for 1975 than for 1976 (.980 for 1975, 1.00 for 1976, and 1.01 for 1978). The pooled t estimate over orchards and years was .994 ± .007. The natural stand t estimate using this model was .937, which was significantly different from an outcrossing rate of 1. ($X^2 = 7.21$, 1 df).

The variances on the t_i estimates were also calculated without the variance on a_i and compared to those which included the a_i variance. The use of the variance on a_i caused a very small increase in the total t_i variance. Over the range of sample sizes used in calculating a_i (2164 to 812), an increase in sample size alone did little to decrease the variance on t. On the other hand, the value of a_i itself had a strong effect on the precision of the t estimate. This is apparent from the fact that a_i is the probability that an outcross genotype cannot be differentiated from a maternal genotype for a given clone. As a result, variances on t for different clones may vary widely depending on the genotypes of the clones. Consequently, the best way to increase the precision of t estimates is to use as many loci, with as many alleles, as possible.

Discussion

All the methods used to measure the rate of outcrossing produced equivalent results. The pooled estimates of t were .992 for the single locus model, .996 for the multilocus model, and .982 using unique markers. None of these estimates was significantly different from 1, or complete out-

Table 2. — Single-locus estimates of the proportion of outcrosses (t) in the progeny of two loblolly pine seed orchards in two seed crop years.

		Locus							Weighted mean over loci		
Year	Orchard	GDH	LAP	PGI	GOT	6PGD	PGM-1	PGM-2	t^2	SE_t	X^2 (df)[1]
1975	LSG	1.035	0.924	0.961	0.955	0.981	--	0.984	0.972	0.012	6.93(5)
	HSG	0.917	0.940	1.000	0.950	0.987	--	--	0.964	0.017	2.54(4)
Pooled over orchards									0.969	0.010	0.13(1)
1976	LSG	1.011	1.027	1.046	0.980	1.018	1.021	0.945	1.020	0.011	5.48(6)
	HSG	1.123	0.957	--	0.939	0.958	--	--	0.994	0.024	9.75(3)*
Pooled over orchards									1.016	0.010	1.02(1)
Pooled over years									0.992	0.007	10.05(1)*

[1] [2] Heterogeneity test of heterogeneity of t estimates over loci.
[2] Pooled using Fisher's weighted variance method.
[3] Significantly different from $t = 1$, $p < 0.05$.
* $p < 0.05$.

Table 3. — Estimates of frequency of outcrosses (t) in the progeny of loblolly pine seed orchard clones (pooled over clones) based on the multilocus model.

Seedcrop	Orchard	Sampled no. clones	Samled no. ramets	Sampled no. seed	t	SE_t	X het(df)[1]
1975	LSG	13	24	2,164	0.985	0.014	21.44(12)*
	HSG	11	14	1,325	0.975	0.015	7.52(10)
	Pooled over orchards			3,489	0.980	0.010	0.18(1)
1976	LSG	13	24	2,131	1.011	0.012	14.86(12)
	HSG	12	12	872	0.978	0.021	11.89(11)
	Pooled over orchards			3,003	1.00	0.010	1.93(1)
1978	LSG	4	4	289	1.020	0.038	2.32(3)
	HSG	3	3	264	1.010	0.030	3.76(2)
	Pooled over orchards			553	1.014	0.024	1.24(1)
TOTAL (pooled over years)				7,045	0.994	0.007	2.79(2)

[1] Fisher's weighted variance heterogeneity test for heterogeneity among clonal estimates.
* Heterogeneity among estimates significant at $p < 0.05$.

crossing. Since the multilocus estimate was based on 24 clones in 1975 and 25 clones in 1976, approximately 50 percent of all the clones in the orchards, it is reasonable to assert that this is a good approximation of the general outcrossing rates in these orchards. These results agree with the smaller sample of ADAMS and JOLY (.988, 1980 a). They are also very close to FRANKLIN's (1968) estimate for the upper crowns of wild stands (.983). Since cones in this study were sampled at random with respect to crown position, these estimates should be an average of the entire tree crown. The fact that these seed samples yield estimates similar to FRANKLIN's for upper crowns suggests that the relatively low density of trees in seed orchards, and open areas between trees, may be the explanation for the lower rates of selfing and selfed seed production in seed orchards compared to the lower crowns in FRANKLIN's old-field loblolly stand.

The methods used, while yielding equivalent results, require different assumptions. Estimates based on unique markers require the assumption that the markers are indeed unique; that, in fact, no markers from other sources will enter the pollen pool. This is more likely to be the case in a relatively discrete population, such as a seed orchard, than in a sample of trees taken from a continuous natural stand. On the other hand, the presence of pollen contamination from outside the orchards could lower the estimates of outcrossing. In these orchards, all alleles found in the orchards were also found in natural stands outside the orchards. The only exception was LAP2-3, found in clone 36, which had an estimated outcrossing rate of .992 based on this unique marker. No doubt this marker as well was found outside the orchards, but it was not detected in our sample. Another difficulty with the unique marker method is that rare alleles and allelic combinations can be found in only a few of the clones studied; this is a disadvantage if an overall population estimate of t is desired.

The single locus method requires the assumptions that pollen pools are homogeneous over all maternal plants, and that the rate of outcrossing is independent of maternal genotypes. As described above, at least in some instances (LSG 1975), the rate of outcrossing varied by clone. Also in these seed orchards, significant differences in the gene frequencies in the pollen pools of different clones were observed (FRIEDMAN, in preparation). If many clones of each genotype are examined, these effects might be expected to average out over genotypes. If the number of clones per genotype is relatively few, however, heterogeneity among different single locus estimates is likely to occur. In this case, often only a few clones possessed a given maternal genotype. The effects of differential self-fertilization by clone, as well as different gene frequencies in pollen pools by clone, are the most likely explanations for the heterogeneity among estimates based on different loci which was observed to be statistically significant in the HSG orchard in 1976.

The assumption of no differential self-fertilization by clone is not required when the multilocus model is used to estimate outcrossing on a clonal basis. These clonal estimates were then pooled to obtain a population estimate. The assumption which is added to those of the single locus model, that the loci used are independent, is easily tested. The multilocus model does also require the assumption of pollen pool homogeneity, but violation of this assumption should have a smaller effect on multilocus estimates than on the single locus estimates (SHAW and ALLARD 1981).

The single locus and multilocus estimates can be compared to obtain an estimate of other forms of inbreeding besides selfing (SHAW and ALLARD 1982). However, in clonal seed orchards there should be no family structure, and indeed, in these orchards estimates are equivalent for the single locus and multilocus models. These results are in agreement with those of SHAW and ALLARD (1982) when comparisons were made between the single locus and multilocus estimates in seed orchards of Douglas-fir.

Based on results from the single-locus model, the level of outcrossing increased significantly between years after

the orchard was reduced in density. The multilocus model yielded a similar trend but the difference was not significant. The outcrossing estimates are so close to 1 that the differences would have little practical significance, even if they were statistically significant. The possibility that factors in orchard management may cause increased levels of outcrossing is supported by the fact that average outcrossing estimates in the background stands of loblolly were lower than those found in these seed orchards. As more studies of this type are done in seed orchards, the effects of number of clones, density, design, species, and pollen production inside and outside the orchards are likely to be further elucidated. The one example mentioned above of decreased outcrossing with increasing proximity of ramets of the same clone suggests that present practices of separating ramets, of the same clone in seed orchards are effective. At the high levels of outcrossing found in this study, the presence of self-fertilized progeny is not a problem in the crops from these seed orchards. However, selfing may still be a problem, however, due to its effect on the production of filled seed.

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Serotaxonomical Investigation of the European Pine Species

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Summary

Studies on serological similarity of European subgenus *Pinus* pine species have disclosed two groups of species. The first group includes four serologically very similar taxa i.e. *Pinus sylvestris, P. mugo, P. uliginosa* and *P. uncinata*. With it *P. nigra* and *P. leucodermis* are linked, but exhibiting significant serological specificity from the *sylvestris-mugo-uliginosa-unicinata* group. The second group of serologically similar species includes *P. brutia, P. eldarica* and *P. pinaster*. The three species are joined loosely by *P. halepensis* and more loosely by *P. canariensis* and *P. pinea*. Two species from subgenus *Strobus, P. cembra* and *P. peuce* are serologically very different from the studied species of subgenus *Pinus*.

Key words: Subgenus *Pinus*, serological diversity, hybridization, taxonomy, proteins.

Zusammenfassung

Serologischen Untersuchungen nach, weisen die europäischen Arten der Untergattung *Pinus* zwei verschiedene Gruppen auf; die erste von ihnen enthält vier serologisch sehr ähnliche Taxa: *Pinus sylvestris, P. uncinata, P. uliginosa* und *P. mugo*. Mit dieser Gruppe sind auch *P. nigra* und *P. leucodermis* eng verwandt. Diese sind aber serologisch von den vier oben erwähnten Arten ziemlich verschieden. Die zweite Gruppe mit serologisch ähnlichen Arten schließt *P. brutia, P. eldarica* und *P. pinaster* ein. *Pinus halepensis* zeigt eine gewisse Ähnlichkeit mit diesen Arten, *P. pinea* und *P. canariensis* können als gesondert stehende Arten der zweiten Gruppe betrachtet werden.

Die zwei europäischen Arten der Untergattung *Strobus, P. cembra* und *P. peuce*, sind von den oben genannten Arten serologisch sehr verschieden.

1. Introduction

Genus *Pinus*, the most numerous one in the *Pinaceae* family, presents a number of interesting taxonomical problems. The genus includes over 100 species and speciation processes continue to take place particularly intensely in Mexican and Central American mountains and less obviously so in South-East Asia. Hybridization irregularities in the genus are of interest from the taxonomic point of view. Many pine species even belonging to distant taxonomic groups cross easily with each other forming a num-