Verifying Controlled Crosses in Conifer Tree-improvement Programs[1])

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Summary

Controlled crosses are basic to the breeding strategy of most applied tree-improvement programs, but a crossing program must be carefully conducted in order to prevent pollen contamination and avoid mislabeling. The validity of controlled crosses can be assessed by comparing allozyme genotypes of seeds with those expected from the genotypes of the putative parents.

We analyzed seedlots from 43 two-parent crosses of Douglas fir *(Pseudotsuga menziesii* var. *menziesii* (Mirb.) Franco] and 30 two-parent crosses of loblolly pine (*Pinus taeda* L.) . A surprisingly high proportion of these crosses (30.2% in Douglas-fir and 33.3% in loblolly pine) were *Invalid,* because two or more progeny differed from expectation. Errors were primarily on the paternal side of the cross (*e.g.*, pollen contamination). Most *Invalid* crosses could have been detected by analyzing 6 to 10 allozyme loci in samples of as few as five seeds. Because the high levels of error observed in this study may exist elsewhere, we suggest that all applied tree-improvement programs could benefit from surveys of the genetic integrity of breeding populations.

Key words: Controlled crosses, allozymes, tree breeding, Douglas-fir, loblolly pine, genetic markers.

Zusammenfassung

Kontrollierte Kreuzungen sind die Grundlage von Züchtungsstrategien der meisten angewandten Züchtungsprogramme. Kreuzungsprogramme müssen jedoch sorgfältig durchgeführt werden, um eine Pollenkontamination zu verhüten und um einer falschen Kennzeichnung der Kreuzungspartner vorzubeugen. Die Durchführung von kontrollierten Kreuzungen kann überprüft werden, indem Allozymgenotypen von Samen mit den Genotypen der vermeintlichen Eltern verglichen werden.

Wir analysierten Samenproben von 43 Kreuzungen von *Pseudotsuga menziesii* var. *menziesii* (Mirb.) Franco und 30 Kreuzungen von *Pinus taeda* L.. Ein überraschend hoher Anteil dieser Kreuzungen (30,2% bei Douglasie und 33,3% bei Weihrauch-Kiefer) wurde inkorrekt durchgeführt, da zwei oder mehr Nachkommen von den Erwartungn abwichen. Die Fehler lagen primär auf der Elternseite der Kreuzungen (z. B. Pollenkontamination). Die meisten „fehlerhaften" Kreuzungen hätten bei der Analyse von 6 bis 10 Allozym-Loci in Proben von weniger als 5 Samen entdeckt werden können. Weil die in dieser Untersuchung beobachteten hohen Fehlerquoten auch in anderen Untersuchungen existieren können, wird vorgeschlagen, daß alle Baumzüchtungsprogramme von einer Kontrolle der genetischen Identität der Züchtungspopulationen profitieren können.

Introduction

The breeding strategies of most intensive tree-improvement programs involve controlled crossing of selected parents. Until recently, it was necessary to assume that these crosses were made with little or no error, since no accurate method was generally available to test their validity. It is now possible to assess the accuracy of controlled crosses rapidly and efficiently (Adams, 1981, 1983) by electrophoretically resolving large numbers of simply inherited genetic markers (allozymes) in conifer seeds (*e. g.*, Rudin and Ekberg, 1978; Adams and Joly, 1980; Eckert *et al.*, 1981; Conkle *et al.*, 1982; El-Kassaby *et al.*, 1982).

In this paper we 1) discuss the use of allozymes to test the validity of controlled two-parent crosses, 2) show how to use the array of allozymes observed in seed tissues to help diagnose the cause of errors in controlled crosses, and 3) survey the accuracy of controlled crosses in a sample of applied tree-improvement programs for Douglas-fir [*Pseudotsuga menziesii* var. *menziesii* (Mirb.) Franco] and loblolly pine (*Pinus taeda* L.).

General Approach to Assessing Validity of Controlled Crosses

Assaying variable allozyme loci in seed tissues is a straightforward way to determine the validity of controlled crosses. The allozyme genotypes of the putative parents are determined either directly, by assaying vegetative tissues in the parent trees (Mitton *et al.*, 1979; Neale *et al.*, 1984) or indirectly, by inferring parental genotypes from progeny arrays in seed samples (Adams, 1983). (Seeds used to determine parental genotypes should come from sources other than the cross to be tested.) These genotypes are compared with those of the progeny embryos. If the progeny genotypes differ from expectation, the cross is invalid. If the progeny genotypes match expectations, the validity of the cross may still be in doubt, since other parents could carry the same alleles as the putative parents. However, the ability to discriminate valid from invalid crosses increases as the number and variability of loci analyzed increase.

Inferring parental genotypes from conifer seeds is simplified by the presence of the haploid (1n) nutritive tissue (megagametophyte). The diploid genotype of a tree can be inferred from the alleles in a sample of megagametophytes. Errors in genotype identification occur only when the megagametophyte sample does not include both allelic variants at heterozygous loci. But, for a sample of n megagametophytes, the probability (p) of misidentification at any one locus is less than $(1/2)^{n-1}$. For example, p is less than 0.03 when n = 6 and less than 0.01 when n = 8.

Analyzing the validity of controlled crosses in conifers is aided by the ability to determine the haploid genotypes of the male and female gametes forming each embryo. Because the megagametophyte and egg of a conifer seed are genetically identical, pollen sperm genotypes can be inferred when both megagametophyte and embryo genotypes are known. Thus, it is possible to tell whether a cross is invalid, and also whether errors in the seed parent, the pollen parent, or both are involved. The collective eggs and pollen that form the viable embryos of a cross comprise, respectively, the egg and pollen pools, the gamete pools.

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Classifying the Validity of Controlled Crosses and Their Gamete Pools

Given a sufficiently large sample ($n > 20$ seeds), it is possible to use allozyme analysis both to test the validity of crosses and to place invalid crosses and their gametic pools in categories useful for diagnosing causes of error. The classification sytem used in this study is summarized in *Table 1*; examples of its use are provided in the Appendix. (Categories A1 through A5 and B1 through B4, described below, refer to the categories summarized in *Table 1*.)

Testing the validity of two-parent crosses requires two types of data for each cross: 1) the genotypes of at least one, but preferably both, putative parents at several variable allozyme loci; and 2) gametic allozyme arrays at the same loci, based on a large sample of seeds, for the egg and pollen pools of the cross. In analyzing a cross, we determined whether the allelic (allozyme) arrays of each gametic pool were error-free — that is, whether they could be the products only of the putative parents. Gametic pools whose allelic arrays corresponded to those expected from the genotypes of the putative parents were assumed to be error-free and classified *Acceptable* (category A1, *Table 1*).

Two types of observation indicated error in gametic pools: 1) the presence of alleles not found in the putative parents (unexpected alleles) or 2) segregation of alleles at two or more loci that were heterozygous in the putative parents at ratios significantly different ($p < 0.05$) from 1:1. Significant deviation at two loci was required to establish error because heterozygotes occasionally do not segregate in an expected 1:1 ratio, as a result of chance or of selective disadvantage of particular alleles (Rudin and Ekberg, 1978; Adams and Joly, 1980; Neale *et al.*, 1984). Requiring significantly distorted ratios at at least two loci, a conservative approach to declaring a cross invalid, increases the probability that crossing error was the cause, rather than segregation distortion.

Four categories were used for gametic pools showing evidence of errors in a cross (*Table 1*): 1) *Contaminated* (category A2) — where at least two parents, one of which may have been the putative parent, contributed to the pool; 2) *Single wrong parent* (*SWP*, category A3) — where a single parent *other* than the putative one contributed to the pool; 3) *Multiple wrong parents* (*MWP*, category A4) — where at least two parents, *none* a putative parent, contributed to the pool; and 4) *Questionable* (category A5) — where one parent (*presumably* the putative parent) was the primary contributor to the pool, with a few gametes contributed by at least one other parent.

Classifying a gametic pool as *Contaminated* or *Questionable* must be arbitrary. In this study, the presence in the sample of two or more gametes with unexpected alleles at one or more loci placed the gametic pool in the *Contaminated* class, while detection of one gamete with unexpected alleles made the pool *Questionable*. While serious contamination cannot be ruled out in a *Questionable* pool, the simplest explanation is that only a small percentage of gametes in the pool were contaminants. These distinctions, although arbitrary, can be very useful in diagnosis. For example, a few *Questionable* gamete pools in a sample of otherwise *Acceptable* pools may indicate relatively minor contamination problems in seed or pollen processing, while many *Contaminated* pools would indicate major problems in maintaining parent-tree identification, seed or pollen processing, or pollination techniques. Knowing whether pol-

Table 1: — System for classifying the validity of the gametic pools (A) of controlled two-parent crosses (B), based on allozyme analyses of large seed samples[1])

Classification	Interpretation
A. Gametic pools (egg or pollen)	
Genotype of putative parent known	
No errors detected	
1. *Acceptable*	There is no evidence that any parent other than the putative parent contributed to the gametic pool.
Errors detected:	
2. *Contaminated*	*At least* two parents contributed to the gametic pool, one of which *may* have been the putative parent.
3. *Single wrong parent (SWP)*	Only one parent, but not the putative parent (i.e., one with a different genotype) contributed to the gametic pool.
4. *Multiple wrong parents (MWP)*	Two or more parents, none of which was the putative parent, contributed to the gametic pool.
5. *Questionable*	One parent, *possibly* the putative parent, primarily contributed to the gametic pool; infrequently gametes were contributed by at least one other parent.
Genotype of putative parent unknown	
No errors detected	
6. *Single parent (SP)*	No more than one parent contributed to the gametic pool.
Errors detected:	
7. *Multiple parents (MP)*	At least two parents contributed to the gametic pool.
B. Crosses	
No errors detected in gamete pools	
1. *Valid*	There is no evidence that other than the putative parents were involved in the cross.
2. *Credible*	For the gametic pool for which the genotype of the putative parent is known, no evidence suggests that the putative parent is not the true one. There is no evidence that more than one parent contributed to the gametic pool for which the genotype of the putative parent is unknown.
Errors detected in one or both gamete pools	
3. *Invalid*	The genetic composition of the progeny, and thus the parentage of the cross, differ substantially from expectation.
4. *Suspect*	Except for a very small percentage of seed, the genetic composition of the progeny is as expected; limited seed or pollen contamination of an otherwise valid cross *may* have occurred.

[1]) At least 20 seeds per cross are required. Criteria for classification are described in the text.

len or egg pools, or both, are *Questionable* or *Contaminated* helps to isolate sources of error.

When both of the gametic samples (pollen and egg pools) of a cross were *Acceptable*, the cross was declared *Valid* (category B1, *Table 1*). If one or both gametic pools indicated identification errors of types other than *Questionable*, the cross was declared *Invalid* (category B3). If one gametic pool was *Questionable* and the other *Acceptable* or *Questionable*, the cross was classified as *Suspect* (category B4).

The ability to classify gametic pools is greatly reduced when genotypes of putative parents cannot be determined independently. The only distinction that can be made in these cases is whether one or more parents were involved in their formation. When only a single parent was detected, the pool was classified *Single parent* (*SP*, category A6). When multiple parents were indicated, the pool was classified *Multiple parents* (*MP*, category A7). Since the genotype of at least one parent of each cross in our study was determined from independent seed samples, only one gametic pool of any cross was classified *MP* or *SP*. All crosses with a *MP* pool were declared *Invalid*. Crosses with a *SP* pool were classified *Invalid* or *Suspect* if the corresponding gametic pool contained errors. Crosses with one pool *SP* and the other *Acceptable* were declared *Credible* (category B2, *Table 1*), indicating that no errors were detected in either pool, but that information needed to evaluate the cross was incomplete.

Materials and Methods

Seedlots from 43 putative two-parent crosses of Douglas-fir and 30 of loblolly pine were obtained from seven cooperating organizations (designated Organizations A through G). All crosses were made in conjunction with applied tree-improvement programs. All loblolly pine crosses were between clones in seed orchards, as were 22 Douglas-fir crosses. Because crosses are commonly made among wild trees in many Douglas-fir tree improvement programs (Silen and Wheat, 1979), seedlots of 21 wild-tree crosses were obtained for comparison.

For each cross, parental genotypes were determined from progeny allozyme arrays in independent seedlots (i.e., not from the cross being tested). In most cases, both parents were genotyped, but in 6 of the Douglas-fir and 12 of the loblolly pine crosses, independent seed samples were available from only one parent (the pollen parent in all but two cases). Since we suspected that most crossing errors would be found in pollen pools, we analyzed these crosses despite the lack of independent information on seed-parent genotypes.

Genotypes of the Douglas-fir parents were inferred from samples of at least eight megagametophytes, with the exception of 5 of the 63 trees, in which only six or seven megagametophytes were sampled. Each megagametophyte was scored at 10 loci that coded allozyme variants in eight enzyme systems: *Pgm1* and *Pgm2* (phosphoglucomutase); *Lap1* and *Lap2* (leucine aminopeptidase); *Got3* (glutamate-oxaloacetate transaminase); *Cat* (catalase); *Glyd* (glycerate dehydrogenase); *Idh* (isocitrate dehydrogenase); *Dia2* (diaphorase) and *6-Pgd1* (6-phosphogluconate dehydrogenase). Electrophoretic procedures were reported in Neale *et al.* (1984) and Merkle and Adams (1987). Banding patterns and genetic analyses of all enzymes except LAP and CAT were reported in El-Kassaby *et al.* (1982) and Neale *et al.* (1984). Mendelian inheritance of LAP and CAT has been confirmed by Adams (unpublished). Samples of at least 10 megagametophytes were used to infer the genotypes of the loblolly pine parents, which were scored at six loci: *Gdh* (glutamate dehydrogenase), *Lap2*, *Pgi2* (phosphoglucose isomerase), *6-Pgd1*, *Pgm1*, and *Pgm2*. Electrophoretic procedures, banding patterns, and genetic analyses are reported in Adams and Joly (1980).

Table 2. — Validity of controlled two-parent crosses in Douglas-fir and loblolly pine.

Species/ organization	Total Crosses	Total Parents	Mean sample size per cross: Seeds	Mean sample size per cross: Loci	Type of cross/classification[1]: Error-free *Valid*	Error-free *Credible*	Errors detected *Invalid*	Errors detected *Suspect*
Douglas-fir[2]								
A	16	21	26.4	9.2	9	2	2	3
B	6	12	24.0	10.0	4	0	2	0
C	11	18	24.0	9.9	5	0	5	1
D	10	17	27.5	8.5	6	0	4	0
Total	43	68			24	2	13	4
%					60.5		30.2	9.3
Loblolly pine[3]								
E	10	14	29.6	6	4	6	0	0
F	10	16	28.5	6	3	5	2	0
G	10	11	30.0	6	2	0	8	0
Total	30	40[4]			9	11	10	0
%					66.7		33.3	

[1]) See text for descriptions of the classifications.
[2]) Crosses were made among seed orchard clones by organizations A and B, and among trees in wild stands by organizations C and D.
[3]) All crosses were among seed-orchard clones.
[4]) One parent was common to crosses made by organizations E and G.

Both embryos and megagametophytes were assayed electrophoretically in seeds from controlled crosses. Sample sizes ranged from 22 to 29 (mean 25.7) seeds per cross in Douglas-fir and 21 to 30 (mean 29.4) in loblolly pine. Gametic genotypes were determined for the same loci that were scored in megagametophytes of the independent seed samples. However the weak expression of *Pgm2* in Douglas-fir resulted in loss of this locus in one set of 10 crosses. Occasional poor resolution of allozymes also resulted in missing data at one or more loci in some controlled-cross samples. If the number of scorable observations at a locus in either embryos or megagametophytes fell below half the seeds sampled, data for that locus were deleted from analysis. Overall, loss of data was very minor. The average number of loci analyzed in the Douglas-fir crosses was 9.2, and no loci were lost from analysis in the loblolly pine crosses. The number of scorable observations at any one locus fell below 90% of the total seeds sampled per cross in less than 3% of the cases.

Results and Discussion

A surprisingly high proportion of the crosses sampled were *Invalid* (*Table 2*), including 30.2% of the Douglas-fir and 33.3% of the loblolly pine crosses. In addition, 9.3% of the Douglas-fir crosses were *Suspect*. *Invalid* or *Suspect* crosses were distributed relatively evenly among crosses sampled from the four Douglas-fir organizations, but 8 of the 10 *Invalid* crosses in the loblolly pine samples were confined to a single organization (Organization G).

The proportion of *Invalid* crosses was higher in the Douglas-fir crosses made in wild stands (43%, Organizations C and D, *Table 2*) than in those made in seed orchards (18%, Organizations A and B, *Table 2*), although the difference was not significant ($X^2_{(1)} = 2.04$, $P > 0.10$). Some difference might be expected, considering the inherent difficulties of making crosses between tall trees in wild stands, often

separated by many miles. The high rate of error in crosses made in the much more favorable conditions of seed orchards is much more surprising and far from acceptable.

Most errors in both Douglas-fir and loblolly pine crosses occurred on the paternal side of the cross. Of 57 egg-pool samples for which the genotype of the putative seed parent was known, only 7% were inconsistent with the expected genotype, whereas 34 % of the 71 pollen-pool samples for which the genotype of the putative male parent was known differed from expectation. Pollen pools which contained errors were most commonly classified as *Contaminated* (all problematic pools from loblolly pine and five from Douglas-fir). *Contaminated* pollen pools can result from poor pollen-isolation techniques, from unintentional application of pollen from more than one parent in multiple pollinations, or from accidental mixing of pollen from different parents before pollination. Contamination by accidental mixing is especially likely to occur in seed orchard crosses if clones are mislabeled so that ramets of different clones are given the same identification. Mislabeling of seed orchard ramets has been reported in both loblolly pine (Hunter, 1977) and Douglas-fir (Adams, 1983).

No gametic pools were classified *Multiple wrong parents* (*MWP*) in this study. Five pollen pools in Douglas-fir were classified *Single wrong parent* (*SWP*). In contrast to *Contaminated*, *SWP* indicated that isolation was achieved in the bagging of female strobili and the pollen of a single parent was applied. Nevertheless, an identity error occurred during either labeling or application of pollen. As with *Contaminated*, *SWP* errors in seed orchard crosses can result if ramets are mislabeled and all pollen applied comes from one or more identically mislabeled ramets.

The other five Douglas-fir pollen pools that contained errors, and for which the genotypes of the putative parents were known, were classified as *Questionable*. In two crosses, the egg pools also were inconsistent with expectation; this finding suggested that the unexpected alleles in the pollen arose from seed contamination resulting from ramet mislabeling or seed-handling errors during extraction or electrophoretic analysis. In fact, any *Questionable* pollen pool could result from seed contamination, even if no errors are detected in the corresponding egg pool, since there is at least a fair chance that a seed contaminant might carry an unexpected allele from the pollen yet match the expectation from the genotype of the seed parent. Crosses in which both gametic pools were *Contaminated* probably also resulted from seed contamination. The genotypes of both putative parents were known in 22 of the *Invalid* or Suspect crosses found in the two species. Five (23%) of the errors in these crosses could be attributed to seed contamination. Thus, while errors on the paternal side seem to have been responsible for most *Invalid* or *Suspect* crosses detected, errors on the maternal side may also have been a significant factor in the genetic integrity of the controlled crosses.

To detect invalid crosses without classifying them, smaller sample sizes than those used in this study are sufficient. Uncovering invalid crosses when few seeds are sampled depends entirely on detection of unexpected alleles, which occur only rarely in *Questionable* gametic pools, but may occur rather frequently in the other categories of error. When the two species were considered together, 28 gametic pools for which the genotypes of the putative parents were known contained errors; six of these pools were classified *Questionable* and the rest either *Contaminated* or *SWP*. Unexpected alleles were detected in the first five seeds sampled in 82% of the *Contaminated* or *SWP* gametic pools and in the first 10 seeds sampled in 86% of those pools. Only two of the six *Questionable* gametic pools, however, would have been detected with samples of five seeds; this number would have increased only by one had 10 seeds been sampled. If the total number of seeds that can be assayed is limited, sampling a few seeds from each of many crosses probably is just as efficient for detecting *Suspect* crosses (and certainly more efficient for detecting *Invalid* crosses) as sampling many seeds from a few crosses. *Questionable* pools will always be difficult to detect, even with moderate sample sizes.

Conclusions

Sample sizes and number of loci required to detect errors in controlled crosses depend greatly on the degree of polymorphism in the marker loci. Both Douglas-fir and loblolly pine exhibit a great deal of genetic variability in allozymes, and 6 to 10 moderately variable loci were quite adequate for the analyses in this study. A similar number of variable loci would probably suffice for other conifers with high levels of variability (*e.g.*, the conifers listed in Hamrick *et al.*, 1981; Adams 1983). A two-stage sampling procedure seems the most efficient for surveying many crosses. In the first stage, genotypes of putative parents would be inferred from independent seed samples or determined directly by assaying vegetative tissues, and five seeds would be analyzed per cross. For detailed analysis of *Invalid* crosses in the second stage, 20 to 25 additional seeds per cross would be sampled, so that the crosses could be classified and possible sources of error identified.

Although validity of controlled crosses can be evaluated without genotypes of either putative parent, and our classification system could be extended to cover such cases, ability to detect and diagnose errors is substantially compromised. For this reason, we strongly recommend that every effort be made to independently determine the genotype of at least one parent in every cross analyzed.

While some error must reasonably be expected in operational controlled pollination and seed handling, the high level revealed in this study probably is much greater than anticipated. One should not conclude from our limited data set, however, that high error rates in controlled crosses are the norm for conifer tree-improvement programs. The crosses analyzed in this study were among the earliest in loblolly pine and Douglas-fir operational tree breeding, and with experience, technique probably has improved. A data set recently provided by Dr. Yousry El-Kassaby (pers. comm.) showed that error rates in operational controlled crossing programs can be considerably less than the average in our study. He detected no errors in 89.3% of 149 Douglas-fir crosses, using 6 allozyme loci and 10 seeds per cross.

Our data do show that lack of control in identity of genetic materials can be a serious problem in tree-improvement programs. Undetected, invalid crosses should not be ignored, because they could severely reduce anticipated genetic gains. We feel that all applied tree-improvement programs could benefit from surveying the integrity of genetic materials in breeding populations and continued monitoring of these materials in future generations. Allozymes are the best tool for this at present. Allozymes also can be used to certify the identity of parent trees, clones, and seedlots for other breeding and research applications

(RUDIN and LINDGREN, 1977; ADAMS, 1981, 1983; BROWN and MORAN, 1981).

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Appendix

Examples of use of the error-classification system in testing validity of controlled crosses

To illustrate the classification system in *Table 2* and its use in diagnosing causes of error, allelic arrays at five representative loci are given for the gametic-pool samples of six Douglas-fir crosses (*Table A1*). Only alleles expected on the basis of the genotypes of the putative parents were found in the gametic pools of cross 34 × 35. In addition, all heterozygous loci in the parents segregated in the gametic pools at ratios not significantly different from 1:1. Therefore, both the egg and pollen pools met the criteria for the *Acceptable* classification, and cross 34 × 35 was classified as *Valid*.

The egg pool of cross 65 × 66 was also classified as *Acceptable*. However, in addition to bearing at least one expected allele at each locus in the pollen-pool sample, three gametes carried an unexpected allele (allele 4 at locus *Lap1*). Thus, at least two pollen parents must have been involved in this cross; it is plausible that one was parent 66. Further evidence that tree 66 was not the lone pollen parent is the lack of allele *Glyd-3* in the pollen-pool sample. Since tree 66 was heterozygous for *Glyd-2/Glyd-3*, these alleles should segregate in a 1:1 ratio. Several explanations are consistent with the alleles observed in the pollen pool; the simplest and most consistent with the intended cross is that pollen from tree 66 was contaminated with pollen from one or more other parents. The pollen pool therefore was classified as *Contaminated* and the cross as *Invalid*. The proportion of contaminants (i.e., gametes with unex-

Table A1. — Allozyme analysis of controlled two-parent crosses of Douglas-fir, showing genotypes at five loci (*Pgm1, Lap1, Glyd, Idh, Dia2*) of the putative parents of six crosses, the allelic arrays in the corresponding gamete pools, and the validity classification of each gamete pool and cross.

			Parental genotypes (P) and allelic arrays of gamete pools (GP)																						
			Pgm1				*Lap1*				*Glyd*			*Idh*				*Dia2*			Gametes with unexpected alleles	Classification	
Cross			P[2]	GP[3]			P	GP			P	GP		P	GP			P	GP			Gamete pool	Cross
♀ × ♂	n[1]			2	3	4		2	3	4		2	3		2	5	7		2	4			
34 × 35	24	♀:	2/3	12	12		2/4	14		10	2/2	24		5/5		24		2/4	11	13	0	*Acceptable*	*Valid*
		♂:	3/3		24		2/2	24			2/2	24		5/7		11	13	2/4	13	11	0	*Acceptable*	
65 × 66	28	♀:	3/3		28		2/4	13		15	2/2	28		2/5	18	10		2/4	5	9	0	*Acceptable*	*Invalid*
		♂:	3/3		28		2/3	9	16	3*	2/3	28		2/5	18	10		4/4		14	3	*Contaminated*	
18 × 19	28	♀:	3/3		28		2/3	14	13	1*	2/3	9	18	5/5		28		4/4		28	1	*Questionable*	*Suspect*
		♂:	2/3	11	17		2/2	27		1*	2/2	28		2/2	27	1*		4/4		28	1	*Questionable*	
26 × 27	24	♀:	2/3	13	11		2/3	14	10		2/2	24		5/5		24		2/4	8	12	0	*Acceptable*	*Invalid*
		♂:	2/3	9	15		2/3		11	13*	3/3	24*		5/7		17		2/4	12	8	24	*Single wrong parent*	
19[4] × 18	28	♀:		13	15			28				28			28					28		*Single parent*	*Credible*
		♂:	3/3		28		2/3	18	10		2/3	16	12	5/5		28		4/4		28	0	*Acceptable*	
40 × 41[4]	24	♀:	3/3		24		2/3	9	15		2/2	21		2/5	11	13		4/4		21	0	*Acceptable*	*Invalid*
		♂:		3	19	2		14	9	1		13	8		5	16			6	15		*Multiple parents*	

[1]) Number of seeds sampled in each cross. The number of observed gametes may be less than n in some cases because of occasional poor allozyme resolution for individual enzymes and seeds.

[2]) Parental genotypes are given in shorthand notation. For example, 2/3 under *Pgm1* refers to genotype *Pgm1—2/Pgm1—3*.

[3]) Each column is headed by an allelic designation, with observed numbers of that allele in each gamete pool given in the body of the table.

[4]) Independent seed samples were not available for determining genotypes of these parents.

*) Alleles that were unexpected from the genotype of the putative parent.

pected alleles) detected in a sample must be considered a minimum estimate of contamination in a gametic pool; many contaminants may go undetected, especially if the alleles carried by the putative parents are fairly common in other potential sources of gametes. Indeed, the lack of *Glyd-3* in this sample suggests that contamination in the pollen pool of cross 65 × 66 was much greater than the three contaminants detected would indicate.

In cross 18 × 19, both gamete pools were *Questionable*, so the cross was classified as *Suspect*. If no error occurs in sampling for electrophoresis, *Suspect* crosses probably result from relatively minor pollen or seed contamination of otherwise valid crosses. If the initial sample is small, additional sampling may be warranted to rule out major contamination.

When none of the expected alleles at a locus is found in a gametic-pool sample, it can be concluded that the putative parent was not involved in the cross. This was the case for several loci in the pollen pool of cross 26 ×27 (*Table A1*). Since the alleles at all loci in the pollen were consistent with expectations for a single parent, the pollen pool of this cross was classified *SWP* and the cross, *Invalid*.

The last two crosses in *Table A1* illustrate crosses where no independent information on the genotype of one putative parent existed. For cross 19 × 18, there was no independently determined genotype for the seed parent, 19; but apparently only one parent contributed to the ovule pool, so it was classified *SP*. Since the pollen pool of this cross was *Acceptable*, the cross was declared *Credible*. No independent information was available on the genotype of the pollen parent (41) of the cross 40 × 41. In this case, the alleles in the pollen pool indicated multiple parentage, so this pool was classified *MP*, and the cross *Invalid*.

Selection of Wood Density and Diameter in Controlled Crosses of Coastal Douglas-fir

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Summary

Analyses of wood density and diameter in full-sib progeny of coastal Douglas-fir indicated additive genetic variance as the only important and significant genetic source of variation after 12 growing seasons. Individual tree heritability for wood density estimated by cores in a full-sib progeny trial of Douglas-fir was high (0.90). Pilodyn estimates were also high and correlated well with core estimates ($r_A = -0.95$). The efficiency of correlated response for half-sib family selection on the wood density core estimates by using the Pilodyn measure was 93%. Individual tree heritability for diameter was 0.23.

A strong negative correlation was shown to exist between wood density and diameter growth ($r_A = -0.53$). Index selection was used to highlight the options and trade-offs that can be made in the light of this adverse correlation.

Conservative options would restrict the loss in wood density or seek to improve both traits at the expense of maximising gain in any one trait. Less conservative options would allow that a loss in wood density was acceptable to gains in volume and overall dry weight.

Key words: Douglas-fir, wood density, diameter, Pilodyn, index selection

Zusammenfassung

Die Analysen der Holzdichte und des Durchmessers in Vollgeschwister-Nachkommenschaften der Küstendouglasie (*Pseudotsuga menziesii* (Mirb.) Franco) zeigten, daß die additive genetische Varianz die einzige wichtige und signifikante genetische Variationsursache nach 12 Vegetationsperioden war. Der individuelle Heritabilitätsschätzwert für die Holzdichte, ermittelt an Bohrspänen von Vollgeschwister-Nachkommenschaften der Douglasie, war hoch (0,90). Die Pilodyn-Schätzwerte waren ebenfalls hoch und eng mit den Bohrspan-Schätzwerten korreliert ($r_A = -0,95$). Die Effizienz für die Selektion von Halbgeschwister-Familien bei Verwendung der korrelierten Meßwerte von den Bohrspänen und dem Pilodyn-Verfahren war 93%. Die Einzelbaum-Heritabilität für den Durchmesser war 0,23.

Eine enge negative Korrelation wurde zwischen der Holzdichte und dem Durchmesserwachstum nachgewiesen ($r_A = -0,53$). Die Index-Selektion wurde benutzt, um die Selektionsoptionen und die Handelsgebräuche, die im Hinblick auf diese negative Korrelation getroffen werden können, herauszustellen. Konservative Optionen würden den Verlust bei der Holzdichte oder die Versuche, beide Merkmale zu verbessern, auf Kosten des maximierten Gewinnes bei jedem Einzelmerkmal beschränken. Weniger konservative Optionen würden ermöglichen, daß ein Verlust in der Holzdichte hinsichtlich der Gewinne bei Volumen und mittlerem Trockengewicht akzeptabel wäre.

Introduction

The timber of Douglas-fir is higly prized for structural uses, pulp and veneer. It is straight-grained, moderately light to moderately heavy (wood density of 430 to 450 kg/m³) and of intermediate durability (Cown, 1976). Wood density is an important trait because of its close relationship to the strength, quality, and yield characteristics of pulp products (Barefoot *et al.*, 1970), and the strength and structural properties of clear-wood products (Barrett and Kellogg, 1984). With the emphasis on growth and yield traits in genetic improvement programmes and the increasing proportion of juvenile wood from fast-grown plantations the importance of wood density and its bearing on quality has often been emphasized (Zobel and Kellison, 1978).

Wood density is not a single property, but is a complex of characteristics such as percentage of summerwood, cell-

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