matographs with integrators that give both quantitative and qualitative determinations of cortical monoterpenes. Since one is almost always able to separate clones by isozyme analysis or GLC of monoterpenes, both are useful techniques. However no studies with isozymes or monoterpenes have attempted to distinguish between all possible combinations of clones in a seed orchard. The use of isozymes should be considered when monoterpene analysis does not show ramets to be different. Because so may more loci can be studied using isozymes, the more lengthy procedure of isozyme analysis is justified to obtain more precision in making a determination. Hence, monoterpene analysis can be viewed as giving positive separation of clones but not necessarily positive similarity of the genetic make-up of ramets.

The large differences in monoterpene composition found among some ramets of the presumed same clone in the Olustee seed orchard clearly demonstrate that a few scions were mislabeled when establishing the seed orchard. In analyzing the clone bank ramets, it is apparent that some scions were also mislabeled when establishing the clone bank. Both of these plantings are used for advanced generation breeding and the establishment of high-gum-yielding seed orchards. The findings of this study indicate the necessity for roguing ramets of questionable identity in the seed orchard and returning to the original seed orchard or ortet to certify correct clone bank ramets. Within a year or two after establishment, seed orchards or clone banks that are to be used in tree breeding programs should be subjected to some kind of certification to avoid errors in the breeding program and in the establishment of commercial orchards. Errors at this stage are often perpetuated and magnified since all scions for a new planting may be taken from one incorrectly labeled ramet.

Myrcene and limonene are often minor monoterpenes (less than 6% of the total) (Gansel and Squillace, 1976) and are most useful in separating clones when one has a low content and the other a high content. The ramets in the commercial seed orchards fell into this category, and separations were straightforward. Increasing confidence in phenotype separations can be obtained with an increasing number of monoterpenes simultaneously separating the same ramet combinations. Furthermore, new research indicates one should sample five buds in determining a tree phenotype (Kossuth and Muse, 1986). A difference of 10% is a most conservative guide but is especially necessary when only one monoterpene is sufficiently different enough to obtain a separation.

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The Role of Reproductive Phenology upon the Mating System of a Douglas-fir Seed Orchard

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(Received 29th December 1986)

Abstract

The relations between reproductive phenology and the mating systems of a Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco] seed orchard were studied using allozyme polymorphisms at six loci. Seeds were analysed from cones of 28, 84, and 35 trees, representing three non-overlapping early, intermediate, and late reproductive phenology classes, respectively. Significant differences ($P < 0.05$) were observed among the three maternal gene pools, but not among the three pollen pools Significant change of gene fixation was observed between the parental (negative F) and filial (positive F) generations for each of the three reproductive phenology classes, indicating the presence of some form of inbreeding. Estimates of outcrossing rates for the intermediate class were higher than those obtained for either the early or late classes, indicating maximum panmixia during the height of flowering.

Key words: Pseudotsuga menziesii, seed orchard, reproductive phenology, outcrossing.

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Zusammenfassung

Die Beziehung zwischen Reproduktions- und Paarungssystemen einer Douglasiensamenplantage wurde anhand von Isoenzympolymorphismen an sechs Genloci untersucht. Saatgut wurde von 28, 84 und 35 Bäumen untersucht. Diese Bäume wurden drei nichtüberlappenden Blühperioden (früh, mittel und spät) zugeordnet. Signifikante Unterschiede (P 0,05) wurde zwischen den drei samenelterlichen, jedoch nicht zwischen den drei Pollen-Genpools beobachtet. Signifikante Veränderungen der Genfixierung wurden festgestellt zwischen den Parental- (negative F-Werte) und den Filialgenerationen (positive F-Werte), und zwar jeweils für die drei Blühperioden. Dieses Ergebnis läßt somit auf eine gewisse Inzucht schließen. Schätzungen der Fremdbefruchtungsrate waren für die mittlere Blühperiode höher als für die beiden anderen Guppen, was während der Hauptblühzeit maximale Zufallspaarung (Panmixie) nahelegt.

Introduction

Synchronization of the phenology of reproductive strobili among different plus tree clones or families is a fundamental need for any successful seed orchard operation (Wolssner and Franklin, 1973; Weir and Zobel, 1975). Failure of synchronization would have a detrimental effect on two of the seed orchard's basic requirements, namely, panmictic equilibrium and minimum selfing (El-Kassaby *et al.*, 1984). It has been demonstrated for several coniferous species that both time and temperature (summarized as heat sums) have a significant effect on flowering time (Kramer and Kozlowski, 1979).

Variation in flowering time has been studied for several tree species, both in natural stands, in provenance trials (Sarvas, 1962, 1968; Andersson, 1965; Griffith, 1968; Critchfield, 1980; Chung, 1981; Nilsson, 1981), and in seed orchard populations (Polk, 1966; Eriksson *et al.*, 1973; Jonsson *et al.*, 1976; O'Reilly *et al.*, 1983; El-Kassaby *et al.*, 1984; Griffin 1984). Depending upon climatic variability among plus tree origins, time differences of reproductive phenology are sometimes found.

Supplemental mass pollination (Denison and Franklin, 1975; Bridgwater and Trew, 1981; El-Kassaby *et al.*, 1984; El-Kassaby and Ritland, 1986a) and the use of water spray cooling treatment (El-Kassaby *et al.*, 1984) were suggested as measures to overcome and control any genetic differences in flowering time in seed orchards. Results from a combination cooling — noncooling treatment on a Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco] seed orchard showed a significant change in male and female strobili flowering time (Fashler and El-Kassaby, 1987). Longer pollination periods of 26—29 days were observed in the uncooled years, which might produce a continuum of smaller orchard breeding subpopulations over time. This also should increase the chance of selfing specifically in the early and late flowering periods because of decreased density of flowering trees during these times. By contrast, the cooling treatment reduced the pollination period to 16—19 days, which is predicted to improve panmixis and increase the frequency of outcrossing, in addition to its main function as a contamination preventive (Fashler and Devitt, 1980; El-Kassaby and Ritland, 1986b).

The objective of this paper is to determine the effect of reproductive phenology upon the mating system of trees in a Douglas-fir seed orchard. If synchronization among-tree reproductive phenology exists, then (1) the outcrossing rate should be homogeneous among early, intermediate, and late classes, and (2) pollen gene frequencies should be homogenous among the three classes. A possible consequence of a lack of such homogeneity of pollen gene frequency is the appearance of some effective selfing (Ritland, 1985) in seed progeny derived from random outcrossing within each phenological class. This can arise only if such heterogeneity of pollen gene frequency is correlated with any heterogeneity of ovule (maternal) gene frequency.

Materials and Methods

Material for this study was obtained from the 3.4 ha, high-elevation Douglas-fir seed orchard of CIP Inc., Victoria, B. C. The orchard population consists of combined clonal/seedling material representing 80 plus trees selected from elevations of between 450—1000 m on southern Vancouver Island and the south coastal mainland of B.C. Trees were planted in a randomized incomplete block design replicated 16 times. The average ages of the clonal propagules and seedlings are 17 and 14 years, respectively. The maximum tree height is approximately 8 m.

An overhead, solid-set, irrigation system was used in the orchard between January and April, 1983, to provide a cooling treatment during reproductive bud development. This treatment (1) delayed flowering until after the local pollen flush and (2) apparently helped synchronize reproduction because flowering occurred rapidly in all trees following cessation of the treatment.

In September 1983, during a bumper cone crop, cone samples were collected from 28, 84, and 35 trees representing early, intermediate, and late reproductive bud phenology classes, respectively. Trees were classified on the basis of their reproductive phenology history. The rank order of bud opening time of trees did not change in the orchard over years, despite the among year variation in pollination period (El-Kassaby *et al.*, 1984). Although the cooling treatment shortened the female reproductive phenology bud burst period to only 19 days in 1983, the different classes still showed the same relative order in bud opening time (data not given). Trees with overlapping reproductive phenological classes (i.e., between early and intermediate or intermediate and late) were excluded from sampling. For each sampled tree, the crown was stratified into four sections prior to cone collection: north (N) and south (S) aspects and upper (U) and lower (L) crown. The identity of all cone lots and subsequent seedlots was retained for these trees. Cones were air dried at room temperature; seeds were extracted, dewinged and cleaned by hand, and stored at 3° C until further use.

The genotype of each tree was inferred for six allozyme loci by electrophoretic assay of the haploid megagametophyte of 32 seeds per tree with equal numbers (8) from each crown segment (i.e., UN, US, LN, and LS). Given family size k, the probability of inference of homozygous maternal parentage given the parent is actually a heterozygote is $(1/2)^{k-1}$. With this large number of seeds per tree, the probability of misclassifying a heterozygote is very close to zero. The genotypes of the corresponding diploid embryos of the 32 seeds per tree were simultaneously derived. Because the haploid genotype of the megagametophyte determines the maternal contribution to the embryo, it was possible to infer the genotype of the pollen gamete based upon the genotype of the embryo.

The electrophoretic procedures, staining recipes, and enzyme nomenclature followed methods reported elsewhere (El-Kassaby *et al.*, 1982a). The enzyme systems used were: esterase (EST) E.C. 3.1.1.1., phosphogluco-isomerase (PGI)

Table 1. — Allele frequencies for ovule (maternal parents), progeny (viable embryos), and pollen (outcrossing pollen) gene pools, for early, intermediate, and late reproductive phenological classes. The two most common alleles for each locus, with 95% confidence intervals, are given (all loci are triallelic).

LOCUS/ALLELE		EARLY			INTERMEDIATE			LATE		
		Ovule	Progeny	Pollen	Ovule	Progeny	Pollen	Ovule	Progeny	Pollen
EST-1	1	0.661 ± 0.102	0.616 ± 0.022	0.566 ± 0.047	0.577 ± 0.064	0.516 ± 0.013	0.475 ± 0.019	0.371 ± 0.116	0.409 ± 0.020	0.466 ± 0.032
	2	0.214 ± 0.102	0.221 ± 0.022	0.219 ± 0.030	0.196 ± 0.059	0.222 ± 0.013	0.232 ± 0.016	0.386 ± 0.152	0.331 ± 0.025	0.262 ± 0.028
PGI-2	1	0.995 ± 0.019	0.965 ± 0.009	0.925 ± 0.019	0.940 ± 0.036	0.925 ± 0.007	0.929 ± 0.011	0.843 ± 0.087	0.882 ± 0.015	0.921 ± 0.017
	2	0.003 ± 0.015	0.034 ± 0.009	0.075 ± 0.019	0.054 ± 0.034	0.063 ± 0.007	0.070 ± 0.011	0.156 ± 0.087	0.117 ± 0.015	0.079 ± 0.017
G6PD	1	0.518 ± 0.132	0.515 ± 0.019	0.476 ± 0.035	0.589 ± 0.057	0.530 ± 0.011	0.458 ± 0.019	0.514 ± 0.134	0.531 ± 0.018	0.501 ± 0.032
	2	0.393 ± 0.148	0.412 ± 0.021	0.470 ± 0.036	0.357 ± 0.061	0.426 ± 0.011	0.506 ± 0.019	0.472 ± 0.135	0.448 ± 0.018	0.463 ± 0.032
6PGD-1	1	0.964 ± 0.048	0.942 ± 0.012	0.927 ± 0.018	0.893 ± 0.044	0.893 ± 0.008	0.898 ± 0.012	0.943 ± 0.055	0.926 ± 0.011	0.893 ± 0.020
	2	0.018 ± 0.035	0.027 ± 0.008	0.036 ± 0.013	0.036 ± 0.028	0.042 ± 0.005	0.046 ± 0.008	0.014 ± 0.028	0.029 ± 0.008	0.052 ± 0.014
PGM	1	0.946 ± 0.059	0.929 ± 0.012	0.921 ± 0.019	0.887 ± 0.045	0.894 ± 0.008	0.896 ± 0.012	0.900 ± 0.074	0.902 ± 0.013	0.900 ± 0.019
	2	0.018 ± 0.035	0.029 ± 0.008	0.040 ± 0.014	0.053 ± 0.033	0.055 ± 0.006	0.057 ± 0.009	0.095 ± 0.073	0.081 ± 0.012	0.061 ± 0.015
IDH	1	0.892 ± 0.079	0.886 ± 0.014	0.851 ± 0.025	0.768 ± 0.064	0.802 ± 0.010	0.842 ± 0.014	0.957 ± 0.047	0.902 ± 0.012	0.826 ± 0.024
	2	0.054 ± 0.060	0.058 ± 0.011	0.090 ± 0.018	0.060 ± 0.038	0.076 ± 0.007	0.096 ± 0.003	0.029 ± 0.040	0.057 ± 0.010	0.101 ± 0.019
N		28	761		84	2551		35	943	

E.C. 5.3.1.9; glucose-6-phosphate dehydrogenase (G6PD) E.C. 1.1.1.49; 6-phosphogluconic dehydrogenase (6PGD) E.C. 1.1.1.44; phosphoglucomutase (PGM) E.C. 2.7.5.1; and isocitrate dehydrogenase (IDH) E.C. 1.1.1.42. The six loci scored were EST-1, PGI-2, G6PD, 6PGD-1, PGM, and IDH. The mode of inheritance and lack of linkage of these enzyme loci was reported in EL-KASSABY *et al.* (1982a,b).

The relationship between observed (H) and expected (h) frequencies of heterozygotes in both of the parental generation and their progeny (viable embryo stage) was used to measure the extent of gene fixation in parent and progeny trees. WRIGHT's (1922) fixation index (F) was calculated for each locus using the formula $F = 1 - (H/h)$, where H is the observed frequency of heterozygotes and h is the expected frequency of heterozygotes under panmixia ($h = 1 - \Sigma p_i^2$ where, p_i; is the frequency of the ith allele at the locus). The variance of F was calculated from the inverted information matrix (KENDALL and STUART, 1979) for p and F for each locus.

Single- and multilocus estimates of outcrossing rate (t) and outcrossing pollen gene frequencies (p) for each reproductive phenology class were estimated using the multilocus mixed mating model for gametophytic conifer data of RITLAND and EL-KASSABY (1985). Significant differences ($P < 0.05$) in allele frequency and outcrossing rate among the three reproductive phenology classes were determined for each locus by comparing the bounds of the 95% confidence intervals.

Results and Discussion

Estimates of allele frequencies and their 95% confidence intervals for ovules (maternal trees), progeny (viable embryos), and pollen (outcrossing pollen) gene pools for the three (early, intermediate, and late) reproductive phenology classes are listed in *Table 1*. The maternal gene pools differed significantly ($P < 0.05$) in eight of the 36 possible pairwise comparisons: at EST-1(1), PGI-2(1,2), and IDH(1) (*Table 1*). This percentage of significant comparisons (22%) is higher than that expected by chance (5%), indicating the presence of three genetically distinct female breeding subpopulations over time, within the seed orchard.

By contrast, significant differences ($P < 0.05$) among the three outcrossing pollen pools was observed only between the early and the other two reproductive phenology classes at EST-1(1) (*Table 1*). The smaller sample size of the early pollen pool relative to the other two classes could be the cause of the single-locus difference observed at EST-1(1). This observed difference among the three pollen pools thus is likely due to chance. In general, pollen production occurred over a longer period than ovule receptivity.

For the progeny allele pools, with the exception of G6PD(1,2) and 6PGD-1(2), all allelic frequencies showed significant differences ($P < 0.05$) among the three reproductive phenology classes (*Table 1*). Significant differences among the progeny gene pool are expected due to the observed differences among the ovule (maternal) gene pools and the partial dependence of the progeny gene pool upon the maternal gene pool, as well as the much larger sample size.

When the ovule (maternal) and progeny (viable embryos) allele pools were compared within each of the three reproductive phenology classes, no significant differences were observed, with the exception of PGI-2 (early), indicating that the orchard subpopulations are temporally panmictic. The observed homogeneity between the ovule and progeny allele pools is also an indication that no migration (i.e., contamination) from outside the orchard has taken place (EL-KASKABY and RITLAND, 1986b) and further emphasize the effectiveness of the cooling treatment as a practical method for reducing pollen contamination (FASHLER and DEVITT, 1980). Differences in allele frequency between progeny and maternal parent trees were observed in a Douglas-fir natural stand (EL-KASSABY *et al.*, 1981), in *Pinus radiata* (MORAN *et al.*, 1980), and in *Eucalyptus delegatensis* (MORAN and BROWN, 1980). These differences at PGI-2 could be based upon differences in outcrossing pollen pools (not applicable here), due to post-mating selection, or chance.

Estimates of WRIGHT's fixation index F (WRIGHT, 1922; F measures excess of homozygosity above Hardy-Weinberg expectation) and their 95% confidence intervals are presented in *Table 2*, for both the maternal trees and their progeny for each reproductive phenology class. In the adult trees (maternal), both EST-1 and G6PD showed significant F for the late reproductive phenology class (*Table 2*). The estimates of F varied within and among the three reproductive phenology classes but most F values did not differ from zero. For the progeny, significant F values were observed at EST-1 for the three reproductive phenology classes and at PGI-2 for the late class. Such a consistent pattern of excessive homozygosity at EST-1 locus was also reported in Douglas-fir (SHAW and ALLARD, 1979, 1982a) and in loblolly pine (*Pinus taeda* L.) (ROBERDS and CONKLE, 1984). The cause of this pattern was not discussed in the case of Douglas-fir; however in loblolly pine, ROBERDS and CONKLE

Table 2. — Estimates of WRIGHT's fixation indices for early, intermediate, and late reproductive phenology classes, with 95 percent confidence intervals. Sample sizes are same as those given in *Table 1.*

LOCUS	EARLY		INTERMEDIATE		LATE	
	Parents	Progeny	Parents	Progeny	Parents	Progeny
EST-1	-0.068 ± 0.323	0.153 ± 0.062*	-0.010 ± 0.178	0.219 ± 0.032*	-0.397 ± 0.263*	0.215 ± 0.054*
PGI-2	-0.001 ± 0.318	-0.031 ± 0.094	-0.049 ± 0.258	0.039 ± 0.041	0.029 ± 0.348	0.093 ± 0.078*
G6PD	-0.317 ± 0.321	-0.034 ± 0.063	-0.025 ± 0.190	-0.032 ± 0.035	-0.335 ± 0.310*	0.016 ± 0.058
6PGD-1	-0.019 ± 0.378	0.041 ± 0.077	0.029 ± 0.193	0.028 ± 0.035	-0.051 ± 0.392	0.020 ± 0.057
PGM	-0.035 ± 0.398	-0.030 ± 0.069	-0.037 ± 0.196	0.010 ± 0.032	-0.111 ± 0.420	0.061 ± 0.069
IDH	-0.089 ± 0.398	0.014 ± 0.059	-0.170 ± 0.210	0.002 ± 0.031	-0.031 ± 0.368	-0.005 ± 0.051
Mean	-0.095 ± 0.143	0.025 ± 0.028	-0.039 ± 0.082	0.048 ± 0.014*	-0.194 ± 0.138*	0.064 ± 0.024*

*) Rejection of null hypothesis that F = 0.00 at 5% level.

suggested that natural selection may have been the contributing factor.

The average F values for the adult trees and their progeny were negative and positive, respectively (*Table 2*), results similar to those reported by SHAW and ALLARD (1982b) for Douglas-fir in eight natural stands. The negative F values for the adult trees indicate an excess of heterozygotes over those expected under panmixia. Natural selection and competition among different trees could favor multiple heterozygotes. The long life cycle, enormous fecundity, and resulting high pre-reproductive mortality of Douglas-fir makes this scenario more likely.

The progeny F values on the other hand showed positive F values, indicating that some inbreeding was practiced by parents in spite of the randomization of seedlings and/or ramets of the same ortet within the orchard. The finite population size (80 plus trees) and the presence of some clonal/seedling trees originating from the same ortet (plus tree) in the orchard population permitted mating among relatives as well as selfing. The different types of mating within the orchard population and their effects upon the observed levels of selfing were discussed in RITLAND and EL-KASSABY (1985).

Estimates of single-locus ($\hat{t}$) and multilocus ($\hat{t}_m$) outcrossing rates for progeny collected from the three reproductive phenology classes are presented in *Table 3*. Single-locus estimates for all classes ranged between 0.653 to 0.999 and all were higher than 0.8 when estimates based on EST-1 locus were excluded (*Table 3*). The EST-1 locus consistently showed lower estimates for $\hat{t}$, as SHAW and ALLARD (1979, 1982a) found for the same species. Within each reproductive phenology class, single-locus estimates of outcrossing rate differed significantly ($P < 0.05$) from each other, even after the EST-1 locus was excluded from the comparisons (*Table 3*). Significant differences among single-locus estimates of outcrossing rate have been reported for Douglas-fir (SHAW and ALLARD, 1979, 1982a; EL-KASSABY *et al.*, 1981), *Picea glauca* (KING *et al.*, 1984), *Pinus ponderosa* (MITTON *et al.*, 1981), and *Pinus radiata* (MORAN *et al.*, 1980). This observed variation is an inherent problem of all single-locus estimates due to their sensitivity to violations in the mixed mating model's assumptions (FYFE and BAILEY, 1951; BROWN and ALLARD, 1970; EPPERSON and ALLARD, 1984; RITLAND, 1985).

Table 3. — Single-locus ($\hat{t}$) and multilocus ($\hat{t}_m$) estimates of outcrossing rates for early, intermediate, and late reproductive phenology classes (95% confidence intervals).

Locus	Early	Intermediate	Late
EST-1	0.737 ± 0.073	0.713 ± 0.038	0.653 ± 0.054
PGI-2	0.904 ± 0.147	0.989 ± 0.043	0.871 ± 0.101
G6PD	0.999 ± 0.071	0.999 ± 0.039	0.807 ± 0.077
6PGD-1	0.933 ± 0.099	0.931 ± 0.047	0.902 ± 0.092
PGM	0.899 ± 0.104	0.899 ± 0.050	0.903 ± 0.089
IDH	0.906 ± 0.089	0.999 ± 0.039	0.903 ± 0.084
$\hat{t}^{\dagger}$	0.942 ± 0.042	0.971 ± 0.019	0.873 ± 0.039
$\hat{t}_m^{\dagger\dagger}$	0.938 ± 0.033	0.968 ± 0.014	0.893 ± 0.032

†) Minimum variance mean after excluding EST-1 locus.
††) Excluding EST-1 locus.

Table 4. — Single-locus and multilocus estimates of outcrossing rate averaged over the three reproductive phenology classes, and for the combined population.

Locus	Mean of 3 classes	Combined
EST-1	0.678 ± 0.019	0.702 ± 0.029
PGI-2	0.943 ± 0.028	0.902 ± 0.051
G6PD	0.950 ± 0.021	0.955 ± 0.035
6PGD-1	0.912 ± 0.026	0.916 ± 0.039
PGM	0.800 ± 0.027	0.899 ± 0.040
IDH	0.947 ± 0.023	0.999 ± 0.030
$\hat{t}^{\dagger}$	0.936 ± 0.010	0.925 ± 0.013

†) Excluding EST-1 locus.

Single-locus minimum variance means of $\hat{t}$ were 0.942, 0.971, and 0.873 for the early, intermediate, and late reproductive phenology classes, respectively (*Table 3*). The esterase locus was excluded because it clearly violates model assumptions.

The mean for the intermediate class was higher than those for the early and late classes. This indicates maximum panmixia during the height of flowering. Results from the reproductive phenology surveys conducted between 1976 to 1983 indicate that the number of receptive female and mature male strobili were higher in the intermediate class than the other two classes (EL-KASSABY *et al.*, 1984; FASHLER and EL-KASSABY, 1987), confirming the potential for maximum panmixia in the intermediate class.

The minimum variance means of single-locus $\hat{t}$ for the early and late reproductive phenology classes were very close to the mean single-locus estimate of $t = 0.9$ previously reported for several Douglas-fir natural stands (EL-KASSABY *et al.*, 1981; SHAW and ALLARD, 1982a) and clonal orchard (SHAW and ALLARD, 1982a) but were lower than the 0.971 reported for the intermediate class. The multilocus estimates of outcrossing rate ($\hat{t}_m$) were 0.938, 0.968, and 0.893 for the early, intermediate, and late reproductive phenology classes, respectively (*Table 3*). Once again the intermediate repoductive phenology class showed a higher estimate of outcrossing than those for the other two classes, although they were not significantly different from each other (*Table 3*).

When estimates were performed by pooling the early, intermediate, and late classes together, this "combined estimate" was close to the mean of estimates of outcrossing performed separately for early, intermediate, and late classes (*Table 4*). If there was effective inbreeding caused by genetic differentiation of phenological classes combined with mating only within each class, one would expect the combined estimate of outcrossing to be less than the average estimate over the three classes because of population substructure effects (ENNOS and CLEGG, 1982; ENNOS, 1985; RITLAND, 1985). However, there was no evidence of this effective inbreeding, for both the single-locus and multilocus comparisons (*Table 4*).

A method to detect effective inbreeding caused by consanguineous matings within phenological classes is to compare outcrossing estimates derived from the multilocus method to those from the single-locus method (SHAW *et al.*, 1981). The single-locus unweighted means were similar to those obtained by the multilocus method, indicating the

absence of other types of consanguineous mating in addition to selfing. However, consanguineous matings are expected in this study because the orchard population contains clonal and related seedling material. Probably our comparisons of single-with multilocus estimates tended to underestimate the proportion of selfing caused by consanguineous matings because of the relatively few loci used for the multilocus estimate (RITLAND and EL-KASSABY, 1985).

Although outcrossing rates are generally under genetic control, the mating system of most plant species is also plastic and subject to environmental influences (CLEGG, 1980). The outcrossing rates of several plant species have revealed marked differences under different environmental conditions. Population structure (ENNOS and CLEGG, 1982; ELLSTRAND and FOSTER, 1983), density (ELLSTRAND *et al.*, 1978), age (MORAN and BROWN, 1980), elevation (PHILLIPS and BROWN, 1977), and xerism of the environment (MARSHALL and ALLARD, 1970; HAMRICK and ALLARD, 1972; BROWN *et al.*, 1974, 1978) all have affected outcrossing rates and significant differences were reported. The spatial and temporal variation in the mixed mating system of most forest trees system warrants special attention (BROWN *et al.*, 1975; MITTON *et al.*, 1977; PHILLIPS and BROWN, 1977; MORAN and BROWN, 1980: SHEN *et al.*, 1981; SHAW and ALLARD, 1982a; MÜLLER-STARCK *et al.*, 1983; EPPERSON and ALLARD, 1984; KING *et al.*, 1984; EL-KASSABY *et al.*, 1986). Within year reproductive phenological differences have also been shown to affect the mating structure within seed orchards (ERIKSSON *et al.*, 1973; JONSSON *et al.*, 1976; O'REILLY *et al.*, 1983; EL-KASSABY *et al.*, 1984).

The assumptions of random mating and panmictic equilibrium were shown to be violated in several seed orchards, and management responses were proposed and implemented (BRIDGWATER and TREW, 1981; EL-KASSABY *et al.*, 1984). Results from our studies indicated that the cooling treatment has a substantial effect on reducing the pollination period and improving panmixis (FASHLER and EL-KASSABY, 1987). The high rate of outcrossing for the three reproductive phenology classes reported here could be due to the observed reduction in the pollination period after the application of the cooling treatment.

In conclusion, to effectively use knowledge of a species' breeding system it is also necessary to understand the mechanisms through which the breeding system is maintained. Investigation of reproductive biology, phenology, and pollination ecology are therefore an implicit component of breeding system studies (HAMRICK, 1982; LOVELESS and HAMRICK, 1984).

Acknowledgements

This project was supported by the British Columbia Ministry of Forests, Research Branch, Sect. 88 nos. JS-02-016 and KS-02-012; by CIP Inc. (formerly known as Pacific Forest Products Ltd.); and by a Nat. Inst. of Health (USA) grant to K. R. (University of California, Riverside, USA). The authors are indebted to J. PARKINSON for technical assistance.

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Short Note: Genetics of Chestnut (Castanea sativa Mill.)

II. Uniformity of Isozyme Phenotypes in grafted Orchards

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(Received 12th September 1986)

Summary

Some enzyme systems (LAP, GOT, SKDH, IDH, 6-PGDH, MDH, GDH and G-6-PDH) were studied in bud tissues of European chestnut by means of starch gel electrophoresis. Individual trees of a grafted stand were collected in central Italy. Results reveal the uniformity of the enzyme phenotypes for all of the enzyme systems analyzed. Individual trees can therefore be considered as ramets of the same clone.

Key words: isozymes, grafting, clone, *Castanea*.

Zusammenfassung

Einige Enzymsysteme (LAP, GOT, SKDH, IDH, 6-PGDH, MDH, GDH und G-6-PDH) sind mittels Stärkegelelektrophorese im Knospengewebe von *Castanea sativa* analysiert worden. Einzelne Bäume sind in einem gepfropften Bestand in Mittel-Italien beerntet worden. Ergebnisse zeigen die Einheitlichkeit der Enzym-Phänotypen aller untersuchten Systeme. Die analysierten Einzelbäume können daher als Ramets eines einzigen Klons betrachtet werden.

Introduction

European chestnut (*Castanea sativa* Mill.) is a species which is naturally widespread over the hill and mountain altitude of the entire Italian territory. Because of its economic value, it has always been cultivated and bred for the quality and quantity of the wood and the fruit.

The so-called "varieties" have been developed through grafting and asexual propagation. No certain information is available on the genetic identity of the varieties. The specification of the cultivated varieties is very difficult, because they are usually described and classified only according to their geographic origin. Different varieties, having distinct origin and diverse local names, could be derived from a single variety, which has differentiated morphologically due to environmental factors (Paglietta and Bounous, 1979).

In order to identify (or differentiate) the varieties, numerous studies based on the analysis of morphological characters of the fruit have been carried out (Antonaroli *et al.*, 1983, 1984; Bassi and Sbaragli, 1984; Bassi and Marangoni, 1984; Borghetti *et al.*, 1983). Studies on the genetic structure of chestnut varieties are still lacking. Preliminary studies based on observations of isozymes as environmentally independent traits have been reported by Sawano *et al.* (1984) and Fineschi (1986), but no information has been available on the genetic control of isozyme systems in chestnut species.

The question of whether different individuals belonging to the same variety are genetically identical or not will be discussed in the present study. In fact, it is possible that only a single tree bearing particularly large fruits has been utilized to graft other trees and has therefore founded a so-called "variety". If all trees of a grafted population turn out to possess identical zymograms, they can be considered as individuals of the same clone. This requires that

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