Literatur

EVERS, F. H.: Über bleibende Differenzierung im Mineralstoffgehalt der Nadeln bei verschiedenen Klonen der Fichte (*Picea abies* (L.) H. KARSTEN), Mitt. des Vereins für Forstliche Standortskunde und Forstpflanzenzüchtung 27, 60—65 (1979). — KLEINSCHMIT, J.: Vergleichende Wurzeluntersuchungen an Fichtensämlingen und Fichtenstecklingen. Forstarchiv 49, 69—74 (1978). — LIENERT, G. A.: Verteilungsfreie Methoden in der Biostatistik. Bd. I. Anton Hain Verl. S. 322 ff. (1973). — NAMKOONG, G. V.: Introduction to Quantitative Genetics in Forestry. U. S. Dept. of Agriculture, Forest Service Techn. Bull. No. 1588, 342 Seiten. (1979). — SACHS, L.: Statistische Auswertungsmethoden. Springer Verl. Berlin, Heidelberg, New York. S. 333 ff. (1971). — SAUER, A., KLEINSCHMIT, J. und LUNDERSTÄDT, J.: Charakterisierung von Fichtenklonen (*Picea abies* KARST.) mit Hilfe morphologischer, physiologischer und biochemischer Methoden. Silv. Gen. 22, 173—182 (1973). — SAUER-STEGMANN, A., KLEINSCHMIT, J. und LUNDERSTÄDT, J. K.: Methoden zur Charakterisierung von Fichtenklonen (*Picea abies* (L.) KARST.) Silv. Gen. 27, 109—117 (1978).

Isoenzyme variation among ten populations of Populus trichocarpa Torr. et Gray in the Pacific Northwest[1])

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Summary

Isoenzyme variation was analyzed among ten riparian populations of black cottonwood in the Pacific Northwest. Isoenzymes from 12 enzyme systems were extracted from root tips of rooted cuttings and separated by horizontal starch-gel electrophoresis.

Although the populations were geographically distinct, overall genetic similarity was very high and average within-population heterozygosity (H_S) was much greater than between-population heterozygosity (D_{ST}). Neighboring populations clustered together genetically, but no statistically significant relationship existed between geographic and genetic distance. Gene diversity, measured by both average heterozygosity per locus and the proportion of polymorphic loci, was generally greater in populations representing larger river drainages, although the differences were very slight. The frequencies of four alleles varied with latitude or longitude, but other allele frequencies appeared unrelated to these coarse gradients. Nearly 13% of the loci sampled did not fit Hardy-Weinberg proportions; most deviations were due to a deficiency of heterozygotes.

Results are discussed in terms of potential factors promoting genetic similarity and differentiation among natural populations of black cottonwood.

Key words: Isoenzyme, Geographic variation, Population differentiation, *Populus trichocarpa*

Zusammenfassung

In einer Studie wurde die Isoenzym-Variation von *Populus trichocarpa* in zehn Fluß-Auewäldern im Pazifischen Nordwesten untersucht. Zwölf verschiedene Enzym-Systeme wurden mittels Stärkegel-Elektrophorese analysiert. Zur Gewinnung der Enzyme eigneten sich Wurzelspitzen von frisch bewurzelten Stecklingen am besten.

Trotz geographischer Distanz war die genetische Ähnlichkeit der Populationen bemerkenswert, und die Heterozygotie innerhalb der Populationen (H_S) war wesentlich höher als die zwischen ihnen (D_{ST}). Die genetische Ähnlichkeit war bei Nachbar-Populationen am höchsten, aber es bestand keine statistisch signifikante Beziehung zwischen geographischer und genetischer Distanz. Die Gen-Verschiedenheit, die sich sowohl durch die durchschnittliche Heterozygotie je Locus, als auch durch die Proportion polymorpher Loci ausdrückt, war im allgemeinen in Populationen aus ausgedehnten Fluß-Einzugsgebieten größer, obwohl die Unterschiede insgesamt gering waren. Die Häufigkeit von vier Allelen variierte im Zusammenhang mit geographischer Breite und Länge, aber andere Allelhäufigkeiten schienen von diesen groben Gradienten unabhängig zu sein. Nahezu 13% der erfaßten Loci zeigten Abweichungen vom Hardy-Weinberg Gleichgewicht, zumeist wegen eines Mangels an Heterozygoten.

Die Ergebnisse werden im Hinblick auf mögliche ökologische demographische Faktoren besprochen, die die genetische Differenzierung von natürlichen Populationen von *Populus trichocarpa* beeinflussen können.

Introduction

Black cottonwood (*Populus trichocarpa* TORR. et GRAY) is one of the most successful pioneer species of the West Coast. Large populations dominate many riparian communities from south-central Alaska to southern California and from the Pacific coast to the Rocky Mountains. Scattered individuals and small stands also occupy many disturbed upland sites that are only marginal for conifer growth.

The wide distribution of black cottonwood implies the presence of considerable genetic variation and/or phenotypic plasticity among genotypes. Breeding programs in other parts of the world have already taken advantage of variation in growth rate, rootability, site adaptability, disease resistance and combining ability in hybridization trials (reviewed in SCHREINER 1959, STEENACKERS 1969, WEISGERBER 1975, ZSUFFA 1973). A IUFRO-sponsored international provenance test, begun in 1973, is aimed at elucidating variation patterns in this species and identifying promising seed sources for specific growing areas. First results have shown significant genetic variation among sources from the Pacific Northwest in germination, seedling mortality (WEISGERBER 1976), growth rate, phenology and rust resistance (DIETERICH 1976). Notable variation in growth rate, wood

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characteristics (Reck 1974), leaf shape and surface area (Weber, unpublished data) has also been observed among selected clones.

While few efforts have been made to exploit this variation in its native range, recent attention given to biomass production has encouraged studies investigating the production potential of black cottonwood under short-rotation coppice regimes (Heilman, Peabody, DeBell, and Strand 1972). It was in this context that a genetic program was initiated in the Pacific Northwest to identify superior clones of black cottonwood as well as to develop high-performing hybrid combinations with other species, notably *P. deltoides* (Stettler and Heilman 1980). To improve the longterm effectiveness of this program, an understanding of the population genetic structure of black cottonwood is essential.

A very rapid and efficient method to investigate the genetic structure is to analyze isoenzyme variation (Feret and Bergmann 1976). The objectives of this study, therefore, were to (1) develop laboratory methods for assaying electrophoretic variation in black cottonwood and (2) survey the distribution of isoenzyme variation among and within ten selected populations in the Pacific Northwest.

The development of an isoenzyme methodology for poplars would be useful for several purposes in addition to analyzing population genetic structure. There has been a long history of domestication in this genus with strong emphasis on vegetative propagation, resulting in an ever-changing pool of commercially available clones. Morphological discrimination is often difficult, particularly among clones of related parentage. If sufficient polymorphic loci could be resolved, a more reliable tool would be available for clonal identification. Prolonged domestication has also led to severe reductions of natural stands in some species, e.g. *P. nigra* in Europe, meaning a likely loss of potentially useful genetic diversity. In the efforts currently directed at their genetic conservation (Koster, personal communication), isoenzyme methods might help in allocating resources most sensibly (Brown 1978, Rudin 1976).

Materials and Methods

Ten populations were chosen to sample variation associated with different river drainages in the Pacific Northwest (*Table 1* and *Figure 1*).

Dormant shoots were collected from the upper crown of 50 randomly selected trees in each population. Sampling was restricted to individuals between approximately four and twenty years old; crown accessibility prohibited sampling older age classes. This procedure may have led to biased estimates of the genetic variation in some or all of the populations.

Table 1. — Specifications of populations studied.

Population	Abbreviation	Latitude	Longitude	Elevation (m)	River drainage
Chilliwack	CHI	49° 05'	121° 56'	61	Chilliwack
Rockport	ROC	48° 28'	121° 37'	67	Skagit
Arlington	ARL	48° 12'	122° 08'	18	Stillaguamish
Monroe	MON	47° 50'	122° 03'	34	Skykomish-Snoqualmie
Index	IND	47° 53'	121° 32'	183	North Fork Skykomish
Snoqualmie	SNO	47° 32'	121° 49'	125	Middle Fork Snoqualmie
Orting	ORT	47° 03'	122° 12'	91	Puyallup
Nisqually	NIS	47° 03'	122° 42'	7	Nisqually
Longview	LON	46° 06'	122° 58'	3	Columbia
Santiam	SAN	44° 44'	123° 03'	61	Santiam

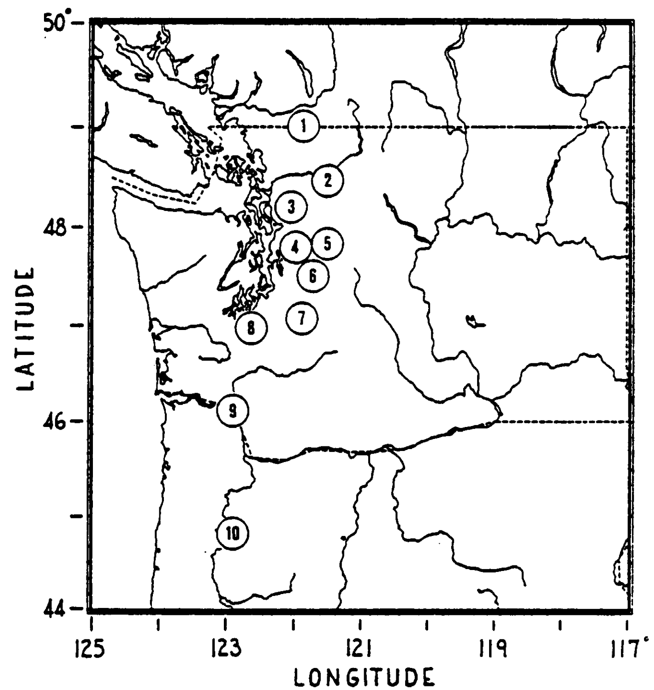

Figure 1. — Location of populations studied. (1) Chilliwack, (2) Rockport, (3) Arlington, (4) Monroe, (5) Index, (6) Snoqualmie, (7) Orting, (8) Nisqually, (9) Longview, (10) Santiam.

Shoots were rooted in Perlite for two to three weeks. The ten most vigorous root tips were then macerated in a cold extraction buffer and stored at −5° C. Zymogram patterns were not altered but enzyme activity decreased if storage exceeded two weeks at this temperature. Detailed methods including recipes for buffers and stains are given elsewhere (Weber 1980)*).

Twelve enzyme systems were assayed by horizontal starch-gel electrophoresis. Electrophoresis was carried out at 50 milliamp, 200—250 V, until the front had migrated 8 cm (3—4 hours). Gels were covered with ice packs which were periodically replaced. Optimal isoenzyme separation and activity were very dependent on the gel and stain recipes used; isoenzymes in *Populus trichocarpa* × *P. deltoides* hybrids, for example, could not be resolved using these recipes.

Statistical Analysis

Goodness of fit between observed genotype frequencies and expected Hardy-Weinberg proportions was tested by the heterogeneity G procedure (Sokal and Rohlf 1969). A G test was also used to detect heterogeneity in single locus frequencies among populations.

The methods described by Nei (1975) were used to compute average heterozygosity per locus (H), average gene diversity within (H_S) and between (D_{ST}) populations, total gene diversity (H_T) and a coefficient of gene differentiation (G_{ST}). The proportion of polymorphic loci was determined for each population as another measure of gene diversity. Any locus with an alternate allele frequency $\geq$ 0.05 was considered polymorphic.

Simple linear regression was used to investigate the relationships between (1) population allele frequency estimates (transformed to arcsin $\sqrt{P}$) and latitude, longitude

*) Reprints of methods and recipes are available upon request from the senior author.

and elevation, and (2) genetic and geographic distance among populations.

Genetic similarity between pairs of populations was estimated according to ROGERS (1972). Cluster analysis (unweighted pair group method using arithmetic averages, or UPGMA) was performed on the matrix of Rogers' similarity values.

Results

Nomenclature for enzymes, loci and alleles is given in *Table 2.* The allele most commonly observed among trees in a trial study was arbitrarily designated the A allele. The origin (0) and migration front at the conclusion of electrophoresis (100) are the reference points for computing relative migration (Rf). Patterns of observed, or expected (not all LA-1 genotypes were observed), zymograms are drawn in *Figure 2.* Although data on the segregation of these bands is not available, we feel confident in using the terms loci, allele and genotype. Identical band patterns were observed when several ramets of the same clone were tested and these patterns were consistent from day to day.

Eleven of the 18 loci were polymorphic in at least one population *(Table 3).* Although statistically significant differences existed among populations at 10 of these loci, allele frequencies were homogeneous over the bulk of the populations *(Table 4).* Results from linear regressions, however, suggested that the frequencies of some alleles varied with latitude (EST-2-A, β = 3.84, $P < .03$, r^2 = .49; PGI-2-A, β = −3.40, $P < .02$, r^2 = .53; LA-1-C, β = −2.37, $P < .01$, r^2 = .70) and longitude (ACIDP-2-A, β = −5.71, $P < .05$, r^2 = .40; LA-1-C, β = 6.30, $P < .01$, r^2 = .80; PGI-2-A, β = 8.40, $P < .02$, r^2 = .53).

Table 2. — Loci and alleles observed. Refer to *Figure 2* for zymogram patterns.

Enzyme	Abbreviation	Locus	Allele	Relative migration Rf (0-100)
Acid phosphatase	ACIDP	1	A	22
		2	A	39
			B	44
Aspartate amino transferase	AAT	1	A	44
			B	34
		2	A	53
Diaphorase	DIA	1	A	22
			B	15
Esterase	EST	1	A	20
		2	A	33
			B	38
Glucose-6-P dehydrogenase	G6PDH	1	A	55
			B	45
Leucine-amino peptidase	LAP	1	A	66
			B	61
Malate dehydrogenase	MDH	1	A	21
		2	A	31
			B	35
Peptidase leucyl-alanine	LA	1	A	66
			B	72
			C	64
Peptidase phenyl-alanyl-proline	PHAP	1	A	50
Peroxidase	PER	1	A	32 (cathodal)
			B	43 "
Phosphoglucose isomerase	PGI	1	A	31
		2	A	53
			B	44
Phosphoglucose mutase	PGM	1	A	55
		2	A	75
			B	66

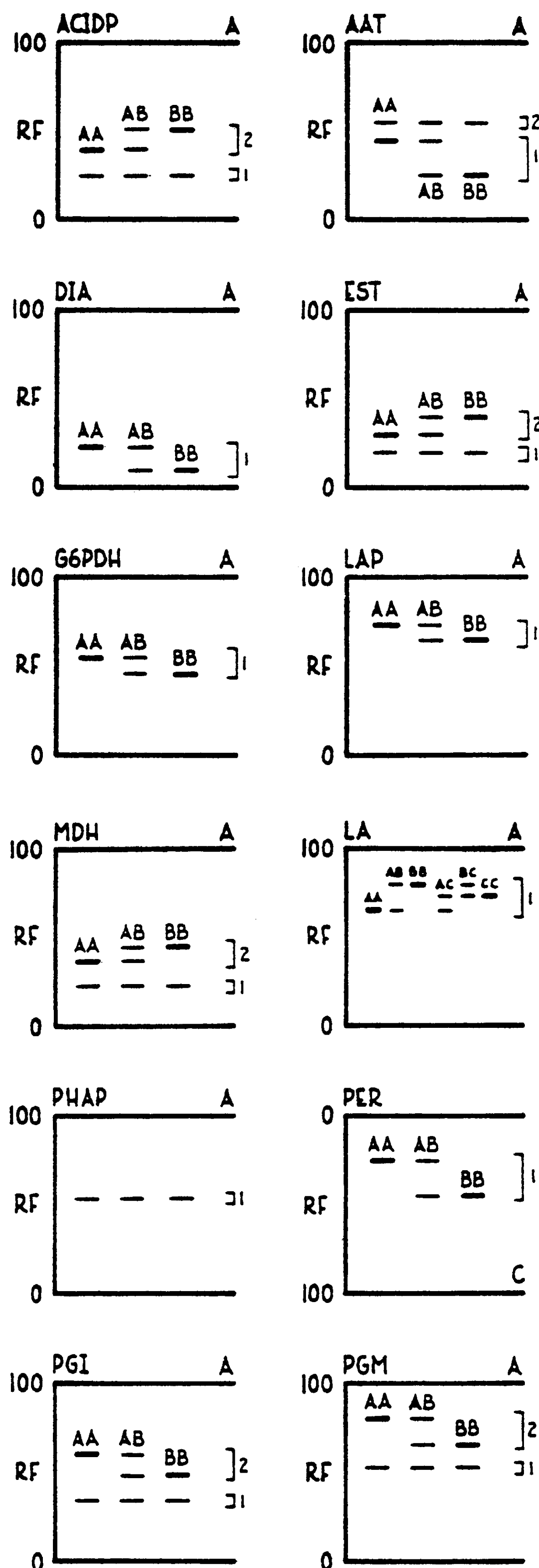

Figure 2. — Observed or expected zymogram patterns. Loci are numbered (1 or 2), and genotypes of polymorphic loci are labeled AA, AB, BB, etc. Rf = relative migration rate (0-100). A = anode, C = cathode. Refer to *Table 2* for abbreviations used and Rf values of alleles.

Gene diversity, estimated by average heterozygosity per locus (H), varied only slightly among populations *(Table 5)*. Nevertheless, some trends may be suggested by these data. The Index (IND) and Chilliwack (CHI) populations, representing two of the smaller river drainages, had the lowest gene diversity. Nisqually (NIS) and Rockport (ROC), both situated on larger drainages, showed greater diversity. Although a correspondence between the size of the drainage and genetic diversity may be expected, Longview (LON), representing the largest drainage, had a relatively low value. The proportion of polymorphic loci, another measure of gene diversity, also tended to increase with the size of the river drainage. The value for the Index population, however, was unexpectedly high. The two measures, therefore, were not always in concordance.

Average within-population heterozygosity (H_S) was much greater than between-population heterozygosity (D_{ST}), with values of .090 and .006 respectively. Total gene diversity (H_T), therefore, was equal to .096 and the coefficient of gene differentiation ($G_{ST} = D_{ST}/H_T$) was .063. In other words, only about 6% of the total gene diversity was attributable to gene differences between populations, while almost 94% of the total gene diversity was found, on the average, within any one population.

Nearly 13% of all loci sampled among the ten populations deviated significantly from expected Hardy-Weinberg proportions *(Table 6)*, and in almost all cases this was due to an excess of homozygotes. Of the 23 significant deviations, 17 were found among the five most southern populations.

Overall genetic similarity, estimated by Rogers' coefficient, was very high among the populations *(Table 7)*. For example, the genetically most distant population pair, Longview-Monroe, still had a similarity value of 0.9298. A dendrogram of population affinities, based on these coefficients, is given in *Figure 3*. Two major clusters are evident, the set of more northern populations and a group of five southern populations plus Arlington. These clusters are not discrete metapopulations, of course, but the grouping indicates that some underlying geographic component may influence the genetic similarity pattern. Although the dendrogram suggests a correspondence between geographic and genetic distance, the relationship is not statistically significant.

Table 3. — Allele frequency estimates and sample sizes for polymorphic loci. Population designations are given in *Table 1*. Nomenclature for enzymes, loci and alleles is given in *Table 2*. Sample sizes varied between 40 and 50.

Enzyme-Locus-allele	CHI	ROC	ARL	MON	IND	SNO	ORT	NIS	LON	SAN
ACIDP-2-A	0.97	0.95	0.97	0.93	0.95	0.93	0.91	0.95	1.00	0.98
ACIDP-2-B	0.03	0.05	0.03	0.07	0.05	0.07	0.09	0.05	0.00	0.02
AAT-1-A	0.47	0.38	0.32	0.61	0.29	0.25	0.24	0.24	0.20	0.20
AAT-1-B	0.53	0.62	0.68	0.39	0.71	0.75	0.76	0.76	0.80	0.80
DIA-1-A	1.00	0.99	0.99	1.00	0.97	0.98	1.00	1.00	0.95	0.99
DIA-1-B	0.00	0.01	0.01	0.00	0.03	0.02	0.00	0.00	0.05	0.01
EST-2-A	0.87	0.85	0.90	0.89	0.99	0.79	0.78	0.82	0.85	0.71
EST-2-B	0.13	0.15	0.10	0.11	0.01	0.21	0.22	0.18	0.15	0.29
G6PDH-1-A	1.00	1.00	1.00	0.96	0.95	1.00	1.00	0.87	1.00	1.00
G6PDH-1-B	0.00	0.00	0.00	0.04	0.05	0.00	0.00	0.13	0.00	0.00
LAP-1-A	0.98	0.96	1.00	0.99	0.92	1.00	1.00	0.99	0.94	0.99
LAP-1-B	0.02	0.04	0.00	0.01	0.08	0.00	0.00	0.01	0.06	0.01
MDH-2-A	0.85	0.86	0.91	0.82	0.92	0.87	0.80	0.81	0.95	0.93
MDH-2-B	0.15	0.14	0.09	0.18	0.08	0.13	0.20	0.19	0.05	0.07
LA-1-A	1.00	0.97	0.96	0.96	1.00	1.00	0.98	0.94	0.98	0.96
LA-1-B	0.00	0.03	0.03	0.03	0.00	0.00	0.01	0.05	0.00	0.01
LA-1-C	0.00	0.00	0.01	0.01	0.00	0.00	0.01	0.01	0.02	0.03
PER-1-A	0.99	0.92	0.77	0.98	0.99	0.66	0.78	0.60	0.58	0.85
PER-1-B	0.01	0.08	0.23	0.02	0.01	0.34	0.22	0.40	0.42	0.15
PGI-2-A	0.89	0.85	0.94	0.98	0.89	0.87	0.97	0.91	0.98	0.99
PGI-2-B	0.11	0.15	0.06	0.02	0.11	0.13	0.03	0.09	0.02	0.01
PGM-2-A	0.99	0.88	0.88	0.97	0.95	0.96	0.97	0.99	0.94	0.96
PGM-2-B	0.01	0.12	0.12	0.03	0.05	0.04	0.03	0.01	0.06	0.04

Table 4. — Heterogeneity G for polymorphic loci and summary of simultaneous test procedure (heterogeneity G) for polymorphic loci. Homogeneous sets of means are underlined (P = 0.05). Frequency of the most common allele (A) decreases from left to right. Population designations are described in *Table 1*.

Locus	G	Degrees of freedom	Probability
ACIDP-2	15.80	9	<0.100
AAT-1	63.16	9	<0.001
DIA-1	17.50	9	<0.05
EST-2	46.48	9	<0.001
G6PDH-1	55.40	9	<0.001
LAP-1	27.89	9	<0.001
MDH-2	22.49	9	<0.010
LA-1	29.99	18	<0.050
PER-1	164.97	9	<0.001
PGI-2	34.30	9	<0.001
PGM-2	22.65	9	<0.010

Locus										
ACIDP-2	LON	SAN	ARL	CHI	NIS	IND	ROC	SNO	MON	ORT
AAT-1	MON	CHI	ROC	ARL	IND	SNO	ORT	NIS	LON	SAN
DIA-1	MON	ORT	CHI	NIS	ROC	SAN	ARL	SNO	IND	LON
EST-2	IND	ARL	MON	CHI	ROC	LON	NIS	SNO	ORT	SAN
G6PDH-1	CHI	ORT	ROC	LON	SAN	ARL	SNO	MON	IND	NIS
LAP-1	ORT	ARL	SNO	MON	NIS	SAN	CHI	ROC	LON	IND
MDH-2	LON	SAN	IND	ARL	MON	ROC	CHI	SNO	NIS	ORT
LA-1	IND	CHI	SNO	ORT	LON	ROC	ARL	MON	SAN	NIS
PER-1	CHI	IND	MON	ROC	SAN	ORT	ARL	SNO	NIS	LON
PGI-2	SAN	MON	LON	ORT	ARL	NIS	CHI	IND	SNO	ROC
PGM-2	NIS	CHI	MON	ORT	SNO	SAN	IND	LON	ROC	ARL

Table 5. — Average heterozygosity per locus (H) and proportion of polymorphic loci. Population designations are given in *Table 1*.

	CHI	ROC	ARL	MON	IND	SNO	ORT	NIS	LON	SAN
'Average heterozygosity per locus' (H)	.07	.10	.09	.08	.07	.11	.09	.11	.09	.09
Standard error (H)	.03	.03	.03	.03	.02	.04	.03	.04	.03	.03
Average n	45.78	47.67	43.72	49.28	45.06	44.94	48.11	46.39	41.06	44.39
Proportion loci polymorphic	0.22	0.39	0.33	0.22	0.39	0.33	0.28	0.39	0.39	0.22

Table 6. — Goodness of fit to Hardy-Weinberg proportions, and deviations from expected heterozygosity. Only significant departures are shown.

Population	Locus	G	df	Probability	Observed-expected heterozygosity
Chilliwack	AAT-1	5.238	1	<0.025	-0.1726
	EST-2	10.682	1	<0.005	-0.1242
	MDH-2	3.990	1	<0.050	-0.0848
Rockport	EST-2	4.103	1	<0.050	-0.0583
Arlington	EST-2	4.549	1	<0.050	-0.596
Monroe	G6PDH-1	4.300	1	<0.050	-0.0368
Index	------				
Snoqualmie	EST-2	4.980	1	<0.050	-0.1118
	PER-1	9.839	1	<0.005	-0.2096
	PGI-2	12.279	1	<0.001	-0.1411
Orting	ACIDP-2	4.704	1	<0.050	-0.0638
	EST-2	9.338	1	<0.005	-0.1654
	MDH-2	4.866	1	<0.050	0.0842
	PER-1	4.045	1	<0.050	-0.1032
	PGM-2	5.995	1	<0.025	-0.0382
Nisqually	ACIDP-2	4.135	1	<0.050	-0.0333
	G6PDH-1	13.448	1	<0.001	-0.1530
	LA-1	11.833	3	<0.010	-0.0738
	PER-1	13.115	1	<0.001	0.2434
	PGI-2	4.549	1	<0.050	-0.0596
Longview	DIA-1	3.86	1	<0.050	-0.0450
	PER-1	26.126	1	<0.001	-0.3539
Santiam	EST-2	11.310	1	<0.001	-0.2035
	PER-1	4.361	1	<0.050	-0.0883

Discussion

The ten riparian populations studied cover a broad geographic range and, yet, very little enzymatic differentiation seems to exist among them (G_{ST} = .063). The relatively high overall genetic similarity *(Table 7)* probably reflects the occurence of periodic gene flow throughout this range.

Black cottonwood is not limited to riparian habitats; natural disturbances and especially man's activities have opened up many potential sites in both riparian and upland communities. The species' rapid growth, early sexual maturity and prolific annual seed production allow it to take advantage of these temporary opportunities. It is likely, then, that the ten populations are not permanently isolated from each other, but periodically linked by gene flow through a network of smaller stands and scattered individuals. Two characteristics of the mating system, anemophily and obligate allogamy, would augment such gene flow. The degree of gene flow in this, and other, pioneer species fluctuates in response to various populations disturbances, so that patterns of genetic similarity observed today could change dramatically over time (Feret 1974). Unfortunately, information on the historical dynamics of these riparian populations and their upland "links" is not available.

In spite of the high overall genetic similarity, the populations were not entirely homogeneous. Some allele frequencies varied with latitude (EST-2-A, PGI-2-A, LA-1-C) and longitude (ACIDP-2-A, LA-1-C, PGI-2-A), while most displayed no geographic pattern of variation.*) Furthermore, neighboring populations tended to cluster together genetically *(Figure 3)*, although the relationship between genetic and geographic distance was not statistically significant. Similar patterns of genetic relatedness and clinal variation have also been reported for populations of *Picea abies* (Lundkvist and Rudin 1977) and *Pseudotsuga menziesii* (Yang, Ching and Ching 1977). Various regimes of selection, either directly or via linkage, migration and genetic drift could produce these patterns (Endler 1977). Linkage studies and more refined environmental data would help to elucidate the importance of selection.

*) It is interesting to note that latitudinal trends were also observed in leaf shape, leaf size, and leaf placement in clones selected from these populations. Data on these and other characters will be published elsewhere.

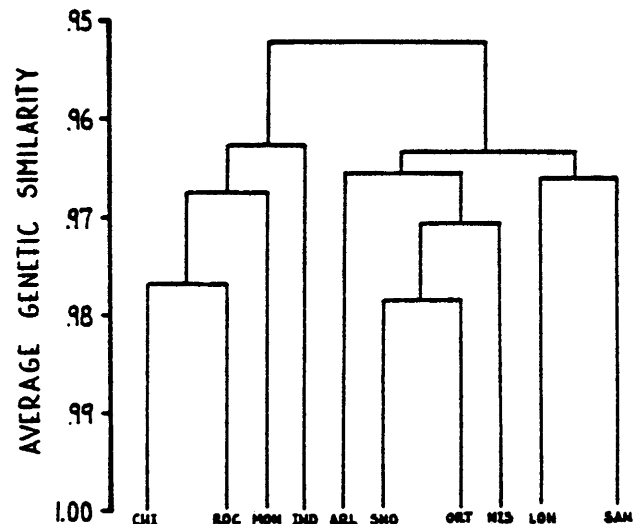

Figure 3. — Dendrogram of populations, based on Rogers' genetic similarity. Population designations are given in *Table 1.*

Table 7. — Rogers' genetic similarity among populations. Population designations are given in *Table 1.*

	CHI	ROC	ARL	MON	IND	SNO	ORT	NIS	LON	SAN
CHI	1.000									
ROC	0.9775	1.000								
ARL	0.9619	0.9739	1.000							
MON	0.9752	0.9614	0.9526	1.000						
IND	0.9680	0.9661	0.9619	0.9545	1.000					
SNO	0.9572	0.9632	0.9684	0.9408	0.9553	1.000				
ORT	0.9573	0.9598	0.9702	0.9553	0.9495	0.9794	1.000			
NIS	0.9478	0.9492	0.9569	0.9404	0.9428	0.9732	0.9692	1.000		
LON	0.9403	0.9482	0.9629	0.9298	0.9477	0.9672	0.9598	0.9626	1.000	
SAN	0.9531	0.9570	0.9667	0.9455	0.9527	0.9661	0.9748	0.9536	0.9670	1.000

The role of drift in this pioneer species may be significant. Temporally and spatially variable disturbances that reduce population size are a normal feature of both riparian and upland stand experience, although their effects may be damped by the large reproductive potential among the survivors. It is unlikely, however, that the genotype distribution in these founder populations reflects the original population variability. Two personal observations support this statement. First, individuals vary in cloning ability; if regeneration occurs primarily by sprouting from stumps or windthrown branches, the founder population may develop from a limited number of clones. Secondly, the mature sex ratio varies among populations; males generally outnumber females which are often clumped in distribution. Even within a stable environment, this unequal sex ratio would favor drift by reducing the effective size of the breeding population (CROW and KIMURA 1970). In an environment subject to periodic disturbance, the seed bank for regeneration may represent the contribution of even fewer females. Random sampling of both gametes and genotypes (seeds, and stump and branch sprouts) may, therefore, significantly contribute to drift and potential differentiation. This differentiation would be retarded, however, by periodic immigration from neighboring populations.

As discussed above, regeneration may involve a limited number of parents (or ramets) producing cohorts of genetically related progeny on newly disturbed sites. If these cohorts are sufficiently distinct genetically, samples drawn across the sites would yield a deficiency of heterozygotes, due to the Wahlund effect (CROW and KIMURA 1970). Almost all cases of within-population departure from Hardy-Weinberg proportions were attributed to a deficiency of heterozygotes *(Table 6)*. While cohort substructure is only one possible explanation for these results, it could dramatically affect the population genetic structure (WRIGHT 1969), and its existence could be readily tested with isoenzyme methods (MULCAHY 1975).

Finally, it must be emphasized that estimates of genetic variation may have been biased by our sampling scheme, which was restricted to individuals between four and twenty years old. It is interesting to note in this connection that the observed average heterozygosity and polymorphism are considerably below values commonly reported for other forest-tree species (HAMRICK 1979). Estimation could have been improved by sampling all age classes in a stratified manner. If older age classes had been included, estimates of genetic variation could have been higher or lower than observed, depending on the extent of disruptive vs. stabilizing selection. With historical information on stand development, this sampling design may provide insight into the relative importance of these two selection modes in black cottonwood populations.

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Literature

BROWN, A. H. D.: Isozymes, plant population genetic structure and genetic conservation. Theoret. Appl. Genet. 52: 145—157 (1978). — CROW, J. F. and M. KIMURA: An Introduction to Population Genetics Theory. Burgess Publishing Co., Minneapolis, Minn. (1970). — DIETERICH, J.: Erste Ergebnisse des internationalen *Populus trichocarpa* Provenienzversuches. Proc. XVI IUFRO World Cong., Oslo. Div. 11: 219—226 (1976). — ENDLER, J. A.: Geographic Variation, Speciation and Clines. Princeton University Press, Princeton, New Jersey (1977). — FERET, P. P.: Genetic differences among three small stands of *Pinus pungens*. Theoret. Appl. Genet. 44: 173—177 (1974). — FERET, P. P. and F. BERGMANN: Gel electrophoresis of proteins and enzymes. *In:* Modern Methods in Forest Genetics. J. P. MIKSCHE (ed.). Springer Verlag, Berlin, Heidelberg, New York. Pp. 49—77 (1976). — HAMRICK, J. L.: Genetic Variation and Longevity. *In* Topics in Plant Population Biology. O. T. SOLBRIG, S. JAIN, G. B. JOHNSON, and P. RAVEN (eds.) Columbia Univ. Press, New York (1979). — HEILMAN, P. E., D. V. PEABODY, JR., D. S. DE BELL, and R. F. STRAND: A test of close-spaced, short rotation culture of black cottonwood, Can. J. For. Res. 2: 456—459 (1972). — LUNDKVIST, K. and D. RUDIN: Genetic variation in eleven populations of *Picea abies* as determined by isozyme analysis. Hereditas 85: 67—74 (1977). — MULCAHY, D. L.: Differential mortality among cohorts in a population of *Acer saccharum (Aceraceae)* seedlings. Am. J. Bot. 62: 422—426 (1975). — NEI, M.: Molecular Population Genetics and Evolution. North Holland Publ. Co., Amsterdam-American Elsevier, New York, Oxford (1975). — RECK, S. G.: Growth and wood characteristics in clones of *Populus trichocarpa* grown in West Germany and their significance for selection. Proc. Joint IUFRO Meeting, Stockholm. S. 02.04.1—3. Session II: 129—143 (1974). — ROGERS, J. S.: Measures of genetic similarity and genetic distance. Studies in Genetics VII. Univ. Tex. Publ. 7213: 145—153 (1972). — RUDIN, D.: Application of isozymes in tree breeding. Proc. IUFRO Joint Meet. Advanced Generation Breeding, Bordeaux. Session 6: 145—164 (1976). — SCHREINER, E.: Production of poplar timber in Europe and its significance and application in the United States. U. S. Dept. Agr., Agr. Handb. 150: 1—124 (1959). — SOKAL, R. R. and F. J. ROHLF: Biometry. W. H. Freeman, San Francisco (1969). — STEENACKERS, V.: L'état actuel de la sélection et de la création des clones de peuplier résistantes aux diverses maladies. Second World Consultation on Forest Tree Breeding. Washington, D. C., August 7—16. Section III. 914 (1969). — STETTLER, R. F. and P. E. HEILMAN: Evaluation and genetic improvement of black cottonwood for short-rotation coppice culture. U. S. Dept. of Energy (Grant #ET-78-G-01-3065). Annual Report, 23 pp. (1980). — WEBER, J. C.: Isoenzyme variation among ten populations of *Populus trichocarpa* (TORR. et GRAY) in the Pacific Northwest. M. S. thesis. University of Washington, Seattle, Wash. (1980). — WEISGERBER, J.: Das waldbauliche Verhalten nordamerikanischer Pappelarten und ihre Eignung für den Anbau in Deutschland. Forst- und Holzwirt, Bd. 30, S. 293—343 (1975). — WEISGERBER, J.: Anzucht und bisherige Entwicklung des internationalen *Populus trichocarpa*-Herkunftsversuches; Beobachtungen des Teilversuches in Hann. Münden. Proc. XVI IUFRO World Cong., Oslo. Div. II: 192—197 (1976). — WRIGHT, S.: Evolution and the Genetics of Populations. Vol. 2, The Theory of Gene Frequencies. Pp. 290—344. University of Chicago Press, Chicago (1969). — YANG, J.-C., T. M. CHING, and K. K. CHING: Isoenzyme variation of coastal Douglas-fir. I. A study of geographic variation in three enzyme systems. Silvae Genet. 26: 10—18 (1977). — ZSUFFA, L.: A summary review of interspecific breeding in the genus *Populus* L. Proc. Meet. Can. Tree Improve. Assoc. 14 (2): 107—123 (1973).