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Annual Report 1998-99 (FY99)

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* Planned Projects

Project Submission

FY98 Report

INTRODUCTION

Message from the Director

This report details Laboratory accomplishments covering the period October 1998 to October 1999, and corresponds to the Federal 1999 fiscal year (FY99).

The National Forest Genetic Electrophoresis Laboratory (NFGEL) was established in 1988 as part of the National Forest System of the USDA-Forest Service. The focus of the lab is to address genetic conservation and management of all plant species using a variety of laboratory techniques including DNA analyses. NFGEL services are provided to managers within the Forest Service, other government agencies, and non-government organizations for assessing and monitoring genetic diversity. We work closely with research institutions to incorporate the latest technological advancements into our program.

To date, we have studied 20 different gymnosperm and 20 different angiosperm species. Angiosperms studied include woody species (such as aspen, cottonwood, and oaks) as well as grasses, shrubs, and forbs. Land management questions we study include issues of genetic diversity and structure, taxonomy, and plant identification. Our work supports tree improvement programs, conservation of plant species (particularly threatened, endangered, and sensitive species), and restoration efforts.

FY99 was a full and successful year for NFGEL.

· We processed five isozyme projects in the area of silviculture and tree improvement. Project accomplishments included identifying contaminated sugar pine seedlots, determining the origin of an isolated limber pine stand as being planted by native Americans, determining the timing of bag application and removal in pollination of loblolly pine, and characterizing the genetic diversity and relatedness in blister rust resistant sugar pine seedcrops.

· Two projects addressing conservation and restoration were also carried out in the isozyme laboratory. We characterized the genetic variation in the sensitive plant *Sisyrinchium sarmentosum* and postulated the effects of grazing on the conservation of the species, and we determined the origin of some taxonomically unknown cottonwood stands in the Pacific Northwest.

· Finally, we delved into the area of forensics. Here we applied DNA technology with the goal of matching a tree branch back to a tree. The DNA evidence will be used in a legal case to reconstruct the scene of a fire.

Mission and Purpose

The mission of NFGEL is to provide state-of-the-art molecular genetic information to the National Forests and other cooperating agencies for the evaluation and protection of our nation's genetic resource. Techniques used will be the minimum necessary to resolve the genetic question at hand.

The purpose of the Laboratory is to analyze molecular genetic markers (proteins and DNA) in plant material submitted by Forest Service employees and those from other cooperating entities. NFGEL provides baseline genetic information, determines the effect of management on the genetic resource, supports the tree improvement program, and contributes information in the support of conservation and restoration programs, especially those involving native and TES (threatened, endangered, and sensitive) species. Our services include project proposal development, sample design and collection strategy formulation, protein and DNA marker electrophoresis, data interpretation and analysis, and reports of results and management implications.

Overview

NFGEL projects were processed to meet a variety of management objectives. Project results were used to guide restoration and conservation projects, and assist in silviculture and tree improvement activities.

During this report year, we processed nine projects utilizing isozyme markers obtained with a combination of starch gel electrophoresis and isoelectric focusing. Reports for seven of these projects follow. Two of the FY99 projects (Project #82 - ponderosa pine source identification, and Project #80 - slash pine genetic diversity) remain in the analysis and reporting stage. We have also completed procedural testing for three FY00 isozyme projects. An update to the FY98 limber pine project (Project #50) is also included in this report. In addition, an update is provided for our forensics work (Project #85) using plant DNA to reconstruct the scene of a fire.

Silviculture and Tree Improvement

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(1) LIMBER PINE: TRAIL MIX OR REMNANT STAND

An isolated 208 acre stand of limber pine (*Pinus flexilis* James*)* on the Custer National Forest, Dickinson, North Dakota was studied to determine if the stand has a possible cultural origin. The stand is within the Limber Pine Research Natural Area at the lower timberline of Southwestern North Dakota where Cannonball Creek and the Little Missouri River converge. This area has 23 prehistoric sites of early peoples and it has been suggested that they may have established this site from limber pine seed that was used for food or trade. From increment borings used to map age distribution patterns, it appears that this stand has a single point of origin, one that corresponds to archeological evidence of Native American habitation. The North Dakota population is the only occurrence of limber pine in North Dakota and is a State endangered plant.

Genetic variation within and among five limber pine populations was measured at 23 allozyme loci using vegetative bud tissue collected from a total of

550 trees. Three hundred trees were sampled from the North Dakota population, 99 trees from an isolated stand near Terry, MT, 51 trees from an isolated stand in the Black Hills of South Dakota, and 50 trees from each of two stands located in the continuous range of limber pine (Pryor Mountains and Crazy Mountains of Montana).

Diversity values for the North Dakota stand are consistently low compared to all other stands. Percent polymorphic loci (99%) equals 39.1% in the North Dakota stand, 43.5% in South Dakota, 60.9% in Terry, MT, 73.9% in Crazy Mountains, and 78.3% in Pryor Mountains. Mean number of alleles per locus among the populations is 1.7, 1.7, 2.0, 2.2, and 2.3, respectively. Expected heterozygosity (H_e) values range from a low of 0.114(.039) in North Dakota to a high of 0.196(.046) in Crazy Mountains. H_e in the isolated South Dakota and Terry, MT stands are 0.145(.042) and 0.188(.048), respectively. The isolated stands are genetically differentiated (carry many genetic differences from each other) (Fst=0.099), whereas the two main range stands are genetically similar to each other (Fst=0.007).

All three isolated limber pine stands studied contain less genetic variation than the stands sampled out of the species continuous range. The North Dakota stand of limber pine contains substantially less genetic variation compared to other isolated stands. Even though, for example, the North Dakota stand is made up of thousands of trees, it still has only 80% of the genetic variation found in the South Dakota stand which is comprised of less than sixty trees.

From the genetic data, it is apparant that the North Dakota limber pine stand has experienced a time of very small population size (bottleneck) and likely expanded rapidly after the bottleneck. The North Dakota population is more similar to Montana limber pine than South Dakota limber pine, and therefore the data does not support the hypothesis that seed from the Black Hills was used to establish the North Dakota stand. The genetic data are consistent with a human origin of the North Dakota population. The extremely low levels of genetic diversity relative to other isolated stands suggests a founding population size of less that 20. However, the possibility of this stand being a remnant cannot be ruled out using genetic data alone. Regardless of the origin of this stand, human manipulation likely occurred during its establishment and may account for the observed genetic composition we see today. [NFGEL Project #50 was completed in cooperation with Lars Halstrom, USDA Forest Service, Gallatin NF].

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(2) IDENTIFYING A MIXED SUGAR PINE SEEDLOT

An individual tree seed collection was made from a blister rust resistant sugar pine tree. Seed was placed in a single container. Subsequently, it was suspected that seed from a susceptible sugar pine tree was mixed with more of the resistant tree's seed, and the possible mixed lot placed on top of the pure seed.

Using an isozyme analysis, we genotyped 30 seed (megagametophytes) each from parent trees, and material from the top and bottom of the container.

We determined that the seed near the top was a mix from the two trees (one susceptible and one resistant to blister rust), and the seed toward the bottom was a pure lot from the resistant tree. The resistant seedlot was salvaged by removing the upper mixed seed layer. [NFGEL Project #81 was completed in cooperation with David Alicea, USDA Forest Service, Region 5, Placerville Nursery, Placerville, CA].

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(3) Timing of Bag Application and Removal in Controlled Mass Pollination

[The following was presented at the 25th Southern Forest Tree Improvement Conference, New Orleans, LA, July 11-14, 1999. FE Bridgwater[1], DL Bramlett[2], and VD Hipkins. [1] USDA-Forest Service, Forest Science Lab, Texas A & M University, College Station, TX 77843-2585; 2 USDA-Forest Service (Retired), Southern Research Station, Juliette, GA 31046. NFGEL Project #66].

Abstract

Controlled mass pollination (CMP) among outstanding parents is one way to increase genetic gains from traditional wind-pollinated seed orchards, but the economic success of CMP depends on both genetic gains and costs. CMP has been shown to be cost-effective (Bridgwater et al. 1998, The Forestry Chronicle 74(2):1-5) even when costs were adjusted for risk (Byram and Bridgwater 1999, These Proceedings).

Both of these studies assumed that CMP was 100% effective. That is, there was no pollen contamination during the CMP process that would reduce the expected gains from mating outstanding parents. This assumption is not always met under operational conditions due to variable strobilus development and the limited amount of time to conduct CMP. We isolated strobili in various stages of development with and without subsequent CMP and estimated pollen contamination in the seeds produced. We found that pollen contamination can occur when bagging is delayed after female strobilus developmental stage 3, but that CMP may reduce the percentage of seeds produced from contaminating pollen to negligible levels even when bagging is delayed until stage 4.

We found that removing bags immediately after CMP when strobili were at optimum receptivity resulted in substantial (41%) contamination. Leaving bags for two days after pollination reduced the contamination percentage to 0.0%. The worst contamination (61%) occurred when female strobili were bagged at stage 3, pollinated once at maximum receptivity followed immediately by bag removal.

Laboratory Methods and Materials

Selected treatments for two crosses (81069 x 81056 and 81069 x 71022) that produced sufficient numbers of filled seeds were sent to the National Forest Genetics Electrophoresis Laboratory (NFGEL) in California for paternity analysis to determine the level of contamination for each treatment. Seeds were genotyped at 22 isozyme loci for 1,013 megagametophyte/embryo pairs. Both unambiguous and cryptic contamination levels were estimated.

Acknowledgments

We wish to thank Weyerhaeuser Co. and their Lyon's, GA seed orchard staff for their contribution to this study.

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(4) Genetic Diversity in Blister-rust Resistant Sugar Pine Seed Crops

COOPERATORS

This was a cooperative study between the California Department of Forestry and Fire Protection (CDF) (project initiator: Laurie Lippitt) and the USDA Forest Service National Forest Genetic Electrophoresis Laboratory (NFGEL). Robert W. Westfall, USDA Forest Service, PSW, Davis, CA provided mating system analysis and report of findings for the embryo data and contributed to the interpretation of all data sets. This work was conducted from funds redirected from the CDF' FY97 Cooperative Forest Health Management Grant (Control No. G-5-97-20-032).

STUDY OBJECTIVES AND FINAL CONCLUSIONS

The overall study goal is to characterize the genetics of select Mountain Home sugar pine. Project objectives and final conclusions follow.

OBJECTIVE 1

Question

Compare the genetic diversity among Mountain Home clusters. Would genetic diversity levels decrease if seed from a subset of Mountain

Home trees were used to produce seedlings? How much diversity is lost when number of trees are restricted?

Answer

Mountain Home clusters contain comparable amounts of genetic diversity and are genetically similar to each other. Genetic diversity does decrease if subsets of trees drops much below 10 (based on data from both the parents and progeny). As number of trees gets very small, alleles are lost which results in the loss of overall genetic diversity (about a 25% to 30% decrease in some estimates). Using a subset of trees from Mountain Home is ok unless those numbers get very small (less than 5 trees is too small; between 5 and 10 trees will also result in some loss of diversity).

OBJECTIVE 2

Question.

Compare the Mountain Home group of sugar pine with sugar pine from all of seed zone 534. Is genetic diversity lost if only trees from Mountain Home are used? Do resistant seed from outside of Mountain Home need to be added to broaden the genetic base?

Answer

The Mountain Home sugar pine contain similar levels of isozyme diversity than sugar pine from the rest of seed zone 534. Overall genetic diversity is not lost if only trees from Mountain Home are used. Resistant material does contain less genetic diversity as measured with isozymes than susceptible parents at Mountain Home. Although diversity levels are lower in the resistant trees, diversity is not so lacking that it appears to be imperative to bring in seed from outside Mountain Home. However, most populations of a species will include some rare, uncommon, or unique alleles. Therefore, material brought into Mountain Home would add to its genetic base. But again, based on this data, diversity levels currently in Mountain Home do not appear to necessitate the addition of outside material; bringing in material from outside would probably be beneficial, however, but is not necessary.

OBJECTIVE 3

Question

Characterize between year genetic variation in the progeny of Mountain Home sugar pine trees. Is there significant differences between years? Are many individuals contributing to the progeny or fewer? Are genetic differences dependent on the cluster in which the trees reside?

Answer

There is a significant difference among the progeny of Mountain Home sugar pine trees between years. Differences are not due to which cluster a tree resides (there is as much difference between progeny from two trees within one cluster as there is between progeny from two trees residing in different clusters). The major difference among progeny is to which family it belongs (it's mother tree). Few males, on average, are mating with each female (may be as low as 1.4 to 3 males, on average); mother trees are sampling different males; and different males contribute to families between years.

MATERIAL AND METHODS

A total of 101 sugar pine parent trees (50 resistant and 51 susceptible) were genotyped at 25 isozyme loci (Table 1).

Table 1. Number of Mountain Home parent trees genotyped per cluster.

Cluster #	no. individuals	no. resistant	no. susceptible
1	17	11	6
2	17	15	2
3	3	1	2
4	26	10	16
5	14	4	10
6*	16	7	9
10	3	0	3
11	3	0	3
unknown	2	2	0

*contains one individual from cluster 8

Ten seed per tree were prepared for analysis following NFGEL Standard Operating Procedures (SOPs). Samples were electrophoresed using three buffer systems (lithium-borate, sodium borate, and morpholine citrate, pH 6) following NFGEL SOPs. A total of 101 individuals were genotyped at 25 loci using 16 enzyme stains (PGM-1,2; ACO; ME7; PGI-2; ADH-1,2; LAP-1,2; UGPP-1; GOT-1,2,3; TPI-1; G6PD-1,2; FEST-2; MDH-2,4; SKD-1,2; IDH; GLYDH; and 6PGD-1,2).

Seed from 51 seedlots of Mountain Home sugar pine were stratified for 90 days using NFGEL Standard Operating Procedures (Table 2). After stratification, seed was germinated using NFGEL standard protocols. Germination percentages per lot ranged from 0% to 100%. Seedlots collected in 1987 from cluster 4 yielded particularly low numbers of germinated seed. Embryo tissue was prepared for isozyme analysis by dissecting an approximately 1cm-long portion of each embryo into a microtiter plate well containing 0.2M phosphate buffer. Tissue was frozen at -70C until electrophoresis. A total of 1452 embryos were genotyped at 15 loci using 12 enzyme stains (PGM-1,2; ACO; PGI-2; ADH-1; LAP-2; UGPP-1; GOT-1,2,3; TPI-1; MDH-2; SKD-1; IDH; and 6PGD-2). For statistical purposes, seedlots were chosen to provide certain numbers of trees/cluster, years/tree, and years/cluster. The choice of lots to use for the analysis was the best compromise among these factors. The overall goal was to genotype 100 embryos per year per cluster (Table 3).

Table 2. Number of Mountain Home seed lots analyzed as embryos.

					year				#embryos	#embryos
Cluster	**Tree#**	**Resistant**	**'87**	**'88**	**'90**	**'92**	**'94**	**'96**	**prepped/lot**	**prepped/tree**
1	38	Y			X	X		X	30/41/27	98
1	39	Y			X	X		X	27/25/27	79
1	61	Y		X	X	X		X	40/24/31/27	122
1	206	Y		X	X	X		X	46/32/27/27	132
2	166	Y		X	X	X		X	34/33/27/35	129
2	215	Y		X		X		X	35/27/36	98
2	218	Y		X	X	X		X	28/36/26/35	125
2	223	Y		X	X	X			27/38/27	92
4	250	Y	X						32	32
4	304	N	X						2	2
4	320	Y	X						0	0
4	339	N	X						14	14
4	321	N	X						0	0
4	305	N	X						10	10
4	322	Y			X				27	27
4	419	N			X				28	28
4	443	N			X				31	31
4	455	N			X				31	31
6	115	Y		X	X		X		32/27/50	109
6	125	N		X	X	X	X		28/29/35/45	137
6	117	Y	X						17	17
6	317	N	X						32	32
6	328	N	X						16	16
6	330	N	X						26	26
6	329	N	X						7	7
6	327	N	X						4	4
6	408	N			X				27	27
6	467	Y			X				27	27
										1452

Table 3. Number of embryos prepared for analysis per cluster per year.

			Year			
Cluster	**'87**	**'88**	**'90**	**'92**	**'94**	**'96**
'1'	0	86	113	124	0	108
'2'	0	124	107	107	0	106
'4'	58	0	117	0	0	0

'6'	102	60	110	35	95	0

A total of 41 sugar pine trees located throughout seed zone 534 were genotyped at 18 isozyme loci for comparison to Mountain Home material. Seed was provided by the US Forest Service, R5 Sugar pine Blister Rust Resistance Program. Ten seed per tree were prepared for analysis following NFGEL Standard Operating Procedures (SOPs). Samples were electrophoresed on three buffer systems (lithium-borate, sodium borate, and morpholine citrate, pH 6) using 11 enzyme stains (PGM-1,2; ACO; PGI-2; LAP-1,2; UGPP-1; GOT-1,2,3; FEST-2; MDH-2,4; SKD-1,2; GLYDH; and 6PGD-1,2).

Genetic diversity estimates, genetic structure statistics, and cluster diagram were obtained using the Biosys-1 computer program (Swofford and Selander 1989). Ritland's MLTR (Ritland 1986; Ritland 1989) was used to determine variation in pollen frequencies among clusters and trees in clusters. This program estimates outcrossing rates and correlations among progeny pairs, giving us estimates of the genetic relatedness among progenies in families. Variances in heterozygosities (Weir 1996) and in genotypic frequencies were estimated using SAS' GLM (SAS Institute Inc. 1988). Effective population sizes, based on yearly variation in allelic frequencies (Waples 1989), were estimated by SAS' IML runs.

RESULTS and DISCUSSION

MOUNTAIN HOME SUGAR PINE CLUSTERS

Genetic diversity estimates of Mountain Home parental material were calculated using allelic data from 25 isozyme loci (Table 4). Mean number of alleles per locus (A) and percentage of polymorphic loci (P) values are lowest for clusters 3, 10, and 11. However, these clusters only contain three trees each and the low diversity values are probably a reflection of small sample size.

Table 4. Genetic diversity estimates for Mountain Home sugar pine parents.

Group	N	A	P	Ho	He
OVERALL					
Species	99.6 (.6)	2.8 (.3)	84.0	.276 (.049)	.273 (.048)
BY CLUSTER					
1	16.9 (.1)	2.2 (.2)	68.0	.287 (.053)	.274 (.050)
2	16.9 (.1)	2.3 (.2)	72.0	.256 (.055)	.271 (.053)
3	2.9 (.1)	1.6 (.1)	52.0	.280 (.066)	.248 (.053)
4	25.5 (.2)	2.4 (.3)	72.0	.268 (.048)	.275 (.050)
5	13.9 (.1)	2.4 (.2)	76.0	.295 (.053)	.282 (.047)
6	15.8 (.1)	2.3 (.2)	72.0	.267 (.051)	.277 (.050)
10	2.8 (.1)	1.7 (.1)	56.0	.360 (.077)	.305 (.063)
11	2.9 (.1)	1.7 (.2)	48.0	.307 (.077)	.283 (.068)

BY RESISTANCE					
susceptible	50.1 (.4)	2.7 (.2)	84.0	.293 (.052)	.282 (.049)
resistant	49.5 (.2)	2.6 (.3)	80.0	.259 (.047)	.264 (.048)

N = mean sample size per locus

A = mean no. of alleles per locus

P = percentage of loci polymorphic (.99 criterion)

Ho = mean heterozygosity - direct count

He = mean heterozygosity - hdywbg expected

We estimated the genetic structure among clusters by measuring Fst. Fst (the percentage of the total variation measured that is found among, in this case, clusters) is 6.6%. The cluster diagram (Figure 1) shows that clusters 1,2,4,5,6,10, and 11 share 100% genetic similarity to each other. Cluster 3 shows a slight dissimilarity to the remaining clusters and shares over 98% similarity to this group.

---

x cluster 1

x

x cluster 4

x

x cluster 5

x

x cluster 6

x

x cluster 10

x

xxxxxxxxx cluster 11

x x

x x cluster 2

x

xxxxxxxxx cluster 3

****

.92 .94 .96 .98 1.0

Figure 1. Cluster diagram of eight Mountain Home sugar pine clusters using UPGMA

DISCUSSION POINT

We found high levels of diversity (as measured by isozyme variation) in the Mountain Home parents compared to other sugar pine (Conkle 1996). Conkle found expected heterozygosity for a rangewide sample of sugar pine to be .22, while H_e in the Mountain Home samples is .27. Although cluster level heterozygosities (.25-.30) are similar in value to the overall Mountain Home heterozygosity (.27), 'A' and 'P' are lower at the cluster level compared to the overall level. This indicates that there may be loss of alleles within cluster.

Clusters do not appear to be differentiated but instead contain comparable amounts of diversity. However, it should be noted that sample size per cluster is very small in some cases which may bias the results. For example, in the three clusters with sample sizes of 3, diversity values are noticeably low or high relative to the other clusters. Preferred sample size should be on the order of twenty or more individuals per cluster to avoid statistical bias. Cluster 3 shows some differentiation from the other clusters (Figure 1). However, because cluster 3 contains only three individuals, this result may not be very significant. Fst among clusters is only 6.6%, also indicating that the clusters are not differentiated.

There is a marked decrease of genetic diversity in the resistant parents compared to susceptible individuals in all estimators calculated (Table 4). It appears that alleles are being lost in resistant material compared to non-resistant. However, these results could also be the result of a biological sampling problem.

The genetic diversity in Mountain Home was compared to the diversity found in the rest of seed zone 534 (Table 5). Forty one trees located throughout seed zone 534 were used to calculate genetic diversity levels in the zone. Seed zone 534 material was genotyped at 18 isozyme loci and compared to the same 18 loci from the Mountain Home data (a subset of the full 25 loci Mountain Home data set). Of the seven loci in the Mountain Home dataset that were not used in the comparison, four loci were monomorphic, and two loci each contained one alternate allele in low frequency (accounting for the lower diversity values of the Mt Home material in Table 4 compared to those in Table 5).

Table 5. Genetic diversity estimates for Mountain Home sugar pine parents and the rest of seed zone 534. Estimates are based on 18 isozyme loci.

Group	**N**	**A**	**P**	**Ho**	**He**
Mt Home	100.6 (.1)	3.1 (.3)	88.9	.316 (.056)	.312 (.055)

Seed Zone 534	41.0 (.0)	2.9 (.3)	88.9	.293 (.055)	.297 (.054)

The percentage of total variation found between Mountain Home and the rest of seed zone 534 (Fst) is 1.0% (if O%, Mountain Home would share all the same alleles in the same frequency with seed zone 534; if 100%, Mountain Home would have no alleles in common with seed zone 534). Mountain Home shares 99.5% genetic similarity to seed zone 534 (Nei's (1978) unbiased genetic identity).

DISCUSSION POINT

The Mountain Home sugar pine trees analyzed contain similar levels of isozyme diversity as the rest of seed zone 534. Mountain Home trees have higher levels of heterozygosity and mean number of alleles per locus and the same percent polymorphic loci compared to trees outside Mountain Home but still in seed zone 534. Because of the smaller sample size in the seed zone 534 group compared to the Mountain Home group, the generally greater diversity values in Mountain Home may not be very significant. Mountain Home material is not genetically differentiated (different) from the rest of seed zone 534 (they share 99.5% genetic similarity and have a Fst value of 0.01 – both values indicating genetic uniformity as measured by isozymes).

Some estimates of genetic diversity decrease as number of trees decrease (Table 6). Genetic diversity was calculated for subsets of random Mountain Home trees ranging from 101 to 5. The mean number of alleles per locus decreased from 2.8 (.3) in a group of 101 trees, to 1.9 (.2) in a random group of 5 trees. Percent polymorphic loci also decreased from 84% in 101 trees to 64% in a random group of 5 trees. Expected heterozygosity values remained about the same regardless of group size (data not shown).

Table 6. Mean number of alleles per locus (A) and percent polymorphic loci (P) in random subsets of Mountain Home trees.

Number of Trees	A	P
101	2.8 (.3)	84
90	2.8 (.3)	84
80	2.8 (.3)	84
70	2.8 (.3)	84
60	2.8 (.3)	80
50	2.7 (.3)	80
40	2.5 (.2)	80
30	2.4 (.2)	80
20	2.2 (.2)	76
10	2.0 (.2)	68
5	1.9 (.2)	64

DISCUSSION POINT

As numbers of trees decrease to low levels, genetic diversity in two estimators decrease. When tree number gets down to less than 20, alleles begin to be lost (mean number of alleles, and percentage of polymorphic loci decrease substantially from group sizes that remain greater than 20 to 30 trees).

MOUNTAIN HOME SUGAR PINE SEED LOTS

Analyses to characterize diversity among seed crops were done over the entire sample of 1452 embryos (progeny) and grouped by cluster and year (Table 7). Estimated multilocus outcrossing rates were generally high and none were significantly different from 1.0 (meaning that material is outcrossing, not inbreeding). Although single-locus rates were not significantly different from multilocus rates, these were generally lower, suggesting some mating among relatives. In addition, outcrossing rates were not significantly different among clusters and years. However, the proportion of full-sib progeny pairs was significantly heterogeneous (by 95% confidence intervals among pairs) among clusters and years. Part of the high proportion of full-sib pairs was due to variances in allelic frequencies among clusters and years. But were this the entire cause, the population estimate would have been the largest.

The proportions of full-sib progeny pairs are extremely large and suggest that only 1.4 to 3 males, on average, are mating with each female in clusters. Variation among pollen frequencies in families is substantial, so mother trees are sampling different males (Table 8). In addition, genotypes vary among trees in cluster from year to year (see Year*tree(cluster) in Table 8), indicating that different males contribute to families from year-to-year. These data then suggest than pollen flow is very limited (flow is limited in the sense that effective pollination at greater distances is limited, not that physical movement of pollen is limited) and there is detectable variation in male fecundity.

Table 7. Estimates of mating system parameters. Estimates are: tm = multilocus outcrossing rate, ts = single locus outcrossing rate. Parental F is an indicator of inbreeding. P(full-sibs) = the proportion of full-sib progeny pairs.

GROUPING	PARENTAL F	tm (SE)	ts (SE)	P(full-sib)
Population	-0.002	0.996 (0.008)	0.972 (0.020)	0.44
Grouping:				
Cluster	-0.131			
1		0.996 (0.130)	0.996 (0.137)	0.64
2		0.991 (0.072)	0.976 (0.083)	0.34
4		0.983 (0.034)	0.953 (0.078)	0.47
6		1.024 (0.031)	0.978 (0.050)	0.62
Year	-0.163			
87		0.993 (0.051)	0.954 (0.062)	0.72
88		1.011 (0.027)	1.036 (0.051)	0.52
90		0.986 (0.017)	0.972 (0.037)	0.54
92		1.065 (0.031)	0.993 (0.036)	0.60
94		0.979 (0.345)	0.829 (0.298)	0.68
96		0.995 (0.069)	1.011 (0.064)	0.44

Table 8. Proportions of the total for sources of variation in heterozygosities and among allozyme genotypes. Residual variation for heterozygosity

contains the higher order interactions; that for variation among genotypes is the variation among pollen genotypes in half-sib families.

SOURCE	HETEROZYGOSITY	POLLEN GENOTYPES
Loci	0.1930	--
Cluster	0.0000	0.0969
Year	0.0000	0.0536
Cluster*Year	0.0000	0.0582
Tree(cluster)	0.0000	0.5409
Year*Tree(cluster)	0.0011	0.2262
Locus*Cluster	0.0119	--
Locus*Year	0.0000	--
Locus*Tree(cluster)	0.0791	--
Locus*Year*Tree(cluster)	0.0083	--
Residual	0.7066	0.2504

DISCUSSION POINT

Few males, on average, are mating with each female. Genetic diversity among families (allelic frequency in pollen contribution of seedlots) is substantial, so mother trees are sampling different males. Also, different males contribute to families from year-to-year (as indicated by genotypes differing among trees in cluster from year-to-year).

Using estimates of variance components for heterozygosities (Weir 1996) and pollen genotypes, one can determine optimal sample numbers for various levels of sampling, e.g. numbers of clusters, years, trees in clusters, and seed per tree. For the variances among heterozygosities, the optimal numbers are found in Table 9.

Table 9. Optimal sampling numbers.

SAMPLING LEVEL	NUMBER
Loci	15
Years	3
Clusters	25
Trees per cluster	7
Seed per tree	5

The optimal number of loci is the same as those used in the analysis, but these are the polymorphic loci and are a subset of the total number. However, a similar type of analysis performed on ponderosa pine, using 44 loci also showed the optimal number of loci to be about 15. Although there were no differences among clusters, there was variation among clusters by locus (Table 8). Thus, the optimal number of clusters is relatively large and

substantially greater than the number sampled in the study.

Effective population sizes (Ne) were estimated from year-to-year variation in allelic frequencies assuming census population sizes (N) of 20 and 200 (Table 10).

Table 10. Effective population sizes for two census population sizes.

N	Ne
20	8-10
200	40-90

The ranges of Ne's listed are based on the years with the greatest numbers of families and progenies: 1988, 1990, 1992, and 1996. Estimated effective sizes are about ½ to 1/5 of the census sizes. The greater the variation from year-to-year, the lower the Ne:N ratio. These ratios are in line with previous estimates from earlier work (Millar et al. 1996).

DISCUSSION POINT

To capture maximum genetic diversity in families, seed from as many clusters should be used as practical. Seed should encompass collections from several years (three is optimum based on these data), and be collected from at least 7 trees per cluster.

EXTRAPOLATION OF STUDY

The lower the frequency of MGR in a stand, the more important it is to retain non-MGR material (to preserve alleles and overall genetic diversity). Retaining non-MGR individuals is more important as you go north because there is less MGR in the north than in the south.

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(5) Searching for Segregating, Polymorphic Loci in Loblolly Pine Seedlings

In order to obtain co-dominant, segregating, polymorphic markers for inclusion in a loblolly pine research program, we attempted to resolve isozyme loci from 6-week old loblolly pine seedlings. Needle tissue was provided from 129 offspring of a selfed parent. Lab prep methods were modified to accommodate for small amounts of tissue (six different prep methods were tested on a subset of 20 seedlings).

Overall resolution was poor and only one locus out of 25 was scorable (Skd-1 was polymorphic for alleles 1 and 4). Lack of resolution was attributed to an insufficient amount of needle tissue. From prior testing, this parent may be heterozygous at Fest-1,2; Ugpp-1; Got-1,3; 6Pgd-1; Mdh-1; and Skd-1,2. It was decided that further testing can be done when seedlings had grown larger and greater amounts of tissue provided. [NFGEL Project #83 in cooperation with Claire Williams, Dept of Forest Science, Texas A&M University]

Conservation and Restoration

(1) Isozyme Variation in *Sisyrinchium Sarmentosum* (Iridaceae)

(2) Identification and Origin of Isolated Cottonwood (*Populus* sp.) Populations in the Pacific Northwest

(1) ISOZYME VARIATION IN *SISYRINCHIUM SARMENTOSUM* (IRIDACEAE)

[Wilson, B.L., Doede, D. and V.D. Hipkins. 2000. Submitted to Northwest Science. NFGEL Project #76 and #77].

Abstract

Sisyrinchium sarmentosum exhibited very little isozyme variation. The species has apparently gone through a population bottleneck (time of extremely low population size) recently. This may have coincided with the recent origin of the species. Because *S. sarmentosum* has such limited isozyme variation, isozyme variation can not be used to make management decisions regarding *S. sarmentosum* populations.

Introduction

Sisyrinchium sarmentosum Suksdorf ex Greene, Pale Blue-eyed Grass, is endemic to a small area of south central Washington and northern Oregon, in the vicinity of Mt. Adams and Mt. Hood (Gamon 1991, Henderson 1976). Over 90% of known populations occur in the Gifford Pinchot National Forest (Raven 1996). It is characterized by pale blue, rather small flowers with tepals that are not emarginate. It is a duodecaploid (12X) and hybridizes to a limited extent with duodecaploid individuals of *S. idahoense* E. P. Bicknell (Henderson 1976). *Sisyrinchium sarmentosum* reproduces vegetatively, at least over small scales, spreading by rhizomes (Raven 1996).

In 1994, a, grazing exclosure was constructed to protect this species at Cave Creek in the Mt. Adams Ranger District of the Gifford Pinchot National Forest. In 1996 the *S. sarmentosum* population was estimated to be 58% larger in the exclosure, and in 1997 it was estimated to be 8% smaller in the exclosure, but in both years sexual reproduction was estimated to be much higher in the exclosure (Raven 1996, 1997). Possibly this difference could affect the genetic variability of grazed and ungrazed populations. Therefore, the detectable genetic variation of grazed and ungrazed populations of *S. sarmentosum* was assessed using isozyme electrophoresis.

Methods

The study includes plants from the six populations of *Sisyrinchium sarmentosum* that could be relocated and were large enough for sampling (Table 1). Leaves were collected from wild plants and transported to the National Forest Genetic Electrophoresis Laboratory (NFGEL) on ice. In addition, the isozymes from the Little White Salmon River population were analyzed with isoelectric focusing (Acquaah 1992, Westermeier 1997). Variations in foliage color and tepal shape suggested that the Little White Salmon River population might contain *S. idahoense*, *S. sarmentosum*, and their hybrids.

Table 1. *Sisyrinchium sarmentosum* populations used in this study. All populations were located in Skamania County, Washington, in the Mt. Adams

Ranger District of the Gifford Pinchot National Forest in Washington. Except as noted, the major grazing animals were cattle. FS Rd. = Forest Service Road. TRS = township, range, and section (parts of a legal description of property).

Population	Location	TRS	Collection Date	Grazing Status	Sample Size
Cave Creek	north of FS road 8631, ca. 1/2 mile south of the junction of Rds. 8631 & 6820	T5N R9E S11 NE1/4 of NE 1/4			
inside fence			2 July 1998	light for 3 years	35
outside fence			30 June 1998	Intense	35
Cayuse Meadows	ca. 400 feet north of Cayuse Meadow and west of Meadow Creek	T7N R8E S10 SW 1/4 of SE 1/4	24 July 1998	light (big game)	35
Peterson Prairie	northwest section of meadow of this name	T6N R9E S27	9 July 1998	none until fall	35
		SW 1/4 of SE 1/4			
South Prairie	north end of meadow, ca. 0.6 miles south of junction of FS Rds. 860 & 8620.130	T5N R9E S17 NE 1/4	10 July 1998	light to moderate	35
Little White Salmon River	small meadow south of FS Rd. 18, ca. 0.25 miles south of jct. of Rds. 18 and 18.068	T4N R9E S15 SE 1/4 of SW 1/4	19 July 1998	light	35

Sample preparation: Samples were prepared using NFGEL standard operating procedures (Anon. 1995). A leaf sample approximately 8 - 11 cm long was ground in a cold mortar with 400 microliters of a Tris citrate buffer pH 7.5 (Gottlieb 1981). The resulting slurry was transferred to wells in a microtiter plate and stored at -70 ºC. During March 1998, the slurry was prepared for electrophoresis by thawing it and absorbing it onto 3 mm wide wicks prepared from Whatman 3mm chromatography paper.

Electrophoresis. Methods of electrophoresis are outlined in Anon. (1995), and follow the general methodology of Conkle et al. (1982) except that most enzyme stains are somewhat modified. A lithium borate electrode buffer (pH 8.3) was used with a Tris citrate gel buffer (pH 8.3) (Conkle 1982) to resolve the enzymes alcohol dehydrogenase (ADH), fluorescent esterase (FEST), leucine aminopeptidase (LAP), malic enzyme (ME7), phosphoglucomutase (PGM), phosphoglucose isomerase (PGI). A sodium borate electrode buffer (pH 8.0) was used with a Tris citrate gel buffer (pH 8.8) (Conkle et al. 1982) to resolve glutamate-oxaloacetate transaminase (GOT), glucose-6-phosphate dehydrogenase (G6PDH), triosephosphate isomerase (TPI), and uridine diphosphoglucose pyrophosphorylase (UGPP). A morpholine citrate electrode and gel buffer (pH 6.1) (Conkle et al. 1982) was used to resolve diaphorase (DIA), isocitrate dehydrogenase (IDH), malate dehydrogenase (MDH), phosphogluconate dehydrogenase (6PGD). All enzymes were resolved on 11% starch gels. For PGI and TPI, two regions of activity (presumed loci) were observed, making a total of 16 loci surveyed. Enzyme stain recipes for enzymes follow Anon. (1995) except that GOT was stained using the recipe from Wendel and Weeden (1989). Two people independently scored each gel. When they disagreed, a third person resolved the conflict. For quality control, 10% of the individuals were run and scored twice.

Data Analysis. *Sisyrinchium sarmentosum* is duodecaploid (Henderson 1976). Therefore, a single band may represent the activity of enzymes

coded by up to twelve loci on 12 chromosomes. Although most isozyme patterns were simple and invariant, some enzymes exhibited multiple bands (Table 2). Because of lack of crossing studies to determine the inheritance of bands in this species, we were unable to identify specific alleles and loci for certain enzymes. Therefore, gels were scored for banding pattern differences and band presence/absence. This type of data results in a phenotypic instead of genotypic analysis.

Phenotypic diversity measures were calculated from both band presence/absence and multi-band patterns (Table 3). For presence/absence data, phenotypic diversity was measured by a polymorphic index (PI), based on the frequency of occurrence of each band. For multi-band patterns, phenotypic diversity measures include: (1) the number of bands found in each plot, (2) percent of stains that yield more than one band pattern, (3) the average number of band patterns per stain in each plot, and (4) Shannon-Weaver Diversity Index values (Shannon and Weaver 1949). The Shannon-Weaver Diversity Index uses the frequency of each band pattern in each plot. The larger the Shannon-Weaver Index, the more diverse the plot. The distribution of the total variation within and among plots was determined by partitioning the total Shannon-Weaver Diversity Index. Band pattern frequencies and the Shannon-Weaver Diversity Index were calculated using Popgene (Yeh et al. 1997), using the codominant marker and haploid settings. The phenotypic relationships among plots were determined by calculating Hedrick's phenotypic identities (Hedrick 1971) for multi-band pattern data, and by cluster and principle coordinate analyses of Jaccard's Similarity Index for band presence/absence data (Chung et al. 1991, Rolf 1987).

Because isozymes in the Little White Salmon River population were invariant, the samples were analyzed as a single population.

Isoelectric focusing. Methods of isoelectric focusing follow the procedures outlined in Anon. 1994-1998. Polyacrylamide gels were stained for the enzymes ACP, DIA, EST, MDH, PER, and PHI, using the buffers ES5480, FS5080, VG1110, and VG1080 (Anon. 1994-1998), for a total of sixteen stain/buffer combinations. Complicated multi-band patterns were observed for all these enzymes. Observations were recorded on the occurrence of isozyme variation in eleven samples identified as *S. idahoense*, three as *S. sarmentosum*, and twelve as hybrids.

Results

One individual from South Prairie was removed from the analysis because it had unique patterns at 15 of the 16 loci sampled; it was presumed to be of some species other than *S. sarmentosum*.

Five of the six *S. sarmentosum* populations tested using starch-gel electrophoresis were monomorphic for all 16 enzymes examined (Table 2). One of these (Cayuse Meadow) was monomorphic for a TPI1 band pattern found in no other sampled population (Table 3). One population (South Prairie) was polymorphic at two enzymes, PGI2 and TPI2.

The isoelectric focusing gels of the Little White Salmon River population revealed individual variation in EST and PER. Variation was observed within the *S. idahoense, S. sarmentosum,* and hybrid groups in four of the sixteen enzyme/buffer combinations tested. All three groups shared the same variations; no variations were unique to any one groups. The most common isozyme bands were shared among all individual samples in all three groups; the variants were rare bands that occurred in addition to the common set.

Discussion

Isozyme variation in *S. sarmentosum* (as detected in starch gel electrophoresis) is extremely limited. Although the phenotypic analysis generated here differs from the genetic analyses compiled by Hamrick and Godt (1990), the comparison is instructive. *Sisyrinchium sarmentosum* appears to have much less isozyme variation seen in the average narrowly endemic taxon. For example, the 19% polymorphic loci observed among the *S. sarmentosum*

contrasts with the 26% average for endemic species (Hamrick and Godt 1990) and the 80% polymorphic loci observed in the only known population of *Hackelia venusta* (Piper) St. John *sensu stricto* (Harrod et al. MS), a species endemic to the same area of Washington. The variation in *S. sarmentosum* is also lower than that in other *Sisyrinchium* species of the Pacific Northwest (Table 4), and the variation in those species was considered low (Pavek 1989).

Isoelectric focusing of enzymes known to be highly variable and to exist in multiple forms revealed limited variation among individuals but no consistent differences among *S. idahoense, S. sarmentosum,* and their hybrids, in the Little White Salmon population. Identity of individual samples could not be checked because voucher specimens were not collected. Assuming the identifications were correct, this uniformity of isozyme variation across species lines provided further evidence that *S. sarmentosum* was derived recently, and suggested a recent common ancestor with (or within) *S. idahoense.*

Why are *S. sarmentosum* isozymes so nearly invariant? The lack of rare alleles suggests a recent population bottleneck affecting the entire species (Luikart et al. 1998). Perhaps the species arose only recently. *S. sarmentosum* is self-compatible and capable of clonal spread (Henderson 1976), but its isozyme variation is low even for a selfing taxon (Hamrick and Godt 1990); selfing or clonal spread alone is not enough to explain the observed lack of variation. The fact that the Cayuse Meadows population is monomorphic for a TPI band found in no other population suggests that rare cases of long distance dispersal (and therefore population bottlenecks) may structure the genetic variation in this species, but the evidence is slim.

Using isozyme variation (revealed by starch-gel electrophoresis) to answer life history or management-related questions about *S. sarmentosum* was impossible because of the low level of isozyme variation seen in all populations. Did grazing affect isozyme variation? There was no variation to affect. Did the plant reproduce primarily by clonal spread, or by seeds? Because there was so little isozyme variation, it was not possible to determine if samples resulted from clonal spread or sexual reproduction. Once a population enters a bottleneck, it looses genetic variation, no matter how it reproduces; sexual or asexual reproduction by invariant parents yields invariant offspring. What population sizes are necessary to preserve all the isozyme variation present in *S. sarmentosum?* Only general answers proved possible. In qualitative terms, many populations should be preserved because some (like Cayuse Meadows) may have unique alleles. Due to the low frequency of the rare alleles, a large population is necessary to preserve isozyme variation at South Prairie, the only population known to be variable. If rare alleles occur in the other populations, they are extremely rare and can be preserved only if populations remain large. Other markers, such as morphological characters or DNA-based measures (ISSRs, RAPDs, etc.) may detect enough variation to provide a firm basis for management decisions.

Table 2. Band pattern frequencies for *Sisyrinchium sarmentosum* populations. "Band Patterns" are the bands present (1) or absent (0) for each isozyme pattern. "Outside" and "inside" refers to the location relative to the grazing exclosure. * = ungrazed.

Enzyme	Pattern	Cave Creek: outside	Cave Creek: inside	Cayuse Meadows	Peterson Prairie	Little White River	South Prairie	Band Patterns
ADH	A	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1111
DIA	A	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	11
FEST	A	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	11
GOT-1	A	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1
G6PDH	A	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1
IDH	A	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1
LAP	A	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1
MDH	A	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1
ME7	A	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1

6PGD	A	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	11
PGI1	A	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1
PGI2	A	1.0000	1.0000	1.0000	1.0000	1.0000	0.9091	110
	B	0.0000	0.0000	0.0000	0.0000	0.0000	0.0909	111
PGM	A	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	11
TPI1	A	1.0000	1.0000	0.0000	1.0000	1.0000	1.0000	01
	B	0.0000	0.0000	1.0000	0.0000	0.0000	0.0000	11
TPI2	A	1.0000	1.0000	0.0000	1.0000	1.0000	0.9118	1110
	B	0.0000	0.0000	0.0000	0.0000	0.0000	0.0882	1111
UGP	A	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	11

Table 3. Genetic variation in *Sisyrinchium sarmentosum* populations, revealed by isozymes. N = mean sample size/enzyme. %P stains = percent polymorphic stains. P.I. = polymorphic index. S.W. = Shannon-Weaver diversity index. s.d. - standard deviation.

Population	**N**	**# bands**	**# unique bands**	**%P loci**	**Patterns/locus (s.d.)**	**P.I.**	**S.W.**	**% S.W. among populations**
Grazed	104	29	2	18.75%	1.1250 (0.341)	0.1675	0.0225	0.0202
Cave Creek	35	27	0	0.00%	1.0000	0.0000	0.0000	
South Prairie	34	29	2	12.50%	1.1250 (0.342)	0.3261	0.0377	
Ungrazed	70	28	1	0.00%	1.0625 (0.250)	0.3916	0.0363	0.0625
Cave Creek Exclosure	35	27	0	0.00%	1.0000	0.0000	0.0000	
Cayuse Meadows	35	27	1	0.00%	1.0000	0.0000	0.0000	
Peterson Prairie	35	27	0	0.00%	1.0000	0.0000	0.0000	
Little White Salmon River	26	27	0	0.00%	1.0000	0.0000	0.0000	
Total	200	30		18.75%	1.1875 (0.403)	0.3480	0.0387	0.1070

Table 4. Genetic variation in *Sisyrinchium sarmentosum* populations, revealed by isozymes, and in a rare *Iris*. N = mean sample size/enzyme. %P stains = percent polymorphic stains. Pops = number of populations in the study. Loci: see text. S.W. = Shannon-Weaver diversity index. Data for *S. montanum* and *S. septentrionale* reanalyzed as band patterns from Pavek (1989), with all high polyploid populations lumped at *S. montanum.*

Population	N	Pops	Enz.	Loci	%P loci	Patterns/locus (s.d.)	S.W.	Source
S. montanum	36	5	13	24	54.17	1.9583 (1.367)	0.2661	Pavek 1989
S. septentrionale	6	1	13	24	20.83	1.2500 (0.532)	0.1417	Pavek 1989
S. samentosum	200	6	14	16	18.75	1.1875 (0.403)	0.0387	this study
Iris lacustris	229	9	10	22	0.00	1.0000	0.0000	Simonich & Morgan 1994

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(2) IDENTIFICATION AND ORIGIN OF ISOLATED COTTONWOOD (*POPULUS* SP.) POPULATIONS IN THE PACIFIC NORTHWEST

(Excerpts from the NFGEL Project Report prepared by Barbara L. Wilson and Valerie D. Hipkins. NFGEL Project #75 was conducted in cooperation with Paul Berrang, USDA Forest Service, Winema National Forest, Klamath Falls, Oregon and Duane Ecker, USDA Forest Service, Ochoco National Forest, Prineville, Oregon).

MANAGER'S SUMMARY

Question 1: What species is the unknown cottonwood of the Pacific Northwest?

Populus deltoides, probably the Plains Cottonwood, *P. deltoides* var. *occidentalis.*

Question 2: Is it native to the Pacific Northwest?

Isozymes alone can't answer that question. However, odds are the tree is native. Evidence?

It's genetically variable. Therefore, either it's native or it has been introduced repeatedly.

Of the sampled populations, the Montana populations of Plains Cottonwood are most similar to the Unknown cottonwood. Therefore, it's native or was introduced from the populations of the east slope of the Rockies (the nearest sampled populations).

Some stumps are large and therefore old. This suggests either the trees are native or the introductions occurred long ago.

Cottonwood seed is only viable for about two weeks. Therefore, it could not have been introduced repeatedly during early settlement, although it could

have been introduced repeatedly after railroad connections with the east were established in the 1870's.

Interesting incidental observations:

There is evidence of introgression from Black and perhaps Narrow-leaf Cottonwood into the Pacific Northwest populations of Plains Cottonwood.

The Juntura population collected on the Jones ranch appears to be a single large clone of hybrid origin. Large clones are unusual in cottonwood, although common in the related aspens.

ABSTRACT

Some of the cottonwoods in the Pacific Northwest resemble Eastern Cottonwood *(Populus deltoides* var. *deltoides),* Plains Cottonwood *(P. d.* var. *occidentalis),* and Fremont's Cottonwood *(P. fremontii),* but are disjunct from known ranges of those taxa. The Pacific Northwest cottonwoods are sympatric with Black and Narrow-leaf Cottonwoods. Isozyme analysis of all five of these taxa was used to identify the Pacific Northwest cottonwoods to species, to characterize the genetic variation in these taxa, and to determine whether the Pacific Northwest Cottonwoods are native or introduced. Isozyme analysis identified the Pacific Northwest populations as Plains Cottonwood. The Pacific Northwest Cottonwoods were genetically variable and most closely resembled Montana populations of Plains Cottonwood. Introgression from Black and perhaps Narrow-leaf Cottonwood contributed to the variability of the Pacific Northwest Cottonwood. One Pacific Northwest population represented by nineteen samples was probably a hybrid that spread clonally. Isozyme analysis alone could not rule out the hypothesis that the Pacific Northwest cottonwoods were introduced. However, the hypothesis that the trees are native appears more plausible.

INTRODUCTION

Cottonwood is an important component of riparian ecosystems in the Pacific Northwest, and its significance for hydrology and wildlife far exceeds the area it covers. Most of the cottonwood in this area is Black Cottonwood *(Populus trichocarpa* Torr. & Gray), and some is Narrow-leaf Cottonwood *(P. angustifolia* James). However, the drainages of the Snake and Columbia Rivers in eastern Oregon and Washington support cottonwoods that are obviously a different species. The identity and origin of these plants has been a matter of debate. These trees have triangular leaves and flat petioles like those of Eastern and Plains Cottonwood *(P. deltoides* Marsh.) or Fremont Cottonwood *(P. fremontii* S. Watson). The plants may be Plains Cottonwood *(P. deltoides* var. *occidentalis* Rydb.) (Jeffery Braatne, MS), and they differ morphologically from typical Eastern Cottonwood *(P. deltoides* var. *deltoides)* (William Randle, pers. com.). Plains Cottonwood grows from the east slope of the Rocky Mountains east, and Fremont Cottonwood grows in California and the southwest (Little 1971). The range of the unknown cottonwood is disjunct from the known ranges of these two species by 300 to 500 km.

For consistency, the unknown Pacific Northwest cottonwood is referred to as the PNW cottonwood. We use the names Eastern and Plains Cottonwood for *P. deltoides* var. *deltoides* and *P. deltoides* var. *occidentalis,* respectively, and use the name *P. deltoides* when referring to that entire species, including both varieties.

Although historical information on cottonwood in eastern Oregon is limited, most experts agree that its population and range have declined. Land managers in the area are interested in restoring cottonwood to riparian areas of dry eastern Oregon. The PNW cottonwood is well suited for such restoration projects. The PNW cottonwood is more common than Black Cottonwood in the hottest, driest parts of eastern Oregon. Juvenile growth of the PNW cottonwood is much faster than that of Black Cottonwood, making the PNW cottonwood easier to establish on degraded sites. However, land managers are reluctant to use the PNW cottonwood for restoration if it was introduced from the east or southwest.

The origin of PNW cottonwood is controversial. It may be native (Jeff Braatne, pers. com.) but it may be introduced, perhaps from multiple sources (Steven J. Brunsfeld, pers. com.) The PNW cottonwood is certainly not a recent introduction. Sprouts have been observed growing from stumps thought to be over 100 years old (Duane Ecker, pers. com.). If cottonwoods were first established in the Pacific Northwest during early settlement, they must have been introduced as seeds. Introduction of live trees into Oregon by covered wagon was an arduous task, and was initially limited to grafted fruit trees. The Oregon Trail, early railroads, and modern highways all run along rivers that are both sources of cottonwood seed and habitats for seedling establishment. Accidental introduction of cottonwood seed by covered wagon is unlikely because cottonwood seeds have limited viability; seeds of southern populations of *P. deltoides* survive approximately two weeks at ambient temperatures (Farmer 1976). However, rail connections between Oregon and the east were established in 1872, permitting accidental introduction by the light, windborne Plains Cottonwood seeds that must have coated the west-bound trains in spring. In addition, the railroads permitted deliberate introduction by seed or cuttings. During the 1880's, an astonishing variety of plants were available to western horticulturalists through catalogue sales.

This isozyme study was undertaken to clarify the taxonomy of the Pacific Northwest cottonwood, characterize its population genetics, and determine if it is native or was introduced during or after American settlement. Isozymes of the PNW cottonwood were compared with those of Black, Narrow-leaf, Fremont, Eastern, and Plains Cottonwoods. Determining the specific identity of the PNW cottonwoods using isozymes is straightforward. Determining its origin is more difficult. Any possible results of isozyme analysis could be predicted by some hypothesis of either native or introduced origin.

Genetic variation and relationships predicted by various hypotheses about the origin of the Pacific Northwest cottonwoods.

	If genetic variation is high . . .	**If genetic variation is low . . .**
. . . and populations are similar to those of the east slope of the Rocky Mountains . . .	Native: PNW trees are derived from the same ancestors as those of the east slope, and populations have always been of medium to large size. Introduced: PNW populations were introduced repeatedly from the east slope, and some introductions occurred a century ago.	Native: PNW populations are derived from the same ancestors as those of the east slope, but lost variation because they were isolated and very small at times. Introduced: PNW populations were introduced rarely and from the east slope.

. . . and populations are different from those of the east slope of the Rocky Mountains . . .	Native: PNW trees have been isolated from other *P. deltoides* populations, and became different. Introduced: PNW populations were introduced repeatedly, but many of the founders derived from locations other than the east slope.	Native: PNW populations are isolated remnants and have been very small for prolonged periods. Introduced: Cottonwoods were introduced rarely, and source populations were (usually) not on the east slope.

Isozyme analysis alone can not determine if the PNW cottonwoods are native or introduced. However, isozyme analysis may eliminate some of these hypotheses. Then other considerations, including the limited period of viability for cottonwood seed, may help eliminate remaining hypotheses.

METHODS

Leaf samples were collected at various locations in the Pacific Northwest and elsewhere.

Collection localities for cottonwoods used in this study. * = clone bank samples were originally collected from multiple states.

Taxon	**Acronym**	**State**	**Location**	**Date**	**Collector**
Black	C	OR	Big Summit Ranger District, Ochoco National Forest	10 June	
Black	M	OR	near Mitchell, on Highway 26, at mile post 67	15 June	
Black	SA	WA	Satus, along Highway 97, 10 – 18 miles south of Toppenish	18 May	Duane Ecker
Black	W	OR	Santiam Pass, along Highway 20, Willamette National Forest	26 & 27 May	Duane Ecker
Narrow-leaf	F	OR	Fish Lake, Steens Mountains	16 June	Duane Ecker
Narrow-leaf	LAN	MT	Laurel, about 20 miles east of Billings	18 May	Lars Halstrom
Narrow-leaf	PK	OR	on the Burns District of the Bureau of Land Management, west of the Alvord Desert	17 June	Paul Berrang
Fremont	A	AZ	near Flagstaff	16 July	Jonathan Horton
Fremont	L	NV	Lahantan, near Reno	6 May	Paul Berrang
Fremont	R	CA	Red Bluff	5 May	Paul Berrang
Eastern	K	KY	eastern Kentucky	July	Randy Rousseau
Eastern	N	*	clone bank; upper Midwest	July	Rick Hall
Eastern	S	*	clone bank at LSU's Ben Hur Farm; Mississippi delta and south Louisiana	11 May	Mike Stein

Plains	CZ	NE	Chadron; Halsey National Forest	July	
Plains	LA	MT	Laurel, about 20 miles east of Billings	18 May	Lars Halstrom
Plains	PO	MT	Laurel, about 20 miles east of Billings	18 May	Lars Halstrom
PNW	JJ	OR	Jones Ranch, Juntura, Malheur County; population occupying less than 0.4 hectare along abandoned railroad at the Malheur River	20 May	Duane Ecker
PNW	O	OR	Owyhee, Ochoco National Forest	19 May	Duane Ecker
PNW	ON	OR	bridge over the Snake River near Ontario	19 May	Duane Ecker
PNW	P	WA	Plymouth	18 May	Duane Ecker
PNW	SU	OR	Succor Creek State Recreation Area on the Vale District, Bureau of Land Management	19 May	Duane Ecker

Plants in Montana were collected from 0.4 km long stretches of the Yellowstone River. Each sample in the East S population was collected from a different clone in a clone bank. The Oregon populations were sampled from low branches or root sprouts of different, sometimes adjacent trees. For other populations, no information on sampling methods is available. Samples were placed in plastic bags with wet paper towels and kept on ice in the field. All samples except those from Nebraska were healthy on arrival at the National Forest Genetic Electrophoresis Laboratory. The Nebraska samples experienced some freezing damage, presumably due to contact with ice during transit.

Electrophoresis. Methods of electrophoresis are outlined in Anon. (1995), and follow the general methodology of Conkle et al. (1982) except that most enzyme stains are modified. Individuals were genotyped at 20 loci. All enzymes were resolved on 11% starch gels. Enzyme stain recipes for enzymes follow Anon. (1995) except that GOT was stained using the recipe from Wendel and Weeden (1989). Two people independently scored each gel. When they disagreed, a third person resolved the conflict. For quality control, 10% of the individuals were run and scored twice.

Data Analysis. Genetic interpretations were inferred directly from isozyme phenotypes based on knowledge of the generally conserved enzyme substructure, compartmentalization, and isozyme number in higher plants (Gottlieb 1981, 1982; Weeden and Wendel 1989). These cottonwoods are diploid with 19 pairs of chromosomes (Larson 1986, Moldenke 1973, Smith 1943), but may be diploidized ancient polyploids (Bialobok et al. 1976). In cottonwoods, most isozyme variants show Mendelian inheritance (Müller-Starck 1992, Rajora 1990a).

Results were analyzed using Popgene version 1.21 (Yeh et al. 1997). A locus was considered polymorphic if an alternate allele occurred even once. Statistics calculated included unbiased genetic distances (Nei 1978), expected heterozygozity (Nei 1973), expected number of alleles/locus (Kimura and Crow 1970), and gene flow (Nm = 0.25[1/Fs]/Fst; Slatkin and Baron 1989). A dendrogram was generated in Popgene using UPGMA and Nei's unbiased genetic distances. F statistics (Wright 1978) for taxa were generated using Biosys-1, version 1.7 (Swofford and Selander 1989).

RESULTS

All populations were moderately variable.

Summary of genetic diversity measures in cottonwood. All = overall statistics for the study. Pop. = population acronym. N = mean number of

individuals sampled per locus, per population. %P = percent of all loci that are polymorphic. A = average number of alleles per locus. ne = Effective number of alleles (Kimura & Crow 1964). Ap = the average number of alleles per polymorphic locus. Ho = observed frequency of heterozygotes. He = frequency of heterozygotes expected under Hardy-Weinberg equilibrium conditions. Fis = proportion of genetic variation found among individuals within populations. Fit = proportion of genetic variation found among individuals within the total variation of that taxon. Fst = proportion of variation found among populations within the total variation of that taxon. Nm = calculated gene flow between populations within that taxon.

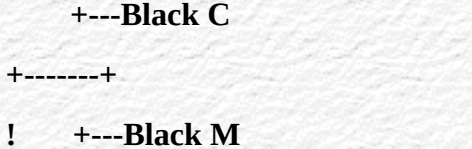
Taxon	Pop.	N	%P	A	ne	Ap	Ho	He	Fis	Fit	Fst	Nm
All cottonwoods		619.0	95%	3.1	1.64	3.21	0.1015	0.3013	0.289	0.679	0.548	0.1966
P. angustifolia		62.5	65%	2.10	1.59	2.69	0.1554	0.2680	0.061	0.430	0.394	0.3819
	F	32.0	45%	1.6	1.39	2.33	0.1741	0.1891				
	LAN	4.5	30%	1.4	1.25	2.33	0.1150	0.1405				
	PK	26.0	40%	1.6	1.34	2.62	0.1462	0.1554				
P. fremontii		97.0	55%	1.9	1.30	2.50	0.0855	0.1471	0.314	0.427	0.165	0.6798
	A	29.0	45%	1.8	1.36	2.67	0.0798	0.1458				
	L	40.0	25%	1.44	1.16	2.60	0.0875	0.0802				
	R	32.5	40%	1.6	1.27	2.62	0.0889	0.1487				
P. trichocarpa		136.0	75%	2.3	1.50	2.62	0.1457	0.2603	0.313	0.443	0.189	1.0851
	C	38.0	50%	1.6	1.30	2.20	0.1433	0.1781				
	M	27.0	55%	1.8	1.35	2.36	0.1197	0.1989				
	SA	38.5	75%	2.2	1.52	2.53	0.1667	0.2698				

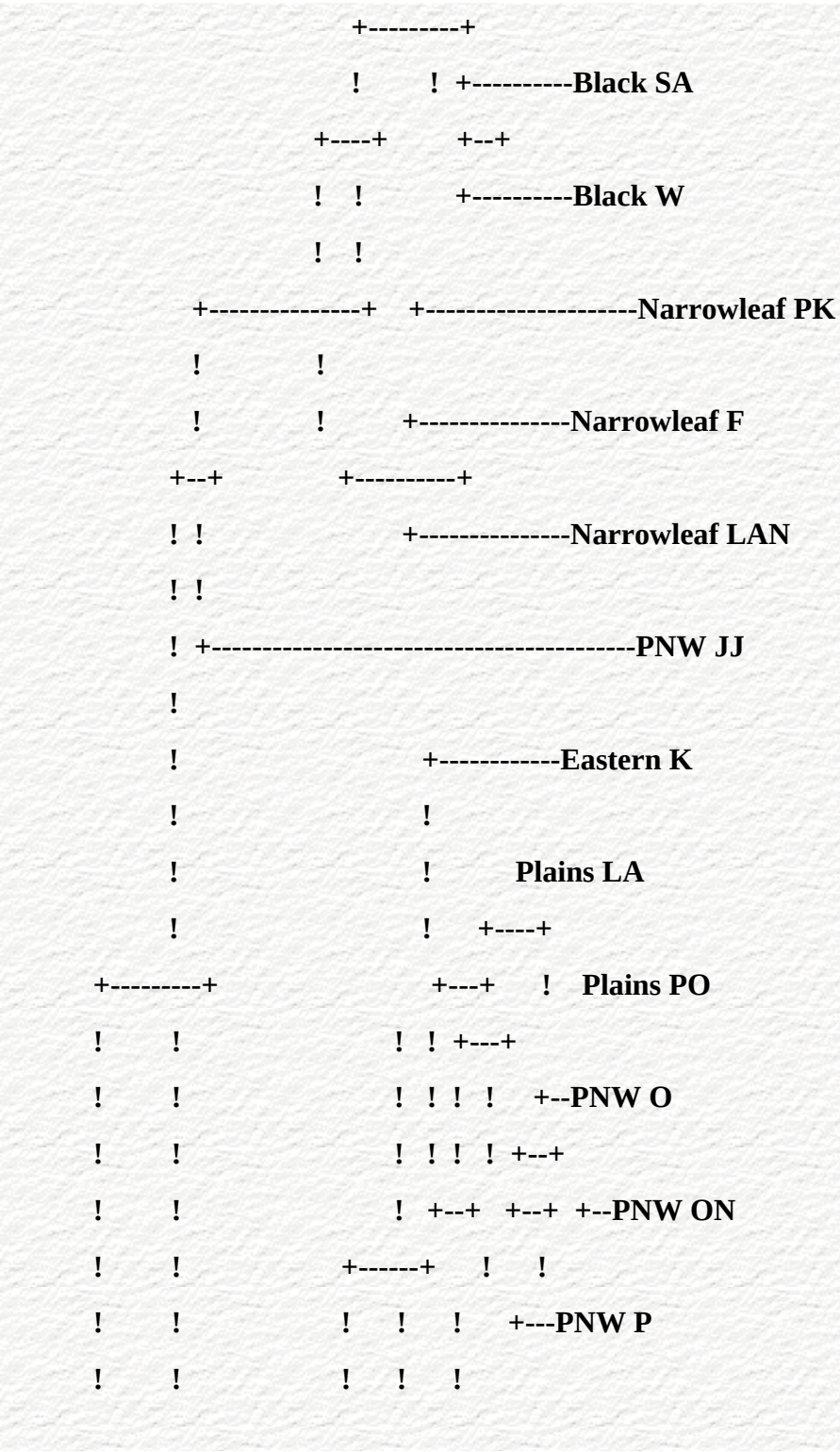
	W	33.0	55%	1.9	1.32	2.64	0.1348	0.1887				
***P. deltoides* – all**		323.0	85%	2.7	1.40	3.00	0.0784	0.2236	0.347	0.685	0.518	0.2268
(without JJ)		304.0	85%	2.7	1.34	3.00	0.0736	0.1944	0.4227	0.6602	0.4209	0.3440
P. deltoides* v. *deltoides		91.5	60%	2.1	1.42	2.69	0.0800	0.2125	0.434	0.647	0.377	0.3354
	K	31.5	45%	1.8	1.29	2.67	0.0537	0.1550				
	N	37.0	50%	1.9	1.37	2.80	0.0958	0.1747				
	S	28.0	20%	1.6	1.23	3.25	0.0955	0.0938				
P. deltoides.* v. *occidentalis		99.5	50%	1.8	1.15	2.60	0.0565	0.1028	0.339	0.528	0.286	1.1888
	CZ	24.5	40%	1.7	1.26	2.75	0.0925	0.1375				
	LA	39.0	30%	1.4	1.11	2.17	0.0437	0.0708				
	PO	36.0	25%	1.3	1.11	2.20	0.0437	0.0681				
PNW		132.0	80%	1.2	1.43	2.81	0.0907	0.2355	0.297	0.629	0.472	0.3124
(without JJ)		113.0	80%	2.4	1.32	2.18	0.0800	0.1798	0.455	0.569	0.210	1.1430
	JJ	19.0	20%	1.3	1.18	2.50	0.1500	0.0870				
	O	29.0	40%	1.6	1.29	2.62	0.0776	0.1637				
	ON	38.0	55%	1.8	1.25	2.45	0.0751	0.1421				
	P	39.0	75%	2.4	1.27	2.80	0.0868	0.1743				
	SU	7.0	40%	1.6	1.14	2.38	0.0714	0.1049				

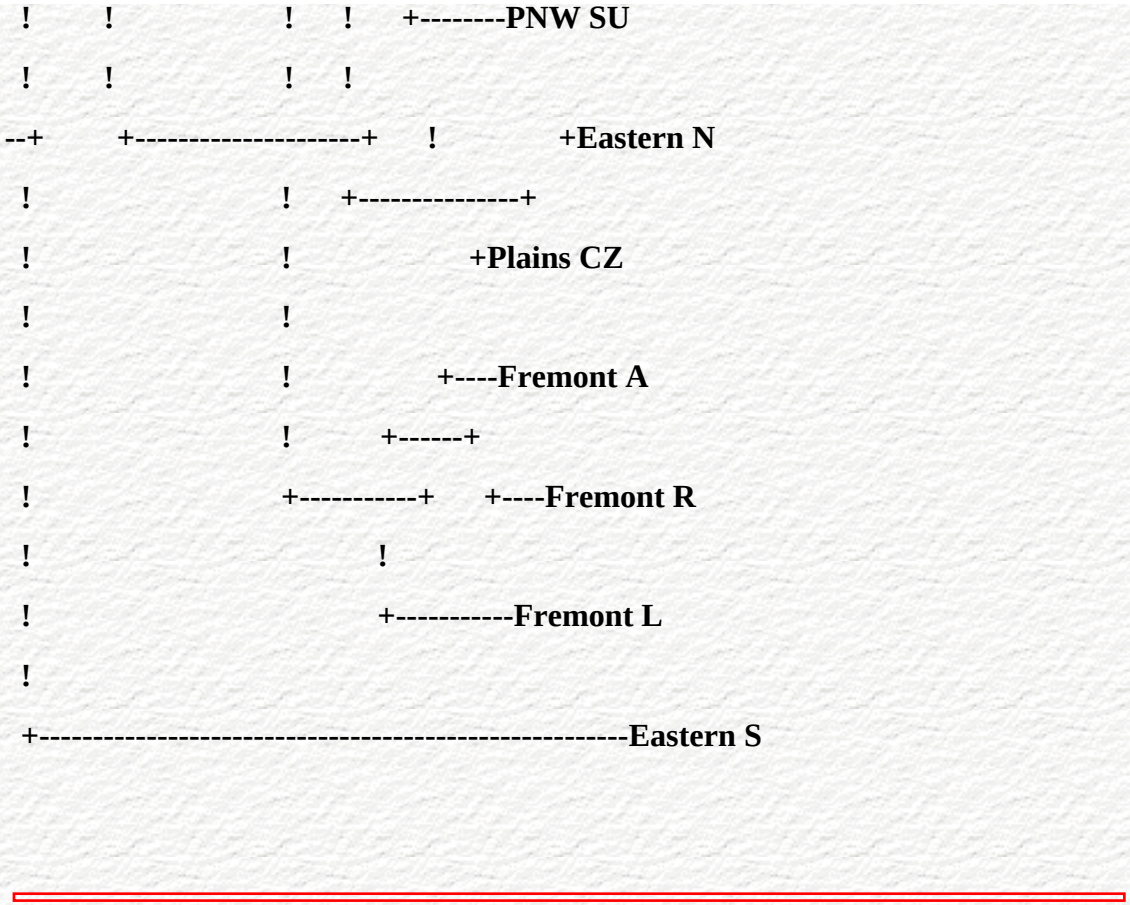
Two Plains Cottonwood populations from Montana (LA and PO) and one PNW population (JJ) were less variable than the others. No fixed differences in allele presence coincided with species boundaries, but extreme differences in allele frequencies separated some taxa. Genetic distances between populations varied widely, but in general populations of the same taxon had identities above 0.85.

Dendrograms based on Nei's genetic distances between populations divided the populations into two clusters, plus two anomalous populations.

Dendrogram of isozyme similarity between cottonwood samples, based on Nei's (1978) unbiased genetic identities.




Black SA
Black W
Narrowleaf PK
Narrowleaf F
Narrowleaf LAN
PNW JJ
Eastern K
Plains LA
Plains PO
PNW O
PNW ON
PNW P



Nei's (1978) genetic distance between populations

One cluster contained Black and Narrowleaf Cottonwoods, and the second contained Fremont, Eastern, Plains, and PNW Cottonwoods. Under the conditions of electrophoresis used in this study, all samples of the highly divergent East S population showed no activity at the UGP-2, TPI-1, TPI-2, and TPI-3 loci, and the PGM allele shared by all southern samples occurred at a frequency of about 0.20 to 0.25 in the more northern populations of *P. deltoides*. Northern populations of *Populus deltoides* (both varieties) plus the PNW Cottonwoods formed a single cluster that was sister to the cluster of Fremont Cottonwood populations.

Ten alleles (at eight loci) were observed in PNW Cottonwoods and not in other populations of *P. deltoides*. Three of these alleles were unique to PNW Cottonwoods, 2 were shared with Black Cottonwood, and five were shared with Black, Narrow-leaf, and Fremont's Cottonwoods. With two exceptions, the alleles observed only in PNW populations were uncommon to rare in the PNW population. The PNW populations ON, P, and SU had high numbers of rare alleles (those with frequency less than 0.1) and that remained true when alleles shared with species other than Eastern or Plains Cottonwoods were removed. .

In general, more genetic variation occurred within than among populations of a taxon (Fst ranged from 0.16 to 0.42), although population differentiation was substantial. Including the divergent JJ population raised the Fst values. Even with the JJ population removed, *P. deltoides* had a high Fst. Hierarchical F statistics show that most of the variation in the cottonwoods is among individuals.

Hierarchical F statistics for cottonwood taxa. Population JJ removed from the data. i = individuals, s = populations, p = species or other taxa, and t = total.

Populations	Fit	Fst	Fpt	Fis	Fsp	Fip
6 groups (Black, Narrow-leaf, Fremont, East, Plains, PNW Cottonwoods)	0.6846	0.5103	0.3328	0.3559	0.2660	0.5272
4 taxa (Black, NLF, Fremont, *P. deltoides)*	0.7051	0.5421	0.3427	0.3559	0.3035	0.5514
Black, NLF, & Fremont Cottonwoods	0.6737	0.5392	0.3631	0.2919	0.2765	0.4877
P. deltoides, 3 groups (East, Plains, PNW)	0.6662	0.4303	0.0772	0.4141	0.3827	0.6383
P. deltoides, (var. *deltoides* and var. *occidentalis)*	0.6734	0.4069	0.1920	0.4494	0.2659	0.5958

Complete multilocus genotypes were produced for 402 individuals. In 267 cases (66%), a given multilocus genotype was represented by a single sampled individual in a population. The mean number of individuals per possible clone was less than three in all populations except the JJ population. For example, in the LAN population of Narrowleaf Cottonwood, the number of individuals/clone equals one. This means that each of the three individuals with complete multilocus genotypes are genetically different (they are not ramets of a single clone). In contrast, the PNW JJ population had 6.33 individuals/clone. The 19 individuals with complete multilocus genotypes are ramets of three different clones. The distribution of multilocus genotypes (potential clones) was not even among the populations. Eleven (78%) of the fourteen potential clones consisting of three or more samples occurred in just three of the populations (Plains LA, Plains PO, and Fremont L). Sixteen of the 19 samples in the PNW JJ population shared the same multilocus genotype, which was scored as heterozygous at three loci (PGI2, UGP1, and UGP2). The two genotypes represented by few samples each differed from the common one at one locus each. The JJ population contained alleles and genotypes that were unusual for *P. deltoides* but occurred in Black Cottonwood.

DISCUSSION

Genetic variation. Few other studies of cottonwood isozymes are directly comparable to this one (Rajora 1989, Rajora and Zsuffa 1990, Webber and Stettler 1981), because most other studies were not designed to report genetic diversity statistics of wild populations. This study revealed higher levels of diversity than had been observed previously in Eastern and Black Cottonwoods. The sample sizes in previous studies of Eastern Cottonwood were lower, but the Black Cottonwood study included larger sample sizes and more populations than this one (Weber and Stettler 1981). Mean genetic diversity measures per population are similar for the two Black Cottonwood studies, but more genetic diversity was detected overall in this study. Two hypotheses could account for this difference. First, NFGEL studies of conifers frequently report higher levels of genetic diversity than do comparable

studies (e.g. Samman et al., MS). The high level of genetic diversity reported in this study may result from the highly standardized laboratory procedures at NFGEL that allow consistent scoring of enzyme variants that differ little in isozyme mobility. Second, the populations for this study were collected from the relatively isolated waterways of eastern Oregon and Washington, and those of the previous study were collected west of the crest of the Cascade Range. The Black Cottonwood populations were therefore differentiated in this study (Fst = 0.18) compared to that of Weber and Stettler (1981; Gst = 0.06). Like the previous study, this one reported an excess of homozygotes in Black Cottonwood, and indeed in all cottonwoods studied (Ho>He).

Population genetic variability was, in general, similar to that of other long-lived woody plants, at both the species and the population level (Hamrick and Godt 1990).

Genetic distances between conspecific plant populations usually exceed 0.9 (Crawford 1990). That is true for most of the cottonwood taxa studied here. However, populations of Eastern and Narrow-leaf Cottonwoods were more distinct that expected.

Identification and taxonomy. Isozyme frequencies were consistent with established cottonwood taxonomy. Black and Narrow-leaf Cottonwood are in taxonomic section *Tacamahaca* Spach, and Fremont, Eastern, and Plains Cottonwood are in taxonomic section *Aigeiros* Duby (Krüssman 1977). The two taxonomic sections coincided with the two clusters of populations identified with isozymes. Although no fixed isozyme differences between cottonwood species were observed, isozyme frequencies clearly distinguished the species of cottonwood. Therefore, isozyme analysis clearly answered one of the basic questions of this study. The Pacific Northwest cottonwood is *P. deltoides,* not an isolated population of Fremont Cottonwood.

Isozyme frequencies did not support traditional taxonomy within *P. deltoides.* They failed to differentiate the sampled populations of *P. d.* var. *deltoides* from Midwestern populations of *P. d.* var. *occidentalis.* The southern sample of *P. deltoides* var. *deltoides,* collected in and near the delta of the Mississippi River, was highly divergent. However, the genetic identity between the Nebraska population *(P. deltoides* var. *occidentalis)* and the northern Midwest sample of *P. deltoides* var. *deltoides* was 0.9861, the second closest in the entire study. The sampled populations of *P. deltoides* are more genetically differentiated than are the highly variable taxonomic varieties. A previous isozyme study united populations of *P. d.* var. *deltoides* and *P. d.* var. *occidentalis* (Rajora 1989). Morphologically, the two varieties are confluent over a large area of the eastern plains and upper Midwest (Gleason and Cronquist 1991). The close genetic relationship between the sample from Nebraska and that from clones gathered from the upper Midwest apparently reflects the biology of these trees more closely than their nomenclature would suggest.

Introgression and clones. Ten alleles occurring in the Pacific Northwest populations were observed in no other *P. deltoides* population. Seven of these alleles also occurred in Black Cottonwood, and some of them occurred in Narrow-leaf and Fremont's Cottonwood. Plains Cottonwood hybridizes naturally with all sympatric species of *Populus,* including Black and Narrow-leaf Cottonwood, and cottonwood hybrids are usually more or less fertile (Bradshaw 1965, Smith 1943). Perhaps these seven alleles occurred in Pacific Northwest populations of *P. deltoides* as a result of introgression from Black Cottonwood.

For the most part, the sampled cottonwoods were the result of sexual reproduction, rather than clonal spread. In each population, the number of genetic individuals may be greater than the reported number of multilocus genotypes, because the samples may differ at loci that were not studied. The low number of individuals per genotype is consistent with the observation that although cottonwoods sprout readily from stumps or root crowns, they rarely spread clonally by suckers (Zsuffa 1976). This contrasts with Quaking Aspen *(Populus tremuloides* Michx.) in which single clones may cover many acres and include dozens or hundreds of stems (Kemperman and Barnes 1976).

The PNW population JJ was unusual. The uniform population occupied less than 0.4 hectare along an abandoned railroad, and the samples came from an area of less than 0.1 hectare. It did not cluster with the other PNW cottonwood populations and sixteen of the individuals shared a single multilocus genotype. All individuals were heterozygous at loci UGPP1 and UGPP2, suggesting that the great similarity among the samples was due to vegetative reproduction, rather than inbreeding. Inbreeding would produce an excess of homozygotes, not uniform heterozygosity (Hartl and Clark 1997), and high levels of heterozygosity are associated with vegetative reproduction in aspens (Cheliak and Dancik 1982). All the individual samples could be ramets of

a single clone. If so, somatic mutation occurred at locus PGI2 and perhaps FEST1. Four alleles observed in all samples from population JJ occur in other species but not in any other sampled population of *P. deltoides*. The sampled JJ population appeared to consist of one genetic individual which was probably a hybrid of *P. deltoides* and either Black or Narrow-leaf Cottonwood, or the descendent of such a hybrid. This population was much more clonal than any other sampled population. Perhaps it was planted, using ramets from a single tree. The JJ population would be unsuitable as a source of propagules for revegetation, if genetic integrity is a goal of the project.

Origin of the Pacific Northwest populations. The PNW cottonwoods were genetically variable and appeared most closely related to the Plains Cottonwood populations from Montana (populations LA and PO). This combination of observations suggest that the PNW cottonwood populations are either native or the result of repeated introductions from the east slope of the Rockies. The variability of the PNW cottonwoods is reduced only slightly if the alleles that may result from introgression are removed from analysis. If the PNW population originated from introductions, they were probably accidental and by seed because deliberate introductions would probably have included sources much further east. Isozyme analysis can not rule out the hypothesis that the PNW cottonwoods were introduced. However, it provides stronger support for the hypothesis that the trees are native.

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Forensics

(1) Using Plant DNA as Evidence in a Fire Investigation

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(1) Using Plant DNA as Evidence in a Fire Investigation

DNA technology has been used as evidence in court cases for over a decade. Yet there is only one case that admitted DNA evidence that came from a plant. As summarized by G. Sensabaugh and D.H. Kaye (Non-human DNA Evidence, 38 Jurimetrics J. 1-16 (1998)): "In *State v Bogan,* a woman's body was found in the desert, near several Palo Verde trees. A detective found two seed pods in the bed of a truck that the defendant was driving before the murder. A biologist performed DNA profiling on this type of Palo Verde and testified that the two pods "were identical" and "matched completely with" a particular tree and "didn't match any of the [other] trees," and that he felt "quite confident in concluding that" the tree's DNA would be distinguishable

from that of "any tree that might be furnished" to him. The jury convicted the defendant of murder, and jurors reportedly found this testimony very persuasive".

Because all living organisms contain DNA, forensic DNA testing can be used on plants as successfully as it has been for human identification. The detection of DNA variation in plant species has application to such cases as homicide, patent infringement, tree and fuelwood theft, and fire investigation. NFGEL is currently participating in a legal case that attempts to use plant DNA to reconstruct the scene of a major fire in California that resulted in millions of dollars of damage, and firefighter injuries. In the laboratory, we were able to extract DNA from tree and branch samples and provide information as to which trees the branches came from. This identification information can then be used to determine liability in the case. This case is currently pending and has a trial date scheduled for spring 2000. [NFGEL Project #85]

STAFF ACTIVITIES

Meetings, Shortcourses, and Workshops

Presentations

1998. V Hipkins. Genetic studies of native plants. Native Plants: Propagating and Planting, Oregon State University, Corvallis, OR, December 9-10.

1999. FE Bridgwater, DL Bramlett, and VD Hipkins. Timing of bag application and removal in controlled mass pollination. 25th Southern Forest Tree Improvement Conference, New Orleans, LA, July 11-14.

1999. V Hipkins. Genetic resource management (or DNA today -- life in the Forest Service). USDA Forest Service Washington Office, Washington DC, July 19.

1999. V Hipkins and L Halstrom. Allozyme variation in an isolated stand of limber pine. Western Forest Genetics Association 1999 Annual Meeting, Flagstaff, AZ, July 27-29.

1999. V Hipkins and R Westfall. Local spatial genetic structure in sugar pine (*Pinus lambertiana*): Implications for the conservation of genetic diversity. Western Forest Genetics Association 1999 Annual Meeting, Flagstaff, AZ, July 27-29.

1999. V Hipkins. Introducing genetic concepts in relation to native plants and revegetation. USDA Forest Service Short Course in Genetic Issues in Using Native Plants for Revegetation, Denver, CO, Sept 27-28.

Posters

1998. V Hipkins, S Carroll, P Guge, and R Meyer. A lab to study native plant genetics. Native Plants: Propagating and Planting, Oregon State University, Corvallis, OR, December 9-10.

1999. V Hipkins, S Carroll, P Guge, R Meyer, and P Skaggs. Genetic resource management at the National Forest Genetic Electrophoresis Laboratory (NFGEL). 25th Southern Forest Tree Improvement Conference, New Orleans, LA, July 11-14.

1999. V Hipkins, S Carroll, P Guge, and R Meyer. A lab to study native plant genetics. R5 Culturist Workshop, Placerville CA, October 6.

1999. L Halstrom and V Hipkins. Limber pine: Trail mix or remnant stand. USDA Forest Service National Silviculture Workshop, Kalispell, Montana, October 4-7.

Attended

1998. Native Plants: Propagating and Planting, Oregon State University, Corvallis, OR, December 9-10 (P.Guge, R.Meyer, V.Hipkins).

Internal Activities

Member of the Eldorado National Forest Incident Purchasing Team (S.Carroll).

Member of the Eldorado National Forest Safety Committee (R.Meyer).

Union Representative - Eldorado National Forest (R.Meyer).

Participated in design, construction, and staffing of NFGEL booth at the County Harvest Fair (R.Meyer).

Professional Activities

Peer reviewer for Canadian Journal of Forest Research and Silvae Genetica (V.Hipkins).

Adjunct faculty member at Northern Arizona University, School of Forestry, Flagstaff, AZ (V.Hipkins).

Western Forest Genetics Association Officer -- three year term (V.Hipkins).

USDA Forest Service Detail (three weeks, July '99) to the Washington Office; Program, Development, & Budget (PD&B), and Forest Management Budget Staff (V.Hipkins).

Schmidtling, RC, E Carroll, and T LaFarge. 1999. Allozyme diversity of selected and natural loblolly pine populations. Silvae Genetica (in press).

Hosted

NFGEL continues to host a variety of visitors. Tours of the facility and operation were provided to (1) Forest Service employees representing four units of the Research branch, the Washington Office, and three Regions of the National Forest System, (2) members of the public, (3) industry, (4) university faculty, and (5) employees from other state and federal government agencies. NFGEL hosted groups from the culturist workshop, Squaw Valley Academy, and St. Mary's College. Our guests also included international visitors from Australia, Korea, Fiji, and Brazil.

Collaborations and Cooperations

NFGEL has developed partnerships with FS Research Stations, Northern Arizona University (Flagstaff), and California Department of Forestry and Fire Protection. The lab continues to cooperate with Bureau of Land Management, US Fish and Wildlife Service, Texas A&M University, the American River College Internship Program in California, and Oregon State University. We also collaborate internally within the Agency to lend expertise in the area of genetics. This takes the form of reviewing contracted work reports on the genetics of mammals, fish, and plants, and on participating in teaching and extension workshops.

Cooperation with the Pacific Southwest Research Station (PSW) has been outstanding since the inception of NFGEL. While NFGEL's protein lab is located at the Placerville Nursery, Camino, CA, the DNA work is carried out in a cooperative arrangement with Dr. David Neale at the PSW Institute of Forest Genetics in Placerville, CA. PSW has shared facilities, equipment, personnel and supplies. NFGEL has reciprocated by providing supplies and equipment to PSW.

CURRENT STAFFING

During FY99, NFGEL was staffed with four permanent full-time, and three temporary part-time employees. NFGEL currently has two vacancies (Associate Director and Data Manager).

Name	Position	Term	E-mail Address
Valerie Hipkins	Director	PFT	vhipkins@fs.fed.us
Suellen Carroll	Lab Manager	PFT	scarroll@fs.fed.us
Pat Guge	Lab Biotechnician	PFT	pguge@fs.fed.us
Randy Meyer	Lab Biotechnician	PFT	rmeyer@fs.fed.us
Andy Skaggs	Data Manager	Temp (10/98-10/99)	
Robert Saich	Lab Biotechnician	Temp (6/99 - 8/99))	
Barbara Wilson	Associate Director	Temp (10/98-9/99)	blwilson@fs.fed.us

FY1999 BUDGET (% Change FY98 to FY99)

Activity	FY98	FY99	% Change
Receipts (in thousands)			
Allocation	290	307	6%
Carryover	0	9.9	
Soft Money	22.7	25.8	12%
Total	312.7	342.7	9%
Expenditures (in thousands)			
Salary (permanant)	149.9	165.3	9%
(temperary)	29.5	58.5	50%
Overhead to ENF	67.3	69	2%
Chemicals/Supplies	26.4	16.5	-60%
Equipment	25.7	10.1	-154%
Travel/Training	5.1	8.6	41%
Awards	4.4	4.4	0%
Fees	1.5	0.5	-200%
Books	0.9	0.8	-13%
Computers	0.6	4.9	88%
Facility Repair	0.5	0.3	-67%
Photos/Slides	0.4	0.5	20%
Postage	0.4	0.4	0%
Office Supplies	0	0.8	--
Furniture	0	1.5	--
Total	312.6	342.1	9%

FY 99 Soft Money

Source	Amount ($)	Percentage
USFWS (Hackelia)	1,500	6
CDF (Sugar Pine)	4,150	16
FS-FSR (Blacks Mtn.)	11,700	64
(Slash Pine)	5,000	
BLM (Cottonwood)	1,500	14
(Perideridia)	2,000	

Total	25,850	100

Project#	Collaborator	Species	Objective	Sample Type	Sample Size	Submission Dates	Preparation Dates	Electrophoresis Dates	Marker System	#Loci
73	Laurie Lippitt, CA Dept. of Forestry	Pinus lambertiana (sugar pine)	Relatedness of rust resistant vs. non-resistant seedlots	Seed (embryos)	1452 embryos	6/12/97	5/6 - 8/5/98	10/6 -11/13/98	Isozymes (starch gel)	16
66	Floyd Bridgwater, FRS-SRS	Pinus taeda (loblolly pine)	Estimate % contaminants in controlled cross treatments	Seed (megs/embryos)	61 seedlots, 1,013 m/e pairs	6/26/97 - 2/2/99	10/17 - 10/30/97 2/19 - 3/3/99	1/6 - 3/11/99	Isozymes (starch gel)	22
81	David Alicea, NFS-R5	Pinus lambertiana (sugar pine)	Seedlot purity identification	Seed	4 seedlots 60 megs	1/21/99	1/26/99	1/27/99	Isozymes (starch gel)	20
76	David Doede, NFS-R6	Sisyrinchium ssp.	Clonal ID; grazing effects on diversity	Leaves	181 indiv.	6/23 - 7/28/98	6/26 - 7/30/98	3/16 - 3/30/99	Isozymes (starch gel)	16
77	David Doede, NFS-R6	Sisyrinchium ssp.	Clonal ID; grazing efects on diversity	Leaves	26 indiv.	6/23 - 7/28/98	6/26/98	4/19 - 4/23/99	Isozymes (isoelectric focusing)	16
82	Mary Francis Mahalovich NFS-R1	Pinus ponderosa (ponderosa pine)	Seed source ID; diversity in seed production area	Seed (megs), buds	1,218 megs, 30 buds	2/3/97 - 3/17/99	3/22 - 4/26/99	4/1 - 6/3/99	Isozymes (starch gel)	26
80	Ron Schmidtling, FSR-SRS	Pinus elliottii (slash pine)	Rangewide diversity	Buds	536 indiv.	1/20 - 2/15/99	2/1 - 3/1/99	6/8 - 7/27/99	Isozymes (starch gel)	20
83	Claire Williams, Texas A & M Univ.	Pinus taeda (loblolly pine)	Screen for polymorphic loci	Needles	129 seedlings	5/25/99	6/8 - 6/29/99	6/24 - 7/9/99	Isozymes (starch gel)	21
75	Paul Berrang, NFS-R6	Populus spp. (cottonwood)	Clarify taxonomic relationships; determine native -vs- non-native	Leaves	684 indiv.	5/5 - 7/16/98	5/6 - 7/17/98	7/29 -10/5/99	Isozymes (starch gel)	19
74	Chris Frisbee, NFS-R4	Lewisia ssp.	Laboratory procedure testing	Leaves	293 indiv.	6/22 - 7/29/99	7/1 - 7/29/99	6/30/99	Isozymes (starch gel)	___
78	Paul Berrang, NFS-R6	Perideridia ssp.	Laboratory procedure testing	Seedlings	178 indiv.	8/28/98 - 7/20/99	9/2/98 - 7/25/99	9/2/99	Isozymes (starch gel)	___
55	Jody Sawasaki, USFWS	Rorippa subumbellata	Laboratory procedure testing	Leaves	150 indiv.	8/15 - 9/1/99	8/18 - 9/3/99	8/17/99	Isozymes (starch gel)	___
85	Dan Nichols, CA Dept of Forestry	Eucalyptus calabasas	Forensics	Leaves, wood	~150 indiv.	4/99 - present	4/99 - present	4/99 - present	DNA (RAPD's)	12

Workload by Region or Agency, FY99

(1) Isozymes (starch gel electrophoresis)

By Project

Region or Agency		Project #	Species	# gels	# days	# weeks
CDF		73	sugar pine	74	13	5.0
USFWS		55	Rorippa	1	1	0.5
Texas A&M		83	loblolly pine	9	2	1.0
FSR-SRS		66	loblolly pine	165	21	11.0
		80	slash pine	74	13	6.0
FS-NFS	-R5	81	sugar pine	3	1	0.5
	-R6	76	Sisyrinchium	35	5	3.0
		75	Populus	78	14	9.0
		78	Perideridia	3	1	0.5
	-R1	82	ponderosa pine	123	16	10.0
	-R4	74	Lewisia	3	1	0.5
NFGEL		--	testing	8	2	1.0

By Agency

Region or Agency			# gels	# days	# weeks
Forest Service					
	NFS		(245)	(38)	(23.5)
		R1	123	16	10.0
		R4	3	1	0.5
		R5	3	1	0.5
		R6	116	20	12.5
	Research				
		SRS	239	34	17.0
CDF			74	13	5.0
USFWS			1	1	0.5
Texas A&M			9	2	1.0
NFGEL			8	2	1.0
TOTAL			576	90	48

(2) Isozymes (isoelectric focusing)

Region or Agency		# gels	# days	# weeks
Forest Service				
	NFS			

R6	16	4	2.0

(3) DNA (RAPDs)

Region or Agency	# days	# weeks
CDF	43	17

*Project 75 and 78 are partially funded by the Bureau of Land Management

CDF=California Department of Forestry and Fire Protection
USFWS=United States Fish and Wildlife Service
FS=Forest Service
FSR=Forest Service Research
SRS=Southern Research Station
NFS=National Forest System
R#=Region

FY00 PLANNED PROJECTS

Project #	Collaborator	Species	Objective	Sample Type	Marker System	Planned Completion Date
75	Paul Berrang, FS-R6	Cottonwood (Populus spp)	determine taxonomic relationships; determine if native	leaves	isozymes (starch gel)	Oct-99
79	Joanna Clines, FS-R5	Collomia rawsoniana	determine genets vs ramets; characterize genetic diversity; conservation management	leaves	isozymes (starch gel)	Nov-99
90	Ron Schmidtling, FSR-SRS	Southern pines (Pinus spp)	determine genetic diversity and phylogeny	buds	isozymes (starch gel)	Dec-99
91	Zhong Chen, NAU	Douglas-fir (Pseudotsuga menziesii)	characterize genetic diversity in douglas-fir that's susceptible or resistant to western spruce budworm	seed	isozymes (starch gel)	Dec-99
87	Rod Stevens, BLM	Port Orford & Alaskan Yellow cedar (Chamaecyparis lawsoniana and nootkatensis)	distinguish species	needles	isozymes (starch gel)	Jan-00
88	Rod Stevens, BLM	Port Orford & Alaskan Yellow cedar (Chamaecyparis lawsoniana and nootkatensis)	distinguish species	needles	isozymes (IEF gels)	Jan-00
72	Dave Doede, FS-R6	Lupine (Lupinus latifolius)	determine taxonomic relationships	seed	isozymes (starch gel)	Feb-00
89	Bob Westfall, FSR-PSW	Sierra grasses (Elymus elymoides and Achnatherum spp)	determine genetic diversity for use with common garden data	seedlings	isozymes (starch gel)	Apr-00
55	Jody Sawasaki, USFWS	Rorippa subumbellata	determine genetic diversity and genetic individuals; conservation management	leaves	isozymes (starch gel)	May-00

84	Vicky Erickson, FS-R6	Quaking aspen (Populus tremuloides)	determine genetic diversity and genetic individuals; conservation management	leaves	isozymes (starch gel)	Jun-00
86	Rod Stevens, BLM	Port Orford & Alaskan Yellow cedar (Chamaecyparis lawsoniana and nootkatensis)	distinguish species	needles	DNA (RAPD's)	Jun-00
74	Chris Frisbee, FS-R4	Lewisia kelloggii	determine taxonomic relationships and genetic diversity; conservation management	leaves	isozymes (starch gel)	Jul-00
78	Paul Berrang, FS-R6	Perideridia spp.	determine taxonomic relationships and genetic diversity; conservation management	seedlings	isozymes (starch gel)	Sep-00
92	Ron Schmidtling, FSR-SRS	Southern pines (Pinus spp)	determine genetic diversity and phylogeny	buds	isozymes (starch gel)	Oct-00

PROJECT SUBMISSION

Projects may be proposed by any Forest Service employee or member of a cooperating agency. Project ideas should be discussed with the NFGEL Director prior to submission. Following preliminary approval, a brief proposal should be submitted summarizing background information, project objectives, and management implications. Projects are prioritized annually by a Steering Committee made up of national geneticists. Final proposals, including study objectives, sampling design, and analysis are developed through close cooperation between NFGEL and our clients. Questions regarding proposal format should be directed to NFGEL.

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