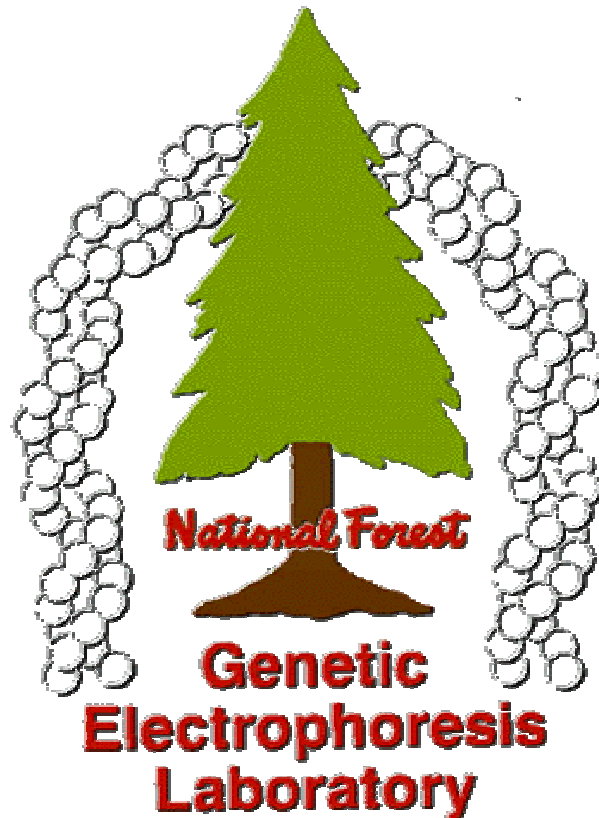


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NFGEL Annual Report 2001 – 2002 (FY02)



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Report prepared December 2002

INTRODUCTION

It is with mixed feelings that we must say goodbye to Suellen Carroll, the NFGEL Lab Manager for the last thirteen years. Suellen joined 'Incident Financial Services' located at the Eldorado National Forest Supervisors Office, Placerville CA in July 2002. Although we are happy that Suellen has moved on to bigger and brighter opportunities, she will certainly be missed at the lab. I have not had the pleasure of working with someone who is as dedicated and dependable as Suellen. She was committed to producing a quality product, and cared deeply about meeting our clients needs. While at NFGEL, she was involved in over 100 projects! She has contributed enormously to the lab and will be missed!



Valerie Hipkins
NFGEL Director
December 2002

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 FY 2002, NFGEL continued to follow its mission 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”. NFGEL performed the following:

- DNA profiling the 13 surviving trees planted under the direction of George Washington growing at Mt Vernon.
- Developing a database for ponderosa pine that will be used to identify off-site plantations. Information will be used to improve overall forest health since offsite stands are generally associated with disease and declining performance.
- Determined the genetic structure of Monterey pine growing along Hwy 1 in CA. Addressed forest health and species conservation issues.
- Determined levels of pollen contamination in Douglas-fir and sugar pine seed orchards. Protects the integrity of reforestation material.
- Determined taxonomy and gene/species conservation strategies for several threatened and endangered species (*Collomia*, *Lupinus*, *Rorippa*, *Hackelia*, *Silene*).
- Determined the genetic diversity and evolution of several pine species growing in the southeast US.
- Determined the genetic structure and species conservation strategies for Quaking Aspen growing throughout the Eldorado National Forest, US Forest Service.
- Genetically characterizing revegetation plant materials by comparing source, indigenous, and rehabilitation site native plants.

Eight reports, including results from 13 projects, follow.

Silviculture and Tree Improvement

- (1) **Evolutionary Relationships of Slash Pine (*Pinus elliottii*) with Its Temperate and Tropical Relatives**
- (2) **Co-dominant, Segregating Polymorphic Markers in *Pseudotsuga menziesii* (Douglas-fir)**
- (3) **Clonal Identification in *Pseudotsuga menziesii* (Douglas-fir)**
- (4) **Supplemental Mass Pollination Efficacy in a *Pseudotsuga menziesii* (Douglas-fir) Seed Orchard**
- (5) **Detecting Segregating Alleles at the CAD Locus in *Pinus taeda* (loblolly pine)**
- (6) **Genetic Diversity in Mexican Populations of *Pseudotsuga menziesii* (Douglas-fir)**
- (7) **Clonal Identification in *Chamaecyparis lawsoniana* (Port-Orford Cedar)**

Conservation and Restoration

- (1) **Quaking Aspen (*Populus tremuloides*) Clones in Northeast Oregon**

Evolutionary Relationships of Slash Pine (*Pinus elliottii*) with Its Temperate and Tropical Relatives

R.C. Schmidling¹ and V. Hipkins²

[This work is a combination of NFGEL Projects 80, 90, 92, 93, and 126].

Abstract

Allozymes in bud tissue and monoterpene contents in xylem oleoresin of slash pine (*Pinus elliottii*) were analyzed from populations across the natural distribution, as well as those from other species in the AUSTRALES pines. Allozyme diversity measures of slash pine were similar to those found in other southern pines. The two slash pine varieties, the slower-growing south Florida variety (var. *densa*) and the more commercial "typical" variety (var. *elliottii*), were not separated in the cluster analysis of allozymes. Variation was continuous from south to north in Florida in slash pine, with no distinct transition between the two varieties. The monoterpene data also showed continuous variation between the two slash pine varieties. Expected heterozygosity declined from south to north, supporting the hypothesis that slash pine resided in a Pleistocene refugium in south Florida or the Caribbean, migrating northward at the close of the ice age. Allozyme frequencies as well as monoterpene compositions of slash pine and its AUSTRALES relatives showed a very close relationship between slash pine and Bahamian Caribbean pine (*P. caribaea* Morelet var. *bahamensis*).

Key Words: *Pinus caribaea*, AUSTRALES, allozymes, monoterpenes

Introduction

Slash pine (*Pinus elliottii* Engelm.) is one of the most commercially valuable pines in the United States. It is commonly planted inside and outside its natural range in the Southeastern US (Boyer and South 1984), as well as in exotic plantings in Asia (Pan 1989), South America (Picchi and Barrett 1967), Africa and Australia (Mullin *et al.* 1978).

Taxonomically, slash pine has obvious affinities with other caribbean hard pines, and before 1952 (Little and Dorman) slash pine and *P. caribaea* Morelet were not considered separate species. Slash pine is now divided into two varieties, the "typical" variety (var. *elliottii* Little and Dorman) and the south Florida variety (var. *densa* Little and Dorman). The transition between the two varieties was mapped in the central Florida peninsula by Little and Dorman (1952)(Figure 1), although Squillace (1966) and Nikles (1966) found no distinct transition between the varieties and considered the variation to be continuous in the peninsula.

There is some evidence for gene exchange between *P. caribaea* var. *bahamensis* (Griseb.) B. and G. and slash pine in southern Florida, based on analysis of cortical monoterpenes (Squillace *et al.* 1977) as well as chloroplast DNA (Nelson *et al.* 1994, Wagner *et al.* 1992). Recently, Dvorak *et al.* (2000) has shown a very close relationship between *P. caribaea* and slash pine using RAPD's.

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Figure 1 here

The purpose of the present study is to further evaluate the patterns of variation in populations from throughout the slash pine range and the nearby tropical and temperate relatives in the subsection AUSTRALES using allozymes and monoterpenes.

Materials and methods

Plant materials

Dormant buds were collected from 17 geographic sources of slash pine across the natural range (Fig. 1 and Table 1). Eleven of the sources were of secondary origin, located in provenance test plantings in south Mississippi (Snyder *et al.* 1967, Doudrick *et al.* 1996). Collection specifications were the same for the recent, *in situ* collections as well as the existing provenance tests: Collections were made from trees in natural stands separated from each other by 100 ft (30 m) or more. One of the collections (LA Land race) was from a seed orchard in central Louisiana, from grafted ramets of trees selected in plantations made during the 1930's. Slash pine does not occur naturally west of the Mississippi, but is widely planted there. Collections were also made of longleaf pine (*P. palustris* Mill.) in Wakulla County FL, from loblolly pine (*P. taeda* L.) in southwest Mississippi, and from Caribbean pine (*P. caribaea* Morelet) on Grand Bahama Island. Buds were collected from approximately 30 trees from each of the populations.

We also made use of monoterpene analysis of oleoresin samples collected by Nikles (1966)³ and Coyne⁴. Samples for both data sets were collected using glass vials inserted into holes drilled into the sapwood at breast-height (Coyne 1965). Collections were made in 26 different populations of eight taxa and hybrids (Table 2). Monoterpenes were assayed using gas chromatography, following procedures and conditions that were nearly identical for both sample sets (Nikles 1966, Coyne and Keith 1972).

Enzyme electrophoresis

Isozyme band patterns were investigated using dormant vegetative bud tissue as the enzyme source material from individual tree collections. Scales were removed from terminal buds and a small portion (8 mm³) of meristem was dissected and submerged in three drops of modified Cheliak and Pitel (1984) extraction buffer (where mercaptoethanol was removed). Samples were frozen at -70C until electrophoresis.

On the morning of the electrophoretic run, extracts were prepared by thawing samples, macerating the bud tissue with a Dremel MultiPro drill press, and absorbing the slurry onto 3mm wide paper wicks. Wicks were inserted into 11% starch gels (Sigma Chemical Co.) that accommodated 30 samples. The preparation and running of the gels are modifications of Adams *et al.* (1990) and Conkle *et al.* (1982). A total of 662 trees were genotyped at 22 isozyme loci using three buffer systems. Buffer system 'LB' (gel and tray buffer 'A' of Adams *et al.*(1990)) was used to resolve enzyme systems leucine aminopeptidase (LAP), phosphoglucomutase (PGM), shikimic acid dehydrogenase (SKD), and diaphorase (DIA). Buffer system 'SB' (a modification of gel and tray buffer 'B' of Adams *et al.* (1990), where the electrode buffer was pH 8.0), was used to resolve enzyme systems triosephosphate isomerase (TPI), catalase (CAT), and glutamic oxaloacetate transaminase (GOT). Buffer system 'MC8' (a modification of gel and tray buffer 'C' of Adams *et al.* (1990), where the stock solution was adjusted to pH 8.0), was used to resolve isocitrate dehydrogenase (IDH), 6-phosphogluconate dehydrogenase (6PGD), phosphoglucose isomerase (PGI), fluorescent esterase (FEST), and malate dehydrogenase (MDH). Running conditions and stain recipes follow Adams *et al.* (1990) and Conkle *et al.* (1982). After the dye marker migrated 8 cm, gels were cut

³ Original data kindly supplied by Dr Nikles.

⁴ John F. Coyne, 1974. Unpublished data on file at the Southern Institute of Forest Genetics, Saucier, MS, USA.

horizontally into four to seven slices, stained and scored.

Statistical analysis

Allozyme data provided several estimates of genetic variation using BIOSYS I (Swofford and Selander 1989): Mean number of alleles per loci (N_s), percent loci polymorphic (P, 100% criterion), and expected heterozygosity (H). Diploid genotypes were also transformed for multivariate analysis using the technique of Smouse and Williams (1982). For each allele at a locus minus one, the value of 0.5 was assigned when the allele was present and 0 when the allele was absent. The score when the allele at the locus is in the homozygous state would be $0.5 + 0.5 = 1.0$, when it is in the heterozygous state, $0.5 + 0.0 = 0.5$. For individuals without the allele the score would be 0. This is equivalent to a measure of the amount of an allele in each individual. Data sets with more than ten alleles can be assumed to have a normal distribution (Smouse and Williams 1982). Transformed allozyme data as well as monoterpene data were analyzed using SAS (1990) multivariate analysis of variance and canonical discriminant analysis.

Results and discussion

Allozyme analysis

Overall, 20 of the 22 allozyme loci were polymorphic in slash pine (91%), with an average of 3.0 alleles per locus. On an individual population basis, an average of 64.8% of the loci in the slash pine populations had more than one allele (Table 1). The average slash pine population had 2.04 alleles per locus and an expected heterozygosity of 0.173. The LA "land race", a collection made in a seed orchard in central Louisiana, had slightly higher than expected allozyme diversity, similar to seed orchard samples in loblolly (Schmidting *et al.* 1999), and longleaf pines (Schmidting and Hipkins 1998).

Although the loblolly and longleaf pines are represented by only single populations, measures of genetic variability are comparable to those previously published (Schmidting *et al.* 1999, Schmidting and Hipkins 1998) (Table 1). Loblolly pine averaged slightly higher than slash pine in allozyme variability, with 2.18 alleles per locus, 72.7% polymorphic loci, and an expected heterozygosity of 0.196. Longleaf pine averaged lower in allozyme variability, with 1.68 alleles per locus, 45.5% polymorphic loci and an expected heterozygosity of 0.107. Allozyme variability in the Caribbean pine was higher than that found in slash pine and more comparable to loblolly pine, with 2.16 alleles per locus, 72.7% loci polymorphic, and an expected heterozygosity of 0.221.

In examining the spatial variation in the frequency of alleles for our data, we found only continuous variation across the range of slash pine. The dividing line between the south Florida variety (*densa*) and the "typical" northern variety (*elliottii*) is not distinct and there is a transition zone where intermediate types occur (Squillace 1966). The presence of this transition zone has been ascribed to hybridization between the two varieties (Mergen 1954). If hybridization were occurring in the transition zone, one might expect greater variability in sources in this zone than in areas north or south of the zone. Squillace (1966) did not find greater variability in this transition zone in morphological traits, rather he found the greatest variability in the south.

Similarly, there was no greater variability in allozymes in the transition zone (Table 1). Population #205 in our study was collected as a "typical" (var. *elliottii*) source from the transition area. Growth and morphology of the resultant seedlings, however, indicated that the source was more closely related to the *densa* variety (Snyder *et al.* 1967). Source #205 is not any more variable than the adjacent sources (Table 1). There does appear to be a decrease in allozyme variability from south to north, however.

Figure 2 here

In a range-wide study of longleaf pine (Schmidting and Hipkins 1998) a distinct decrease in allozyme variability was found from west to east. This was interpreted as evidence for a single refugium for longleaf pine in south Texas or north Mexico during the last Pleistocene ice age maximum (ca. 14,000 years before

present). The loss in adaptively neutral allozyme alleles resulted from stochastic processes occurring during the rapid migration north and east at the close of the Pleistocene.

Expected heterozygosity is probably the best measure of variability in allozymes. If we plot expected heterozygosity versus distance along the proposed migration route for slash pine at the close of the Pleistocene (Schmidting 2000), we see a decrease in heterozygosity with distance from the refugium (Fig. 2). This is consistent with the proposed Pleistocene refugium in south Florida / Bahamas, and a migration northward after the retreat of the ice (Schmidting 2000). There is no discernable increase in heterozygosity at the transition between the varieties.

Continuous variation is also evident in a plot of the first two canonical vectors from the canonical analysis of the transformed allozyme data (Fig. 3). It appears that the continuum includes the Caribbean pine from Grand Bahama (*Pcb*), although *Pcb* is separated from *Ped* by some distance. Longleaf pine is well separated from *Pcb*, *Ped*, and *Pee*. Loblolly is not shown because it is completely off the chart. These results are in accord with those of Dvorak *et al.* (2000).

Monoterpene analysis

The most obvious difference among taxa in monoterpene composition of stem oleoresin is in the proportion of beta pinene and beta phellandrine (Table 2). The northern variety of slash pine (*Pee*) has relatively low levels of beta phellandrine and high levels of beta pinene. The only obvious difference between slash pine and caribbean pine is in the proportion of delta-3-carene, which is very low in both races of slash pine, but can be as high as 14% in *Pcb*. Samples from *Pinus cubensis* contain almost 90% alpha pinene with only small amounts of other constituents. The longleaf pine X slash pine hybrid essentially had only alpha and beta pinene, more similar to pure longleaf pine (Franklin and Snyder 1971) than to slash pine.

A plot of the first two canonical vectors from the monoterpene analysis shows a much closer relationship between the south Florida slash pine (*Ped*) and the Bahamian Caribbean pine (*Pcb*) (Fig. 4) than the canonical analysis of the allozyme data (Fig. 3). Both analyses show a rather distant relationship between longleaf pine and the other taxa, in accord with Dvorak *et al.* (2000).

Monoterpene contents of oleoresin are quite suitable for studies of geographic variation, since they are highly heritable and little affected by environment (Squillace and Wells 1981). This is evident in Table 2 and Figure 4. Two of the *Pcb* samples were collected from provenance tests in South Africa (SA-1 and SA-11), whereas the other samples were collected *in situ* (Nikles 1966). It is apparent that very little difference exists in the monoterpene composition in samples collected on different continents in different climates. Similarly, the Coyne collections were made in south Mississippi, in provenance tests and natural stands. The *Pcc* sample collected there, as well as the *Pee/Ped* transition sample (the same as source #205 in the allozyme analysis) are comparable to the samples collected *in situ*.

Figure 3 here

Figure 4 here

Conclusions

Both the monoterpene and allozyme analyses suggest a very close relationship between slash pine and Bahamian Caribbean pine. Natural hybrids in adjacent plantations of *Pcc* and *Pee* have been reported (Slee 1971). Seed yields of controlled hybrids between slash pine and Caribbean pine are related to the geographic distance among the taxa. For these hybrids, the order of seed yields were: *Pcb X Pee* > *Pcc X Pee* > *Pch X Pee* (Nikles 1995). There may be some influence of longleaf pine on the evolution of *Pee* as suggested by Nikles (1966), but the evidence presented here is not conclusive. Artificial hybrids among most of the southern pines have been made (Snyder and Squillace 1966), including the slash X longleaf hybrid. Natural hybrids between longleaf and slash pine have been reported in north Florida (Mergen 1958), but the evidence is not very conclusive. A group of putative natural hybrids from the same area were determined to belong to one species or the other, with no intermediates⁵.

Recent evidence has shown natural hybridization between *Pch* and *P. tecunumani*, at the far western end of the distribution of Caribbean pine taxa (Dvorak *et al.* 2000). This suggests a closer relationship between the southern pines and the closed-cone pines than was previously thought, and may indicate a Mexican origin for slash and Caribbean pines.

The data presented here show continuous variation from *Pch* to *Pcc* to *Pcb* to *Ped* to *Pee*, which supports a hypothesized migration route from southern Mexico through the Caribbean islands and up the Florida peninsula. During the most recent Pleistocene ice age, about 14,000 years before present, sea level was around 100 meters lower than currently, exposing large areas of the continental shelf. The Bahamas would have consisted of several large islands, rather than the many small islands. Distances between the mainland and the islands would have been less, facilitating migration after the close of the Pleistocene.

The somewhat arbitrary nature of species determination is shown here. One could certainly justify calling the slash pine / Caribbean pine complex one species with five varieties.

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⁵ T. Kubisiak. Unpublished data on file at the Southern Institute of Forest Genetics, Saucier, MS, USA.

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Table 1. Population origins, number of trees sampled per population, mean number of alleles per locus (N_a), percent loci polymorphic (P_l), and expected heterozygosity (H_e) at 22 loci.

Populations Sampled ¹	Lat	Sample size	N _a	P _l	H _e
<i>Pinus elliottii</i> var. <i>densa</i> (<i>P.e.d.</i>)					
11 Big Pine Key, FL	24.6	35	2.18(0.24) ²	63.6	0.197(0.039)
12 Everglades, FL	25.4	31	1.86(0.19)	59.1	0.170(0.041)
2 Collier Co. FL	26.1	30	2.05(0.19)	68.2	0.177(0.040)
21 Okeechobee, FL	27.0	31	2.09(0.20)	68.2	0.189(0.041)
3 Highlands Co., FL	27.6	32	2.14(0.22)	68.2	0.194(0.042)
<i>P.e.d.</i> - <i>P.e.e.</i> Transition					
205 Polk Co., FL	28.1	30	2.05(0.21)	68.2	0.176(0.043)
<i>Pinus elliottii</i> var. <i>elliottii</i> (<i>P.e.e.</i>)					
4 Disney, FL	28.4	35	2.09(0.22)	63.6	0.187(0.044)
5 Marion Co., FL	29.2	35	2.00(0.20)	59.1	0.170(0.041)
6 Baker Co., FL	30.2	35	2.14(0.23)	63.6	0.165(0.037)
203 Baker Co., FL	30.2	29	2.05(0.21)	63.6	0.158(0.035)
61 Wakula Co., FL	30.2	23	1.95(0.18)	63.6	0.169(0.037)
62 Cape San Blas, FL	29.7	31	1.95(0.19)	63.6	0.170(0.039)
207 Monroe Co., AL	31.1	38	2.09(0.20)	68.2	0.174(0.042)
209 Harrison Co., MS	30.5	30	2.00(0.21)	63.6	0.147(0.038)
211 St Tammany, LA	30.6	30	1.77(0.16)	59.1	0.173(0.042)
201 Colleton Co., SC	32.3	30	2.14(0.20)	72.7	0.175(0.038)
70 LA Land Race	-	31	2.23(0.25)	63.6	0.194(0.041)
<i>Pinus elliottii</i> Means			2.04	64.8	0.173
<i>Pinus caribaea</i> var. <i>bahamensis</i> (<i>P.c.b.</i>)					
East Grand Bahama	26.6	32	2.14(0.21)	72.7	0.210(0.047)
West Grand Bahama	26.6	32	2.18(0.21)	72.7	0.233(0.047)
<i>P. palustris</i>	30.2	30	1.68(0.20)	45.5	0.107(0.039)
<i>P. taeda</i>	31.2	32	2.18(0.21)	72.7	0.196(0.047)

¹ Populations with single digit I.D. are from a 4-year-old provenance test located in south Mississippi (Doudrick *et al.* 1996), those with double digit I.D. were collected *in situ*, and those with triple digit I.D. are from a 45-year-old provenance test in south Mississippi (Snyder *et al.* 1967).

Table 2. Monoterpene composition of stem-gum samples from slash pine and related taxa.

Taxa ²	Source ³	n	Component ¹						
			α -Pin	Camph	β -pin	Δ_3 -car	Myrc	Lim	β -phel
			----- % -----						
<i>Pee</i> - MS	2	10	46.5	0.36	34.37	0.01	0.27	0.01	18.5
<i>Pee</i> - MS	2	7	58.2	0.67	34.24	0.36	0.79	0.01	4.7
<i>Pee</i> - FL	1	14	60.7	0.79	21.91	0.01	2.36	1.05	13.2
<i>Pee</i> <i>Ped</i>	2	13	30.1	0.43	5.79	0.09	0.56	0.01	63.0
<i>Ped</i> - 13	1	13	37.4	1.05	2.90	0.30	2.95	1.46	54.2
<i>Ped</i> - 14	1	8	51.2	1.16	4.74	0.23	2.88	1.39	38.4
<i>Ped</i> - 16	1	16	44.1	1.06	5.49	0.16	2.87	1.32	45.1
<i>Ped</i> - 18	1	12	36.8	1.08	3.53	0.42	2.79	1.18	54.3
<i>Pcb</i> - 1 1	15	30.4	1.01	2.55	2.67	3.04	1.44	58.9	
<i>Pcb</i> - SA1	1	10	40.9	0.69	5.36	3.78	2.66	1.14	45.5
<i>Pcb</i> - 3 1	15	39.0	1.00	3.97	3.48	3.29	2.05	47.3	
<i>Pcb</i> - 5 1	17	32.7	0.81	2.10	8.29	3.21	1.38	51.5	
<i>Pcb</i> - 9 1	15	41.0	1.11	3.79	10.65	3.16	1.53	38.6	
<i>Pcb</i> - 11	1	15	26.0	0.77	1.83	9.41	3.17	0.98	57.8
<i>Pcb</i> - SA11	1	12	38.1	0.68	3.91	0.30	3.08	1.43	52.5
<i>Pcb</i> - 17	1	15	36.3	1.45	2.94	0.59	2.87	1.70	54.2
<i>Pcb</i> - 20	1	16	28.1	0.84	2.31	14.64	3.26	1.79	49.0
<i>Pcb</i> - 21	1	16	33.3	0.97	2.56	0.49	2.70	1.26	58.7
<i>Pcc</i> - 1 1	9	62.1	0.72	4.91	2.50	2.01	0.50	27.2	
<i>Pcc</i> - 2 1	12	64.0	1.41	5.55	0.21	2.76	0.41	25.7	
<i>Pcc</i> 2	11	58.9	0.68	5.56	3.77	3.82	3.55	23.7	
<i>Pch</i> - BH	1	12	68.1	0.48	10.81	0.04	2.75	1.08	16.8
<i>Pch</i> - H	1	11	64.4	0.69	6.49	0.05	3.05	1.19	24.1
<i>Pch</i> - N	1	11	65.0	0.72	8.02	0.06	3.40	1.15	26.7
<i>P cubensis</i>	1	10	89.1	0.73	3.63	0.04	2.42	1.01	3.0
<i>Pp X Pee</i>	2	23	54.3	0.17	41.29	0.61	0.01	0.01	3.6

¹ Alpha pinene, camphene, beta pinene, delta-3-carene, myrcene, limonene, and beta phellandrine, respectively.

² *Pee* = *Pinus elliotii* var. *elliotii*, *Ped* = *P. elliotii* var. *densa*, *Pcb* = *P. caribaea* var. *bahamensis*, *Pcc* = *P. caribaea* var. *caribaea*, *Pch* = *P. caribaea* var. *hondurensis*, *Pp X Pee* = *Pinus palustris* X *P. elliotii* var. *elliotii* F1 hybrid.

³ Original source of the data: 1 - Nikles (1966), 2 - Coyne (1974) unpublished data.

Figure captions

Figure 1. Map of the southeastern United States and adjacent Caribbean showing the natural distribution of slash pine (adapted from Critchfield and Little 1966) and sample points for allozyme analysis.

Figure 2. Plot of expected heterozygosity H_e for slash pine allozyme data versus migration distance from a putative Pleistocene refugium near Key West, FL. H_e is also shown for the Bahamian Caribbean pine (*Pcb*), but is not included in the regression. Data for the LA land race is also not included.

Figure 3. Plot of first and second canonical vectors (representing 46% of the total variation) from the multivariate analysis of transformed allozyme data. Loblolly pine is not shown, as it is widely separated from the data shown.

Figure 4. Plot of first and second canonical vectors (representing 92% of the total variation) from the multivariate analysis of monoterpene data.

Co-dominant, Segregating Polymorphic Markers in Douglas-fir (*Pseudotsuga menziesii*)

Project Objective

To obtain co-dominant, segregating polymorphic markers for inclusion in a Douglas-fir research program.

Results

One hundred Douglas-fir trees were genotyped at over 20 isozyme loci (starch gel electrophoresis (4 grandparents, 2 parents, and 94 progeny)).

Five loci (Lap, Fest, Ugpp, Idh, and Pgi-2) were segregating among the progeny. One progeny individual could not be the offspring from the designated parents.

Clients were provided with photographs of gels, a double scored data set, and a copy of the project summary. Data will be incorporated into a Douglas-fir genomic mapping project.

NFGEL Project #101; Contact: Kostya Krutovskii, USDA Forest Service, Pacific Southwest Research Station, IFG-Davis

Clonal Identification in *Pseudotsuga menziesii* (Douglas-fir)

Project Objective

Verify ramets of *Pseudotsuga menziesii* (Douglas-fir) clones. Between two and eleven ramets from each of four clones were submitted for analysis.

Results

Nineteen Douglas-fir trees were genotyped at 20 isozyme loci using starch gel electrophoresis.

CLONE 1

The genotype of ramet 14-13 does not match that of clone 1. Individual 14-13 does not appear to be a ramet of clone 1.

CLONE 2

Sample 2-18-12 (sent 10/16/01) is not the same sample 2-18-12 that was sent previously (6/1/01). These two samples differ at several loci (as confirmed on gel photos). The new sample 2-18-12 matches the genotype of 2-05-09 and both appear to be ramets of clone 2. Samples 2-10-01-west-fork and 2-10-01-east-fork have matching genotypes. However, this genotype does not match the genotype of 2-18-12 and 2-05-09 at over five loci. It appears that 2-10-01-west/east-fork is not a ramet of clone 2.

CLONE UNKNOWN-29-02

Samples 29-02-ground and 29-02-crown have matching genotypes. The genotype does not match that of clone 1, clone 2, or clone 3.

CLONE 3

The 'progeny' individuals (7-58, 23-58, 17-27, 3-119, 10-30, 33-60, 26-54 or 53, 20-81, 30-114, or 14-118) could all be offspring of tree 3-11-42 (if tree 3-11-42 served as an open-pollinated mother tree).

NFGEL Project #122; Contact: Bryan Schulz, Olympic Resource Management

Supplemental Mass Pollination Efficacy in a Douglas-fir Seed Orchard

Project Objectives

To determine the efficacy of supplemental mass pollination methods in a Douglas-fir seed orchard by genotyping meg/embryo pairs from five controlled crosses.

Results

Seed from four parent trees were genotyped using 10 megs per parent. Seed from five controlled crosses were germinated to produce a minimum of 75 meg/embryo pairs. Parent 1 served as the paternal parent, the other three trees are maternal parents.

A total of 399 seed (megagametophyte/embryo pairs) from crosses A, B, C, D, and E were genotyped at 15 isozyme loci. Two of the crosses had two seed contaminants each. The seed contaminants were removed from the percent pollen contaminant calculation. The minimum percent pollen contamination ranged from 11.2 to 53.6%. Undoubtedly, there are also undetected contaminants in the dataset (contaminating pollen that have the same multilocus genotype as those produced by the paternal parent tree #1).

cross	# seed analyzed	seed contaminants		pollen contaminants	
		#	%	#	%
A	63	2	3.2	23	37.7
B	77	0	0.0	31	40.2
C	84	2	2.4	44	53.6
D	77	0	0.0	26	33.8
E	98	0	0.0	11	11.2

NFGEL Project #123; Contact: Jim Reno, Sequim Seed Orchard

Detecting Segregating Alleles at the CAD Locus in *Pinus taeda* (loblolly pine)

Project Objectives

Provide segregating alleles for the CAD loci in loblolly megs.

Results

Segregating CAD alleles were equally detected on both the LB and SB buffer systems, using one half of a megagametophyte (three quarters of a meg gave more intense staining than using half a meg). Some null alleles were difficult to confirm (difficult to distinguish from faint active alleles).



NFGEL Project # 127; Contact: Geoff Gill, USDA Forest Service, Pacific Southwest Research Station, IFG-Davis

Genetic Diversity in Mexican Populations of Douglas-fir (*Pseudotsuga menziesii*)

Project Objectives

To determine levels of genetic diversity in Mexican populations of Douglas-fir. Samples from the USA and Canada will be used as reference populations. A visiting scientist from Mexico will analyze the USA and Canada samples at NFGEL, and receive laboratory and technical training. The scientist will return to Mexico to complete the analysis of the samples from the Mexican populations.

Results

Douglas-fir tissue was received from Idaho, Arizona, and California (one population from each state; 30 individuals per population).

General diversity statistics were calculated. Statistics were as expected except that the Arizona population was significantly low in diversity and slightly divergent.

Diversity statistics for USA populations of Douglas-fir. P=percent polymorphic loci, A=mean number of observed alleles, Ho=observed heterozygosity, He=expected heterozygosity.

Population	N	P	A	Ho	He	Fst
California	59	61.1	1.9(0.9)	0.193(0.23)	0.215(0.25)	
Idaho	56	72.2	2.3(1.0)	0.196(0.24)	0.240(0.26)	
Arizona	59	44.4	1.8(1.1)	0.134(0.22)	0.143(0.23)	
ALL	173	83.3	2.7(1.1)	0.173(0.20)	0.234(0.25)	0.168

Nei's genetic similarities (above the diagonal) among Douglas-fir populations.

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=====
pop ID      CA      ID      AZ
=====
CA          *****  0.920  0.899
ID          *****  *****  0.964
AZ          *****  *****  *****
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NFGEL Project #130; Contact: Dr. J. Jesus Vargas Hernandez, Especialidad Forestal, Colegio de Postgraduados, Montecillo, Mexico. Visiting scientist: Francisco Alberto Dominguez Alvarez.

Clonal Identification in *Chamaecyparis lawsoniana* (Port-Orford Cedar)

Project Objective

Verify ramets and offspring of a *Chamaecyparis lawsoniana* (Port-Orford Cedar, POC) clone. Three ramets of the clone, and 28 putative offspring, were submitted for analysis.

Results

Thirty-one POC individuals were genotyped at 18 isozyme loci using starch gel electrophoresis. Genomic DNA was also extracted from the three ramets and RAPD data obtained using 10 primers.

Both the isozyme and RAPD data show that Ramets 1 and 2 have matching genotypes. The Ramet #3 genotype does not match the genotype of Ramets 1 and 2. This is clearly demonstrated at the PGI-2 isozyme locus.

Lane: 1 2 3 4 5



Lane 1 and 5: Red Pine Controls

Lane 2: Ramet 1

Lane 3: Ramet 2

Lane 4: Ramet 3

From the isozyme data, we are unable to determine which ramet (#1, #2 or #3) served as the maternal parent to the seedlings. Seedling 4-1 is not, however, an offspring of these ramets (all three ramets have a '11' genotype at 6PGD-1, whereas seedling 4-1 has a '22' genotype). Also, we did not pick up enough isozyme variation among the possible pollen parents to make assessment regarding paternity. However, it seems the more pressing issue at this point are the mis-identified ramets.

NFGEL Project # 131; contact: Richard Sniezko, USDA Forest Service – Region 6.

Quaking Aspen (*Populus tremuloides*) Clones in Northeast Oregon

Project Objectives

To further investigate the patterns and levels of genetic diversity within and among aspen populations in Oregon. Data will be combined to the prior NFGEL aspen project, Project #68.

Introduction

Quaking Aspen (*Populus tremuloides* Michx.) appears to be declining in the Blue Mountains of eastern Oregon. Comparison with historical data shows aspen to be absent in many sites formerly occupied, and many existing stands are at risk of extirpation. Factors leading to these changes include fire suppression, conifer succession, and grazing by both livestock and wild ungulates. Declining stands consist of mature to old (ca. 125 year old) trees, and the overstory trees are experiencing extensive mortality from natural causes. Herbivory of sprouts by wild and domestic ungulates is causing high mortality in the younger age classes. Seed set appears to be low, in part because many stands are isolated, and in part because a majority of the surviving stands appear to be male. Once mature old trees are dead and sprouting ceases or is too heavily browsed, the stand can not replace itself and will be lost.

Understanding Quaking Aspen clone structure is crucial to preserving aspen genetic diversity. Management efforts include constructing grazing exclosures, transplanting root suckers and/or nursery stock to augment natural regeneration, and establishing off-site clone banks. Each of these approaches is expensive in terms of time or money, and resources for managing aspens are limited. Therefore, these procedures must be applied efficiently. If each aspen stand is a single clone, preserving a small part of it will preserve its diversity. If each stand contains many clones, more extensive efforts may be required to preserve existing diversity.

This study uses isozyme analysis to identify clones, determine the number of clones in each stand, determine whether stands are most similar to other nearby stands, and assess the genetic diversity of aspen in northeast Oregon.

Materials and Methods

In 1997, leaf tissue samples were collected in 46 stands on 20 river drainages on the North Fork John Day Ranger District, Umatilla National Forest, Morrow and Umatilla Counties, Oregon. Samples were collected along transects made through the long axis of each stand. In 1999, samples were collected in 45 stands on 25 river drainages in the Umatilla and Wallowa-Whitman National Forests, tribal lands of the Confederated Tribes of the Umatilla Indian Reservation, and nearby private property, and from two isolated saplings in an area burned in Oriental Basin in the east side of the North Fork John Day Ranger District. Sampling procedures were similar to those in 1997, but sampling intensity was lower within stands and fewer stands per drainage were sampled.

A total of 17 isozyme loci were scored via starch gel electrophoresis. Analysis was performed on a data set that included one sample per multilocus genotype per stand. Within a stand, all samples with the same multilocus genotype were considered ramets of the same clone. Samples from different stands were considered to be different clones, even if they were identical. The probability of occurrence of each genotype was calculated as the product of the frequencies of all alleles in that genotype. Results were analyzed using Popgene version 1.21.

Results

Of the 789 samples analyzed, there were 232 genetic individuals (or clones). The average probability of occurrence for the complete multilocus genotypes was 2.6×10^{-5} (s.d. 7.1×10^{-5}). Three of the complete genotypes duplicated in different stands may have occurred by chance, as a result of sexual reproduction, but three others may represent remnants of fragmented ancient clones.

The number of clones per stand varied from 1 to 14, with an average of 2.5. In 41 (45%) stands only one genotype was detected. Although sampling intensity was higher in 1997 than in 1999, essentially the same proportion of stands appeared to be monoclonal in each year (46% and 44% in 1997 and 1999, respectively). In 24 (48%) stands with more than one clone, samples of each clone were collected sequentially, indicating that clones may have been separated although adjacent. In the others (52%), clones were interdigitating to a greater or lesser extent. Stands that apparently consisted of a single clone varied in size from 0.01 to 1.4 ha. They averaged smaller than stands with two or more clones. Despite much overlap in size, this difference was significant ($t = -2.15$, $p = 0.035$) over the entire study, but not in either year alone.

Clone structure varied among regions. For example, in the eastern portion of the North Fork John Day Ranger District, stands were smaller, distances between stands were large, 73% of stands consisted of a single clone and the others contained two clones, and the number of clones/sample was 0.18. In the western portion of the same Ranger District, stands were larger and closer together, only 39% of stands consisted of a single clone and the others contained up to 14, and the number of clones/sample was 0.26. Within the western portion of the North Fork John Day Range District, drainages also differed in the number of clones per stand.

As a group, the aspens were genetically variable, with 15/17 (88%) of loci polymorphic and an average of 3.94 alleles per locus (Table). Individual stands, which consisted of only one to 14 genetic individuals, were much less variable, averaging 25.9% polymorphic loci with 1.33 alleles per locus. All stands were variable in the sense that they had at least one polymorphic locus. (In monoclonal stands, the polymorphic locus (loci) was (were) heterozygous in all individuals).

Regions and drainages were genetically similar. Genetic identities among regions were all >0.92 . Genetic similarities among all drainages average 0.9096. Genetic similarities among aspen stands were highly variable and often low, averaging 0.885. Genetic and geographic distances between stands were not related ($r = 0.172$).

The fixation index (F) varied from zero to 0.51 in drainages that contained more than one stand, and from 0.21 to 0.58 in regions, indicating a deficiency of heterozygotes. F_{st} values for stands within regions were high. Overall, differences among individuals within drainages contributed about most of the total variation; drainages and regions were much less differentiated, contributing 6% to 7% each.

Discussion

Aspen stands in northeastern Oregon are diverse. As in northeast North America, the monoclonal Oregon stands are often small (averaging 0.4 ha), and the majority contain two or more clones. Like aspen in the Rocky Mountains, an important proportion (45%) of stands appeared to consist of only one clone, and at least three clones once attained great size, although they are now fragmented. Northeast Oregon aspens include saplings apparently established in the last decade and remnants of ancient clones.

Three genotypes appear to be the fragmented remnants of large, ancient clones. Each of these three genotypes grows in what are now two stands separated by at least 200 m. Morsay Creek clone #1 is especially impressive. Only one genotype was detected in Morsay Creek stands 1 and 2. This genotype is expected to occur rarely by sexual reproduction ($p = 10^{-8}$), so it is more likely that the trees in both stands are ramets of a single clone, even though these two stands are separated by 866 m.

Apparently a single aspen clone became established and spread along Morsay Creek, dying in the middle.

In northeast Oregon, aspen clone structure varies among areas. For example, in eastern portion of the North Fork John Day Ranger District, drainages with good aspen habitat are few, short, and distant from one another. Aspen stands are few and isolated. Most stands (73%) contain only a single clone, and the maximum number of clones per stand was two. Establishment of aspen from seed is episodic, depending on exposure of bare soil at the season when the short-lived aspen seeds disperse, plus adequate precipitation to support seedling growth until roots are long enough. In an area like the eastern portion of the North Fork John Day Ranger District, seed is unlikely to reach the isolated patches of suitable habitat during the rare seasons seedling establishment is possible. Therefore, the establishment of even one clone is unlikely, and an isolated clone of this dioecious species cannot reproduce sexually.

The situation is different in the western portion of the same ranger district. Drainages are longer and contain more aspen habitat. Distances between stands are shorter. Seed is more readily available when environmental conditions allow seedling establishment. The chance of two or more clones becoming established in the same area is greater. More female clones become established, and therefore sexual reproduction is possible and more seed is available when conditions allow seedling establishment. Many stands on the west side of the North Fork John Day Ranger District were diverse, with as many 14 clones per stand.

Even within the western part of the North Fork John Day Ranger District, clone diversity differs among drainages, perhaps due to differences in fire history. For example, the five aspen stands of Morsay Creek has half as many aspen clones as the four stands on Thompson Creek, although approximately the same number of samples were collected in each drainage and the Morsay Creek stands cover a larger area. Other stands in the area were also diverse, such as Matlock Creek (12 clones) and Sugar Bowl Creek (51 clones). We hypothesize that the more diverse drainages have burned relatively recently or frequently, and therefore they have provided more opportunities for establishment of aspen seedlings. We hypothesize that Morsay Creek has had fewer recent fires. Therefore, genetic individuals that have died out have not been replaced and the number of extant clones is low.

Although the extent and diversity of clones within aspen stands varies geographically, all studies of aspen genetic diversity, including this one, agree that *Populus tremuloides* harbors unusually great genetic diversity. The 88% polymorphic loci observed in this study is similar to the values observed elsewhere. The 3.94 alleles per locus is higher than observed before.

Most of the genetic variation observed in this study was individual variation, as has been true in other studies of *Populus tremuloides*. Aspen stands are extremely differentiated; F_{st} values for stands within regions are greater than 0.4 for five regions. However, this does little more than restate the fact that individuals are different; the average number of genetic individuals per stand was 2.5. When clones are grouped into drainages or regions, larger units that could more reasonably be considered subpopulations within the total study, F_{st} values are lower, indicating that populations are only moderately differentiated.

Management Implications

Isozyme analysis of aspen stands in the Umatilla is useful for conservation efforts in three ways. First, the clone maps generated by this study permit efficient collection of ramets from different clones. Phenotypic variation alone had not revealed the extent of many of these clones. Second, the high F_{st} values reveal that stands are highly differentiated genetically. Therefore, conservation efforts

will be successful at preserving local aspen genetic resources only if those efforts are directed at preserving many stands and their component clones. Finally, isozyme analysis can help prioritize aspen stands and clones for preservation. From a genetic standpoint, high priority stands might include the most diverse stands (e.g. Sugar Bowl stand 7) or remnants of ancient stands (e.g. Morsay Creek stands 1 and 2). Of course, ecological as well as genetic issues are involved in prioritizing stands for conservation.

Table. *Populus tremuloides* genetic diversity, by region, using one sample per clone per stand. Clones = number of genotypes detected. %P = percent polymorphic loci. A = number of alleles per locus. Ae = effective number of alleles per locus. Ho = observed heterozygosity. He = expected heterozygosity. F = fixation index = (He-Ho)/He. Fst = differentiation of stands within region. Nm = calculated gene flow among stands. Standard deviation in parentheses. Regions: CTUIR = Confederated Tribes of the Umatilla Indian Reservation; UMA = Umatilla National Forest; WAW = Wallowa-Whitman National Forest. Subregions: NFJD = North Fork John Day Ranger District (east or west side), BAK = Baker Ranger District, HP = Heppner Ranger District, WW = Walla Walla Ranger District, WAV = Wallowa Valley Ranger District.

Region	Subregion	Stands	Clones	%P	A	Ae	Ho	He	F	Fst	Nm
Entire study		91	233	88.2	3.9 (1.8)	1.6 (0.8)	0.162 (0.266)	0.234 (0.258)	0.306	0.49	0.256
Mean		13 (11)	33.3 (39)	70.6 (9.0)	2.4 (0.4)	1.5 (0.1)	0.177 (0.026)	0.238 (0.028)	0.256 (0.053)	0.414 (0.129)	0.423 (0.271)
UMA	HP	7	12	76.5	2.3	1.6	0.206	0.272	0.243	0.488	0.262
UMA	NFJD East	15	19	64.7	2.4	1.5	0.167	0.231	0.279	0.579	0.182
UMA	NFJD West	36	120	64.7	2.4	1.4	0.139	0.193	0.281	0.428	0.334
UMA	WW	11	27	70.6	2.5	1.6	0.205	0.251	0.182	0.409	0.361
CTUIR		9	13	64.7	2.2	1.5	0.154	0.235	0.344	0.504	0.246
WAW	BAK	4	10	64.7	1.9	1.4	0.173	0.217	0.204	0.210	0.939
WAW	WAV	9	32	88.2	3.2	1.7	0.197	0.267	0.264	0.281	0.640

NFGEL Project #84, in combination with NFGEL Project #68; Contact: Vicky Erickson, USDA Forest Service, Umatilla, Wallowa-Whitman and Malheur National Forests.

STAFF ACTIVITIES

Meetings, Shortcourses, and Workshops

Presentations

- 2002. V Hipkins. Applied conservation genetics. Lecture to biology class at Consumnes River College. April 12.
- 2002. RC Schmidting and VD Hipkins. The after-effects of reproductive environment in shortleaf pine (*Pinus echinata*). IUFRO Working Group: Pacific temperate conifers as native and introduced species – genetics and sexual reproduction. Orleans, France, May 27 – 30.
- 2002. V Hipkins. Estimating pollen contamination. Northwest Seed Orchard Association, Eureka, CA. June 18-20.

Posters

- 2002. VJ Erickson, BL Wilson, D Shirley, and VD Hipkins. Genetic diversity and structure of isolated populations of quaking aspen (*Populus tremuloides*) in northeastern Oregon: implications for conservation and management. Western Forest Genetics Association/Northwest Forest Biology Workshop. University of Washington, Pullman, WA, July 15 – July 19.

Attended

- 2002. Western Forest Genetics Association. University of Alberta, Edmonton, Alberta, Canada, July 22 – 25. (V Hipkins).
- 2002. National Genetics Meeting, USDA Forest Service, National Forest System, Lake Hiawasse, GA, April 21 – 26. (V Hipkins)

Professional Activities

- Peer reviewer for Canadian Journal of Forest Research and TAG (V Hipkins).
- Adjunct faculty member at Northern Arizona University, School of Forestry, Flagstaff, AZ (V Hipkins).
- Western Forest Genetics Association Officer – President (fourth year) (V Hipkins).

Internal Activities

- Member of Region 2,4,5, & 6 FFIS Fire Payment Team (S Carroll)
- Member of the National Forest Service Safety Committee (R Meyer)
- Union President – Pacific Southwest Research Station (R Meyer)
- Participated on selection panel for Region 9 Geneticist position (V Hipkins)
- Participated in updates of the NFS Genetic Resource Program Strategic Plan, and National Genetics Program Review (V. Hipkins)

Hosted

NFGEL continues to host a variety of visitors. Tours of the facility and operation were provided to Forest Service employees, members of the public and private industry, university faculty, foreign scientists, and employees from other state and federal government agencies. NFGEL hosted a visiting scientist from Mexico for a six week period, a graduate student from UC Santa Cruz for two days, and two School to Career program members for one day each. .

Collaborations and Cooperations

NFGEL formed collaborations with FS Research Stations, Bureau of Land Management, California Department of Transportation, US Fish and Wildlife Service, University of California at Davis, private companies, and non-profit groups. We also collaborate internally within the Agency to lend expertise in the

area of genetics. This took the form of reviewing contracted work reports on the genetics of mammals and plants.

CURRENT STAFFING

During FY02 (10/1/01 to 10/1/02), NFGEL was staffed with four permanent full-time, and five temporary employees. NFGEL currently has two permanent full-time 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 (10/01 – 7/02)	scarroll@fs.fed.us
Pat Guge	Lab Biotechnician	PFT	pguge@fs.fed.us
Randy Meyer	Lab Biotechnician	PFT	rmeyer@fs.fed.us
Robert Saich	Data Manager	Temp (10/01 – 10/02)	rcaich@fs.fed.us
Barbara Wilson	Associate Director	Temp (10/01 – 12/01)	--
Ricardo Hernandez	Lab Biotechnician	Temp (12/01 – 10/02)	ricardohernandez@fs.fed.us
Joe Mortenson	Lab Biotechnician	Temp (12/01 – 3/02)	--
Josh Seitz	Lab Biotechnician	Temp (12/01 – 8/02)	--

BUDGET

Activity	FY00	FY01	FY02
Receipts (in thousands)			
Allocation	290.0	290.0	343.0
Carryover	0.2	6.9	0.0
Soft Money	26.5	169.8	66.2
Total	316.7	466.7	409.2
Expenditures (in thousands)			
Salary (permanant)	*171.5	**185.7	***198.5
(temperary)	25.6	19.2	29.3
Overhead to Headquarters	59.0	60.0	39.6
Overhead to Site	0.0	10.9	48.5
Chemicals/Supplies	23.6	15.7	29.1
Equipment	3.2	164.2	8.1
Travel/Training	11.6	5.0	5.5
Awards	0.0	2.5	1.3
Fees	0.5	0.3	0.0
Books/subscriptions	1.0	0.0	0.3
Computers (not including FOR	3.7	0.8	0.1
Repair	1.2	0.8	0.8
Photos/Slides/Publications	1.2	1.2	0.6
Postage	0.9	0.1	0.2
Office Supplies	0.2	0.1	0.0
Furniture	0.4	0.0	0.4
Lab Relocation	0.0	9.7	0.0
Total	303.6	476.2	362.3

*does not include \$5.2 in salary due to alternate salary sources

**does not include \$11.7 in salary due to alternate salary sources

*** does not include \$18.0 in salary due to alternate salary sources

FY 02 Soft Money

Source		Amount (\$)	Percentage
FS-NFP (WO)	Conservation and Restoration	15,852	23.9%
FS-R6	Conservation of <i>Perideridia</i>	1,200	1.8%
FS-R5	Conservation of <i>Silene</i>	14,000	21.1%
FS-PSW	Computer FOR	7,000	10.6%
Private Companies	Seed Orchard Management	4,689	7.1%
BLM	Seed Orchard Management	23,482	35.5%
Total		66,223	100.0%

Project Workload, FY02

(1) Isozymes (starch gel electrophoresis)

By Project

Region or Agency	Project #	Species	# gels	# days	# weeks
Forest Service					
SRS	92	<i>Pinus massoniana</i>	52.0	11.0	6.0
R5	100	<i>Populus tremuloides</i>	90.0	12.0	6.0
PSW	101	<i>Pseudotsuga menziesii</i>	24.0	4.0	2.0
R1	103	<i>Pinus ponderosa</i>	6.0	1.0	0.50
FSR-RMRS	104	<i>Viguiera multiflora</i>	10.0	1.0	0.50
FSR-RMRS	106	<i>Erigeron pumilis</i>	23.0	3.0	1.50
FSR-RMRS	108	<i>Crepis acuminata</i>	15.0	2.0	1.00
FSR-RMRS	110	<i>Astragalus utahensis</i>	10.0	1.0	0.50
FSR-RMRS	113	<i>Eriogonum umbellatum</i>	23.0	2.0	1.00
FSR-RMRS	115	<i>Lupinus argenteus</i>	16.0	2.0	1.00
FSR-RMRS	117	<i>Bromus carinatus</i>	7.0	1.0	0.50
FSR-RMRS	119	<i>Vicia americana</i>	7.0	1.0	0.50
SRS	126	<i>Pinus elliotii</i>	12.0	1.0	0.50
PSW	127	<i>Pinus taeda</i>	6.0	1.5	2.00
SRS	128	<i>Pinus taeda</i>	3.0	0.5	0.25
R6	131	<i>Chamaecyparis lawsoniana</i>	12.0	3.5	2.00
FSR-RMRS	132	<i>Atriplex canescens</i>	33.0	4.0	2.00
FSR-RMRS	134	<i>Stipa comata</i>	7.0	1.0	0.50
FSR-RMRS	139	<i>Artemesia tridentata</i>	0.5	0.5	0.25
FSR-RMRS	141	<i>Chrysothamnus nauseosus</i>	0.5	0.5	0.25
CalTrans	98	<i>Pinus radiata</i>	7.0	1.0	0.50
Pope Ind.	122	<i>Pseudotsuga menziesii</i>	3.0	0.5	0.25
Weyhaeuser	123	<i>Pseudotsuga menziesii</i>	54.0	12.0	6.50
BLM	124	<i>Pinus lambertiana</i>	120.0	13.0	6.50
Mount Vernon	129	"Washington trees"	20.0	2.0	1.00
International -Mexico	130	<i>Pseudotsuga menziesii</i>	9.0	0.5	0.50
TOTAL			570.0	82.5	44.0

By Forest Service Region or Agency

Region or Agency	# gels	# days	# weeks
Forest Service			
NFS			
R1	6.0	1.0	0.50
R5	90.0	12.0	6.00
R6	12.0	3.5	2.00
Research			
SRS	67.0	12.5	6.75
PSW	30.0	5.5	4.00
RMRS	152.0	19.0	9.50
CalTrans	7.0	1.0	0.50
Pope Ind.	3.0	0.5	0.25
Weyhaeuser	54.0	12.0	6.50
BLM	120.0	13.0	6.50
Mount Vernon	20.0	2.0	1.00
International -Mexico	9.0	0.5	0.50

(2) DNA

Region or Agency	Project #	species	# DNA extractions	Extraction Method	# PCR reactions	# days	# weeks
FS-NFS-R1	103	<i>Pinus ponderosa</i>	644	FastPrep	--	37	26
FS-FSR-RMRS	105	<i>Viguiera multiflora</i>	25	FastPrep	--	2	2
FS-FSR-RMRS	107	<i>Erigeron pumilus</i>	16	FastPrep	--	1	1
FS-FSR-RMRS	107	<i>Erigeron pumilus</i>	82	FastPrep	--	7	3
FS-FSR-RMRS	109	<i>Crepis acuminata</i>	30	FastPrep	--	4	7
FS-FSR-RMRS	111	<i>Astragalus utahensis</i>	62	FastPrep	--	8	8
FS-FSR-RMRS	114	<i>Eriogonum umbellatum</i>	20	FastPrep	--	2	1
FS-FSR-RMRS	114	<i>Eriogonum umbellatum</i>	42	FastPrep	--	3	1
FS-FSR-RMRS	116	<i>Lupinus argenteus, L. sericeus</i>	80	FastPrep	--	5	7
FS-FSR-RMRS	118	<i>Bromus carinatus</i>	20	DNeasy		2	2
FS-FSR-RMRS	120	<i>Vica americana</i>	20	FastPrep		1	1
FS-NFS-R6/BLM	121	<i>Chamaecyparis lawsoniana, Ch. nootkantensis</i>	98	FastPrep		9	16
FS-NFS-R6/BLM	121	<i>Chamaecyparis lawsoniana, Ch. nootkantensis</i>			77	6	6
Mount Vernon	129	<i>Fraxinus americana</i>	6	DNeasy		3	4
Mount Vernon	129	<i>Fraxinus americana</i>			78	4	9
Mount Vernon	129	<i>Ilex opaca</i>	20	DNeasy		2	4
Mount Vernon	129	<i>Ilex opaca</i>			172	12	6
Mount Vernon	129	<i>Liriodendron tulipifera</i>	8	DNeasy		4	4
Mount Vernon	129	<i>Liriodendron tulipifera</i>			126	4	9
Mount Vernon	129	<i>Morus alba</i>	2	DNeasy		1	1
Mount Vernon	129	<i>Morus alba</i>			28	4	9
Mount Vernon	129	<i>Tsuga canadensis</i>	10	DNeasy		2	4
Mount Vernon	129	<i>Tsuga canadensis</i>			102	12	6
FS-NFS-R6/BLM	131	<i>Chamaecyparis lawsoniana</i>	3	FastPrep		1	1
FS-FSR-RMRS	133	<i>Atriplex canescens</i>	193	DNeasy		15	10
FS-FSR-RMRS	133	<i>Atriplex canescens</i>	43	FastPrep		2	2
FS-FSR-RMRS	135	<i>Stipa comata</i>	62	DNeasy		3	1
FS-FSR-RMRS	140	<i>Artemesia tridentata</i>	24	DNeasy		2	1
FS-FSR-RMRS	140	<i>Artemesia tridentata</i>	12	FastPrep		1	1
FS-FSR-RMRS	142	<i>Chrysothamnus nauseosus</i>	42	DNeasy		4	4

By Region or Agency

Region or Agency	# DNA Extractions	# PCR Reactions	# days	# weeks
Forest Service				
NFS-R6/BLM	101	77	16	23
NFS-R1	644		37	26
FSR-RMRS	773		62	52
Mount Vernon	46	506	48	56

ABI 3100 Markers

Region or Agency	Number of 16 capillary runs	Date interval	Dilute Pre-selective Amplification Rx Product
Mount Vernon, Project 129	25	8/9/02 to 9/27/02	42
Development	28	1/10/02 to 5/30/02	7

BLM=Bureau of Land Management

FS=Forest Service

CalTrans=California Department of Transportation

FS=Forest Service

FSR=Forest Service Research

RMRS=Rocky Mountain Research Station

NFS=National Forest System

R#=Region