Annual Report 1999-2000(FY00)

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INTRODUCTION

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. 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.

The Mission

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

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 agencies. 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.

Message from the Director

This report details Laboratory accomplishments covering the period October 1999 to October 2000, and corresponds to the Federal 2000 fiscal year (FY00).

During this report period we completed fifteen projects using a combination of protein and DNA markers. Our work was used to support silvicultural and tree improvement activities, as well as conservation and restoration programs. We also continued to expand our role in the area of forensics. Our wide range of clients included the USDA Forest Service National Forest System and Research Branch, other federal agencies, and private organizations.

We faced several technical challenges this year. First, there was a global shortage of high quality starch when the primary starch producer could not deliver a suitable lot. This led us and other laboratories from around the world to search for alternate producers. We tested a variety of starch lots and found a company in Canada that produced it to our desired quality. This starch provides the best separation between alleles. The other sources tested were unable to separate alleles well and if used, would have resulted in lower levels of detectable variation. This higher quality starch cost twice as much per kilogram than our previous source, but is required to meet our quality standards. Secondly, the microwave that we used for the last twelve years to prepare our starch gels came to the end of its long, fruitful life. This may seem like a trivial matter as we thought until we tried to replace it. The past twelve years has brought many improvments. It has also brought smaller microwaves. None of

our glassware would fit into the new, microwaves available today. The problem was resolved by purchasing the largest microwave we could find and replacing our glassware with shorter pieces. Newer is not always better.

On a positive note, NFGEL will be relocating our facility to the Institute of Forest Genetics (IFG) in Placerville, California. We anticipate that the move will occur October 1, 2001 (Fiscal Year 2002), contingent upon the completion of renovations at IFG. Benefits of the move include improved access to technology and partners required to build our operation to capacity, and improved ability to perform science-based management in a more cost-effective manner. The newly remodeled lab will provide modern and efficient laboratory space that is handicap accessible and meets all safety standards. Our space at IFG will triple that now available to us at our present location. I look forward to the NFGEL-IFG association and believe that this connection will further our ability to maintain the state-of-the-art technology needed to accomplish our National Forest System mission. I am very grateful to all the people who have supported our move and, with their efforts, made this happen. Thanks to Sheila Martinson, David Neale, Frank Burch, Garland Mason, Ann Bartuska, Hal Salwasser, Sharon Friedman, the NFGEL Steering Committee, and all those who provided comments, offered support for NFGEL and our mission, and helped with groundwork preparations.

Valerie Hipkins
(vhipkins@fs.fed.us)
NFGEL Director

Overview

During this FY00 report year, we processed 12 projects utilizing isozyme markers obtained with a combination of starch gel electrophoresis and isoelectric focusing (IEF), and three projects using DNA techniques. NFGEL projects were processed to meet a variety of management objectives. Nine reports, including results from 13 projects, follow.

Silviculture and Tree Improvement

1. Genetic Variation and Origin of a Ponderosa Pine Seed Production Area
2. Evolutionary Relationships of Slash Pine (*Pinus elliottii*) with Its Temperate and Tropical Relatives
3. Allozyme Variation in Interior Douglas-fir: Association With Growth and Resistance to Western Spruce Budworm Herbivory
4. Clonal Identification in Douglas-fir (*Pseudotsuga menziesii*)

Conservation and Restoration

1. Evaluation of Genetic Diversity in Tahoe yellow cress (*Rorippa subumbellata*)
2. Genetic variation and population structure of the rare California plant *Collomia rawsoniana* (Polemoniaceae)
3. Seed Zones for Eastern Sierra Grass Species

Forensics

1. DNA Profiling and Identity Analysis of Ponderosa Pine Evidence Samples
2. Genetic Profiling of Black Walnut

GENETIC VARIATION AND ORIGIN OF A PONDEROSA PINE SEED PRODUCTION AREA

GENETIC TESTING SUMMARY

NFGEL Projects 70 and 82

Both DNA (chloroplast SSR's) and isozymes (starch gel electrophoresis) studies were carried out to identify the origin of ponderosa pine stands in the Idaho Panhandle National Forest, and to measure genetic diversity in various sources.

- These genetic analyses were not able to provide a conclusive source identification for the Avery SPA. The genetic markers (both DNA and isozymes) were not able to distinguish the submitted sources because all sources contained similar genetic variation. The amount of genetic information was not lacking; the abundant number of markers generated were simply not measuring source differences. As much evidence supported a native origin theory for the SPA as supported a non-local origin (see DNA Summary Report, and Discussion section of the Isozyme Summary Report). However, through the isozyme work, it appears that the SPA could not have originated from a Black Hills source.
- The DNA evidence strongly supports the hypothesis that a Bitterroot source was the origin of Brush Lake 3.
- The DNA evidence is inconclusive on determining an origin source for Brush Lake 2 and 4, and Deer Park. Again, because the genetic information could not tell sources apart well, we were not positively able to identify the origin of these three stands. However, it is likely that Brush Lake 2 and 4, and Deer Park are probably not from a Bitterroot source.
- The Avery SPA, and the Avery Natural Stand (the nearby native stand that is the most likely origin of parental material if the SPA was not planted), contain moderate levels of genetic diversity, typical of other tested sources (both 'native' and 'non-local').
- Although we could not know this before doing the research, isozyme and chloroplast DNA SSR markers are not the optimum tool to use to distinguish sources of Ponderosa Pine from the Idaho panhandle area. Instead, information from genecological research, as well as site indicators (insect/disease problems, broken tops, inferior form), should be used to assess off-site, or non-optimum, origin of suspect plantations. It is possible that other kinds of DNA markers could distinguish sources, but this would require an additional, thorough research oriented study.

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ISOZYME STUDY (NFGEL Project #82)

INTRODUCTION

In 1910, fires wiped out millions of acres of Ponderosa Pine in northern Idaho. Following the burn, plantations were often established using seed from unsuitable sources. These sources where typically either geographically distant (off-site) or elevationally incorrect (local source material from a higher or lower elevation than the planting site). The most commonly used sources were from the Bitterroot National Forest of west-central Montana, but some plantations were established using seed from the Black Hills

National Forest (southwest South Dakota), the Colville National Forest (northeast Washington), or the Wenatchee Natural Forest (central Washington). Many of these planted stands have deteriorated and have subsequently been removed.

A seventy-year-old stand on the Avery Ranger District of the Idaho Panhandle National Forest (IPNF) is performing well. The trees are healthy and produce abundant seed crops. Therefore, this stand is being used as a Seed Production Area (SPA). No records exist to document the origin of this stand, which was established after the 1910 burn. Several stands planted from Bitterroot and Colville sources exist within two air miles of the Avery SPA, but the SPA itself may have regenerated naturally. If it is a natural stand, it is probably closely related to (and probably originated from) a 150-year old stand located at an elevation of 3600 feet on the same ridge where the Avery SPA grows at elevation 4300 feet.

The origin of the trees in the Avery SPA is of concern for two reasons. First, trees of non-local origin (either geographically or elevationally), may deteriorate because they are not well adapted to local environmental conditions. Second, conservation of native genetic resources is a priority for the Forest Service. The effect on genetic diversity of replanting with seed from improved tree stands, rather than unimproved stands like the Avery SPA is also a concern. Therefore, this study uses isozymes to compare the genetic variation of trees from the Avery SPA with those from other possible native and non-local seed sources.

METHODS

Samples consist of approximately 40 seeds collected from at least 10 trees in various Seed Production Areas or natural stands. Also included in the analysis are five single family tree improvement lots (Table 1; Figure 1). Samples from the Colville National Forest consisted of ten small lots per Ranger District, and those from the Black Hills consisted of seven small lots. Each small lot apparently represented seeds collected from a single tree. In 1998 no seed could be obtained from the 150-year-old natural stand thought to be the parent stock of the Avery SPA. Therefore, branch tips (needles and a set of terminal buds attached to about 3-5 inches of stem) were collected at this site. Branch tips were placed on ice in the field and shipped to NFGEL in March 1999.

Forty megagametophytes were prepared from each bulked seed lot, except that four were prepared from each small Colville lot and seven from each small Black Hills lot. Seeds were soaked in 1% H202 for 48 hours. They were then plated, chilled, and allowed to germinate (Anonymous 1995). Seed lots that

developed visible mold growth were soaked in 1% chlorine bleach and replated. Lots requiring treatment for mold included the IPNF lots 7134, 7135, 7180, 7181, 7260, 797, 7305, 7344, 7354, 7356, 7361, 7390, 7396, and 7397, three lots each in Colville 1 and 2 and two in Colville 3, and TI-1, TI-2, and TI-3). After germination began, the whole megagemotophyte was dissected out of the seed and placed into a microtiter plate well containing 150 ul of 0.2 M phosphate buffer (Anonymous 1985) and macerated. Six 2-mm wide wicks prepared from Whatman 3mm chromatography paper were soaked in the slurry, separated into replicate microtiter plates, and stored at -70°C.

For the Avery Natural Stand, scales were removed from terminal buds, and a 2 mm3 section of meristem was submerged in 150 ul of a 1 M Tris extraction buffer, pH 8.0 (Anonymous 1995) in microtiter plate wells, and stored at -70°C. On the morning of electrophoresis, samples were thawed and ground, and the slurry was absorbed onto three 3-mm wide wicks prepared from Whatman 3mm chromatography paper.

Electrophoresis: Methods of electrophoresis are outlined in Anonymous (1995), and follow the general methodology of Conkle et al. (1982) except that most enzyme stains are modified. A total of 26 isozyme loci were resolved using megagametophytes, but only 21 of these were resolved using bud tissue. A lithium borate electrode buffer (pH 8.3) used with a Tris citrate gel buffer (pH 8.3) (Conkle et al. 1982) to resolve alcohol dehydrogenase (ADH), aconitase (ACO), florescent esterase (FEST), leucine aminopeptidase (LAP), malic enzyme (ME), phosphoglucose isomerase (PGI), and phosphoglucomutase (PGM). A sodium borate electrode buffer (pH 8.0) used with a Tris citrate gel buffer (pH 8.8) (Conkle et al. 1982) to resolve catalase (CAT), glutamate-oxaloacetate transaminase (GOT), glucose-6-phosphate dehydrogenase (G6PDH), and phosphogluconate dehydrogenase (6PGD). A morpholine citrate electrode and gel buffer (pH 6.1) (Conkle et al. 1982) was used to resolve diaphorase (DIA-1), florescent esterase (FEST), and malate dehydrogenase (MDH). A morpholine citrate electrode and gel buffer (pH 8.0) (Anon. 1995) was used to resolve fructose-1,6-diphosphate dehydrogenase (FDP), and isocitrate dehydrogenase (IDH). Two loci each were scored for the enzymes CAT, IDH, LAP, 6PGD, PGI, and PGM, and three loci each were scored for GOT and MDH. Sequential electrophoresis (Crawford 1990) using two buffer systems was used to score FEST-2 alleles. All scored isozymes migrated anodally, except that GOT-3 migrated cathodally. All enzymes were resolved on 11% starch gels. Enzyme stain recipes for enzymes follow Anonymous (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.

Most studied isozymes are known to show Mendelian inheritance in ponderosa pine (Linhart et al. 1989,O'Malley et al. 1979). Genetic interpretations were inferred directly from the isozyme phenotype of each megagametophyte, based on knowledge of the generally conserved enzyme substructure, compartmentalization, and isozyme number in higher plants (Gottlieb 1981, 1982; Weeden and Wendel 1989).

Data Analysis Five isozymes resolved in megagametophyte samples but not in buds. Therefore, ADH, CAT1, CAT2, IDH2, and ME were removed from the data set before analysis. Results for the 30 diploid bud tissue samples from the Avery Natural Stand were arbitrarily partitioned into 60 haploid genotypes, to allow comparison with the other samples. Results were analyzed using Popgene version 1.21 (Yeh et al. 1997). A locus was considered polymorphic if an alternate allele occurred at least once. Statistics calculated included unbiased genetic distances (Nei 1978), Nei's (1973) gene diversity statistic (h), effective number of alleles (Kimura and Crow 1964), inferred gene flow ($Nm = ½(1-Gst)/Gst$; McDermott and McDonald 1993), and Gst (Nei 1973).

The sampling method used for this study presents a problem for analysis. The samples within each population were not independent. Population samples were bulked seed lots from at least ten trees (IPNF) or samples from a known small number of trees (Colville, Black Hills). Four to seven megagametophytes were sampled per tree for Colville and Black Hills populations. The number of

megagametophytes sampled per tree for the other samples is unknown but may be four. Each of the four megagametophytes represents a sample of half the genotype of the mother tree. The four megagametophytes from one tree are not independent samples. This fact was ignored in the analysis. Its importance in this study depends on the use made of the data. The same sampling method was used for all the populations except the Avery Natural Stand. Therefore, using these data to compare populations within this study seems reasonable, with two caveats. First, accidentally sampling many megagametophytes from one mother tree in a small bulked sample may cause a population to appear less variable than it is. Second, the bud sample from the Avery Natural Stand represents thirty independent samples, sixty independent haploid genotypes.

RESULTS

The Avery Natural Stand was the only population sampled as dormant buds, rather than as megagametophytes. The five isozymes ADH, CAT-1, CAT-2, IDH-2, and ME resolved using megagametophyte tissue but failed to resolve in the bud samples (Table 2). These five loci were removed from the analysis. Natural populations and possible source populations were genetically variable, and single family tree improvement lots were less variable as is expected from any single family collection (Table 3). The natural stands and Colville stands have more than 90% of their variation within, rather than among populations (Gst < 0.1) (Table 4). The single family tree improvement lots were more differentiated, with about 60% of the variation within family. When the five isozymes that did not resolve in the Avery Natural Stand were included in the analysis, statistics summarizing genetic variability were slightly higher, but similar (data not shown).

Genetic similarities among the natural IPNF stand plus those from the Colville, and Wentachee populations were high, averaging greater than 0.96. These stands formed a large cluster in a dendrogram based on genetic distances (Fig. 2). Populations within this large cluster were little differentiated. At the base of this cluster were the Avery Natural Stand and the Avery SPA. Near the base of the tree was a series of highly differentiated populations, which were the single family tree improvement lots and the Black Hills sample. Divergent characteristics of the Black Hills sample included four alleles (PGM-1 D, and GOT-3 C, F, I, and J) that were observed in no other sample, and two (ACO D and GOT-3 H) that were each observed in only one other population. The tree improvement lots contained three alleles (FDP D, GOT-1 H, and LAP-1 C) observed in no other sample (Table 2). The Avery Natural Stand was moderately variable. Although its variation was slightly lower than average, the statistics were within one standard deviation of average for natural stands (Table 2). It was the basal population in a cluster of putatively native stands, with the Avery SPA (Fig. 2). Only one rare allele (6PGD-2 C) was observed in both the Avery Natural Stand and the Avery SPA.

DISCUSSION

Isozyme analysis was not able to provide a definitive source identification for the Avery SPA.

The Avery SPA may have originated from natural regeneration, perhaps from the Avery Natural Stand. The SPA and Avery Natural Stand are adjacent in the cluster diagram and

the two stands do share one rare allele (6PGD-2 C). Both stands contain moderate levels of genetic diversity. As measured by all estimators, the Natural Stand contains slightly greater levels of diversity than does the SPA (as would be expected if the Natural Stand was the parental source of the SPA). The fact that the Avery SPA is still performing well, after many stands originating from off-site seed have declined, provides additional evidence that the Avery SPA may be of native origin.

Alternatively, samples from the Bitterroot, Colville, and Wenatchee National Forests, possible sources of stands planted after the fires of 1910, were not differentiated from putative natural stands in the Idaho Panhandle National Forest (Fig. 2). Therefore, isozymes can not rule out the hypothesis that the Avery SPA originated from one of these sources. The close association between the two Avery stands in the dendrogram suggests a close relationship (Fig. 2), but the evidence is weak. Population that are basal in a cluster differ from those more centrally located, but may share only the negative characteristic of being different rather than any positive similarity. At 0.9643, the genetic similarity between the Avery SPA and the Avery Natural Stand was not especially high (Table 5). Also, the SPA contains six alleles not observed in the Natural Stand (although all six alleles occur at low frequency in the SPA), and the GOT-1 D allele occurs at a frequency of 41.7% in the Natural Stand, but is absent in the SPA. Given the high frequency of the GOT-1 D allele in the Natural Stand, you would expect to observe this allele in the SPA, if in fact, the Natural Stand served as the parental material. However, sampling and to some degree selection, could account for the absence of these alleles in their respective stands.

The cluster diagram does indicate that the SPA did not originate from the Black Hills source (Fig. 2).

These results were similar to those of the study of simple sequence repeats (SSR's) in the chloroplast DNA of the Avery SPA and possible native and non-local source populations (Echt 1999). That study suggested a native origin for the Avery SPA, but support for that hypothesis was statistically weak because the sampled populations were little differentiated. The Avery SPA contains moderate levels of genetic diversity, typical of other tested sources (both native and 'non-local'). Like the Avery Natural Stand, the genetic variation in the SPA was slightly lower than average, although the statistics were within one standard deviation of average for natural stands (Table 3).

ACKNOWLEDGEMENTS

We thank Suellen Carroll, Patricia Guge, and Randy Meyer of the National Forest Genetic Electrophoresis Laboratory for electrophoresis and gel scoring. We thank the following for providing seeds: Colleen Archibald, Carol Aubry, Tom DeSpain, Jay Dunbar, Blaine Cook, Joseph M. Linn, Aram Eramian, Kevin Naffin, and Mike Poor.

REFERENCES

Anonymous. 1995. National Forest Genetic Electrophoresis Laboratory

Standard Operating Procedures. NFGEL, USDA Forest Service, Camino, California

Conkle, M. T., P. D. Hodgskiss, L. B. Nunnally, and S. C. Hunter. 1982.

Starch Gel Electrophoresis of Conifer Seeds: a Laboratory Manual.
Gen. Tech. Rep. PSW-64. Berkeley, CA: Pacific Southwest
Forest and Range Experiment Station, Forest Service, U.S.
Department of Agriculture. 18pp.

Crawford, D. J. 1990. Plant Molecular Systematics: Macromolecular
Approaches. John Wiley & Sons, New York, New York.

Echt, Craig S. 1999. P70 Summary Report. Unpublished report to the USDA Forest Service,
National Forest Genetic Electrophoresis Laboratory.

Gottlieb, L. D. 1977. Electrophoretic evidence and plant systematics. Ann. Missouri
Bot. Garden 64: 161-180.

Gottlieb, L. D. 1981. Gene number in species of Asteraceae that have different chromosome
numbers. Proc. Nat. Acad. Sci. USA 78: 3726-3729.

Gottlieb, L. D. 1982. Conservation and duplication of isozymes in plants. Science 216: 373-380.

Kimura, M., and J. F. Crow. 1964. The number of alleles that can be
maintained in a finite population. Genetics 49:725-738.

Linhart, Y. B., J. B. Mitton, K. B. Sturgeon, and M. L. Davis. 1981. Genetic variation in space
and time in a population of Ponderosa Pine. Heredity 46: 407-426.

McDermott and McDonald 1993. Ann. Rev. Phytopathol. 31:353-373.

Nei, Masatoshi. 1973. Analysis of gene diversity in subdivided Populations.
Proc. Nat. Acad. Sci. USA 70(12): 3321-3323

Nei, M. 1978. Estimation of average heterozygosity and genetic distance
from a small number of individuals. Genetics 89: 583-590.

O'Malley, D. M., F. W. Allendorf, and G. M. Blake. 1979. Inheritance of isozyme variation
and heterozygosity in Pinus ponderosa. Biochemical Genetics 17: 233-250.

Weeden, N. F. and Wendel., J. F. 1990. Genetic of plant isozymes. pp. 46-72 in Isozymes in
plant biology, eds. D. E. Soltis and P. S. Soltis. Dioscorides Press, Portland, Oregon

Wendel, J. F., and N. F. Weeden. 1989. Visualization and interpretation
of plant isozymes. Pages 5-45 In Soltis, D. E., and P. S. Soltis
(editors) Isozymes in Plant Biology. Dioscorides Press, Portland,
Oregon.

Yeh, F. C., R.-C. Yang, and T. Boyle. 1997. Popgene Version 1.20.
University of Alberta, Edmonton, Alberta.

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DNA STUDY (NFGEL Project #70)

February 4, 1999
Revised July 19, 1999

OBJECTIVES

Determine whether the ponderosa pine seed production area (SPA) is a plantation of non-local seed source or a naturally regenerated stand, and determine the presumed off-site seed source origins of four plantations on the Bonners Ferry Ranger District.

LAB WORK

Bud tissue was collected from 30 to 37 individual trees at each of the following sites: the SPA, a nearby native stand (MLO - most likely origin), the Bitterroot off-site seed source, the Colville seed source, and four plantations near Bonners Ferry (Deer Park, Brush Lake 2, 3 & 4) (Map). Geographically the Colville sources are generally not off-site seed sources per Rehfeldt's genecological research and the PP seed transfer expert system. However, the Colville NF source could be considered off-site if it is from the wrong elevation. If the trees are slower growing or shorter in stature than local PP, but otherwise show no insect/disease problems or broken tops, we might be able to assume that it came from higher elevation than would normally be recommended for the "lower elevation" IPNF planting site. If the Colville source has a mix of typical heights for its age class, but with some trees exhibiting broken tops, sparse crowns, insect and disease problems, and/or twisted stems, then we might be able to assume that it came from too low an elevation off of the Colville for the "higher elevation" IPNF planting site. And even more subtle, if we are dealing with a off-site seed source issue related to elevational transfer, than another likely culprit could be an IPNF source itself, not just the Colville or Bitterroot. The samples were processed at NFGEL to extract DNA, and sent to the lab in Rhinelander, WI for DNA analysis. A subset of the samples was screened at 15 chloroplast (cp) microsatellite (SSR) loci, and 2 loci were found to be polymorphic. These two loci were then genotyped on the full population set of 256 trees.

RESULTS

One cpSSR locus had 2 alleles, the other 5 alleles. These 7 alleles produced 6 different chloroplast haplotypes among the 8 populations. Statistical tests (exact G-tests) were done to evaluate differences in haplotypic distributions between all possible pairs of populations, and probability (P) values were obtained to evaluate whether the observed differences were statistically significant (Table 1). Values <

0.05 indicate that two populations were different, while values > 0.05 suggest they were not different. The P values can also be used as a rough indication of how likely it is that two populations are genetically identical, but it must be kept in mind that the P values only hold for this single set of data, and cannot be extrapolated to other populations, or even be taken as literal interpretations for the study populations. More extensive sampling, or additional DNA marker data, may change the P values. The levels of cpSSR variation observed in ponderosa pine were lower than for any other pine populations examined by this or other laboratories.

INTERPRETATI0N

The data are consistent with the hypothesis that the SPA originated from the adjacent native stand, although this interpretation has only moderate statistical support (P = 59%), and is confounded by the higher P values seen between SPA and BIT, DPK, BL2 and BL3. Additional DNA or isozyme marker genotyping of these and other native and off-site stands are warranted if a more definitive conclusion is needed concerning the origin of the SPA. It appears highly likely, however, that the Bitterroot source was the origin of the Brush Lake 3 plantation, and that the Brush Lake 2 and 4 plantations came from different seed source(s). The very low level of observed ponderosa pine cpSSR diversity may be explained by 1) a very small founder population having given rise to present day ponderosa pine populations in this region of the country, 2) planting practices based on seed stocks having very few pollen parents, or large, bulked collections coming from the same mother trees with very few pollen parents, 3) the sampling scheme used in this study (using presumed Bitterroot and Colville seedlots planted on IPNFs), or 4) a combination of all. Marker genotyping of additional known native populations are needed to determine whether the levels of diversity seen in the MLO stand and SPA are typical.

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

Abstract

[from the paper presented at the conference: Forest Genetics for the Next Millenium, October 8-13, 2000, Durban, South Africa, by Ron Schmidtling (USDA Forest Service, SRS, Southern Institute of Forest Genetics, 23332 Hwy 67, Saucier, MS 39574)].

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*).

Project Objectives

Genotypic data will be used to investigate the natural genetic diversity in the following Southern pines: slash pine (*Pinus elliotii*), *Pinus caribaea*, longleaf pine (*Pinus palustris*), loblolly pine (*Pinus taeda*), slash x *P. caribaea* hybrids, and slash x longleaf pine hybrids. [NFGEL Projects 80, 90, and 93].

Materials and Methods

A total of 790 individual tree collections were submitted to NFGEL for isozyme analysis from 12/1/99 - 2/28/00.

# Individuals	Species	Source	Project#
536	slash pine	17 rangewide populations	80
33	slash pine	Punta Gorda	93
30	slash pine	Bradenton	93
30	slash pine	St Petersburg	93
33	slash pine x *Pinus caribaea*	--	90
32	*P. caribaea* var. bahamensis	west end Grand Bahama	93
32	*P. caribaea* var. bahamensis	east end Grand Bahama	93
30	longleaf pine	Florida	90
32	loblolly pine	southern Mississippi	90
2	slash x longleaf pine	--	93

* An additional 18 slash x longleaf pine hybrids were received on 1/20/00 and prepped within Project #92 (*P. massoniana* and loblolly pine).

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 (2-3 mm2) 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 Conkle et al. (1982). A total of 790 trees were genotyped at 24 isozyme loci using three buffer systems. Buffer system 'LB' was used to resolve enzyme systems aconitase (ACO), leucine aminopeptidase (LAP), phosphoglucomutase (PGM), shikimic acid dehydrogenase (SKD), and diaphorase (DIA). Buffer system 'SB' (where the electrode buffer was pH 8.0), was used to resolve enzyme systems triosephosphate isomerase (TPI), glucose-6 phosphate dehydrogenase (G6PD), catalase (CAT), and glutamic oxaloacetate transaminase (GOT). Buffer system 'MC8' (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 Conkle et al. (1982). After the dye marker migrated 8 cm, gels were cut horizontally into four to seven slices, stained and scored. Data was e-mailed to client on 3/20/00.

Literature Cited

Cheliak, WM and Pitel, JA. 1984. Genetic control of allozyme variants in mature tissues of white spruce trees. J.Hered. 75:34-40.

Conkle, M. T., P. D. Hodgskiss, L. B. Nunnally, and S. C. Hunter. 1982.

Starch Gel Electrophoresis of Conifer Seeds: a Laboratory Manual. Gen. Tech. Rep. PSW-64. Berkeley, CA: Pacific Southwest Forest and Range Experiment Station, Forest Service, U.S. Department of Agriculture. 18pp.

Allozyme Variation in Interior Douglas-fir: Association With Growth and Resistance to Western Spruce Budworm Herbivory

(NFGEL Project #91)

OBJECTIVES

This work was done in cooperation with Zhong Chen, School of Forestry, Northern Arizona University, Flagstaff, AZ, as part of his doctoral degree program. NFGEL's contribution was to assist in genotyping Douglas-fir (Pseudotsuga menziesii) trees located in Arizona and Colorado that were identified as either susceptible or resistant to western spruce budworm, and edit the resulting manuscript.

MATERIALS AND METHODS

Seed was soaked in 1% hydrogen peroxide for 48 hours, rinsed and plated out in petri dishes lined with 1% hydrogen peroxide soaked Kimpack germination paper. Fifteen seed per tree were germinated, and 10 seeds were prepped per individual tree. When seeds opened, embryo tissue was removed, and megagametophytes were ground in 150ul of 0.2M phosphate extraction buffer. Extracts were absorbed onto six 2.0mm wicks. Six replicate trays of wicks per set were stored at -70. Ten percent of the samples were run and scored twice for quality control. Samples were genotyped at 25 loci using three buffer systems..

FINAL PRODUCT

This work was submitted for publication to Canadian Journal of Forest Research by Zhong Chen. (Allozyme variation in interior Douglas-fir: association with growth and resistance to western spruce budworm herbivory, by Zhong Chen, Thomas E. Kolb, Karen M. Clancy, Valerie D. Hipkins, and Laura E. DeWald). The abstract from the submitted manuscript follows.

Abstract: We used starch gel electrophoresis to investigate levels of genetic variation between trees of interior Douglas-fir (Pseudotsuga menziesii (Mirb.) Franco var. glauca) that were phenotypically resistant versus susceptible to defoliation by the western spruce budworm (Choristoneura occidentalis Freeman). We also investigated the association between allozyme variation and tree growth traits. Overall, the phenotypically resistant trees had a lower allelic heterozygosity ($p = 0.020$) compared to susceptible trees. Among 25 loci we examined, the resistant trees also had a higher frequency of the most common alleles ($p = 0.057$) and a higher proportion of homozygous genotypes, especially at loci FEST-1 ($p = 0.004$), ACO-1 ($p = 0.080$), and 6PGD-1 ($p = 0.084$). The higher allelic heterozygosity in susceptible trees was mainly due to their higher proportion of uncommon and/or rare alleles. Compared with susceptible trees, resistant trees had higher average radial growth rates ($p = 0.047$) and less temporal variability in growth rate over 25 years ($p = 0.037$). Mean radial growth rate and average tree heterozygosity were not related at any site ($p = 0.316$). Relationships between temporal variability in growth rate and tree heterozygosity were inconsistent among sites. Our results suggest that phenotypic differences in resistance of interior Douglas-fir to western spruce budworm defoliation are caused by genetic differences among trees.

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

OBJECTIVE

The project objective was to perform clone identification on eight individuals of Douglas-fir. This work was done for Bryan Schulz of Olympic Resource Management, Poulsbo, WA. (NFGEL Project #96)

MATERIAL AND METHODS

Eight individuals of Douglas-fir were submitted for isozyme analysis (starch gel electrophoresis): two putative ramets of clone 1; the suspected parental source of clone 1; two putative ramets of clone 2; two putative ramets of clone 3; the suspected parental source of clone 3.

One to three dormant vegetative buds per tree were dissected. Tissue was submerged into microtiter plate wells containing 150 ul of cold Melody/Neale buffer. Plates were frozen at -70C. On the morning of the electrophoretic run, samples were thawed, macerated with a dremel tool, and absorbed onto three, 3MM paper wicks. Samples were genotyped at 18 loci using three buffer systems. 70% of the samples were run and scored twice. .

RESULTS

Individual trees were genotyped at 18 isozyme loci. Sample identifications have been changed for this report.

Sample	PGM1	PGM2	ACO1	ACO2	FEST	UGPP1	UGPP2	GDH	GOT1	GOT2	GOT3	GLYH	MDH1	MDH3	G6PD	6PGD
1-1	11	12	12	11	22	11	12	11	11	11	14	22	11	11	12	11
2-1	11	12	11	11	22	11	11	11	11	11	11	22	11	11	22	11
1-2	11	12	11	11	22	11	11	11	11	11	12	00	11	11	12	11
2-2	11	33	11	11	22	23	13	11	11	11	11	12	11	12	11	11
1-3	11	22	11	22	22	22	11	11	11	11	11	11	11	11	22	11
2-3	11	22	11	22	22	22	11	11	11	11	11	11	11	11	22	11
x-x-3(?)	11	23	00	00	22	11	11	11	11	11	11	00	11	11	22	11
parent 1	11	12	12	11	22	11	12	11	11	11	14	22	11	11	12	11

*'0' is missing data

CONCLUSIONS

Samples 1-1 and 2-1 are not ramets of the same clone. Their genotypes do not match at five loci (ACO1, UGPP2, GOT3, G6PD1, and SKD).

The genotype of sample 'Parent 1' matches that of '1-1'. It therefore appears that 1-1 is correctly identified as clone #1, and that 2-1 is not a ramet of clone #1 and is misidentified.

Individuals 1-2 and 2-2 are not ramets of the same clone. Their genotypes do not match at eight loci (PGM2, UGPP1 and 2, GOT3, MDH3, G6PD, IDH, and SKD).

Individuals 1-3 and 2-3 do appear to be ramets of the same clone. Their genotypes match at all 18 loci. The wild tree collection (x-x-3?) is not the parent of clone 3. The wild tree genotype did not match the clone 3 genotype at four loci (PGM2, UGPP1, IDH, and SKD).

Evaluation of Genetic Diversity in Tahoe yellow cress (*Rorippa subumbellata*)

INTRODUCTION

Rorippa subumbellata (Roll.), Tahoe yellow cress, is endemic to sandy beaches on the shores of Lake Tahoe in California (El Dorado and Placer counties) and Nevada (Carson City Rural Area, Washoe and Douglas counties). The plants are found where the beach is wide enough to offer a back beach area, out of wave action and behind the highest debris deposit line (Ferreira, 1987). The distribution around the lake edge is patchy, with most occurrences found on the west and south shores in California, where the greatest expanse of beaches occur (CSLC, 1998).
R. subumbellata is a rare plant restricted by both geography and habitat requirements, which periodically undergoes significant fluctuations in number of individuals and sites as a result of naturally and artificially induced phenomena. This species is a Federal candidate for listing under the Endangered Species Act of 1973.

According to a CSLC (California State Lands Commission) Biological Assessment (1998), the number of *R. subumbellata* occurrences in any particular year is strongly related to lake level fluctuations. During periods of low water, additional habitat for *R. subumbellata* is exposed and becomes available for colonization, such as occurred during the 1992-1993 season. When the lake elevation is high, much of the habitat for *R. subumbellata* is inundated, and therefore unavailable for plant colonization. While high lake levels may cause mortality in some
R. subumbellata sites and pose an immediate threat to existing individuals, it may benefit the species in the long term by removing other plant species and opening new habitat when lake levels drop. Unfortunately under current conditions, dam operations alter the historical seasonal fluctuation of the

lake, maintaining higher water elevations during the spring and summer, the growing season for *R. subumbellata*. Substrate disturbance, construction, other development, and recreation are the primary human caused disturbances which have been documented impacting
R. subumbellata and its habitat.

R. subumbellata is a perennial plant which is capable of re-sprouting each season from dormant rootstalks, though it is unknown if rootstalks can survive being inundated for long periods of time. Little detailed information concerning the reproductive biology of *R. subumbellata* is available. Pollinators have not been identified or recorded. The dominant mechanism of site colonization, whether by seed, re-sprouting, or the deposition of vegetative plant material by water processes, has not been determined (CSLC 1998). The longevity and germinative capabilities of the seed of *R. subumbellata* are unknown. Seeds are small (< 1mm) and probably drop down, establishing in close proximity to the plant that shed them. Seed could be dispersed by the wind and wave action of the lake. The CSLC Biological Assessment explains that beaches at the mouths of streams are completely reformed during periods of high spring runoff, such as occurred in 1982, 1983, 1986, and 1997. During such beach forming events, aerial stems and rootstocks of *R. subumbellata* are removed. This material may be deposited around the lake, providing a mechanism for *R. subumbellata* to distribute propagules to other lakeshore locations. At this time there are not data to either support or refute this idea.

The purpose of this investigation is to determine the genetic characteristics of *R. subumbellata*. This study provides information on genetic variability among *R. subumbellata* sites, which will be used to assess long-term population viability under existing Lake Tahoe management scenarios. In accordance with the Scope of Work for Interagency Agreement FWS 14-48-0001-95813, the National Forest Genetic Electrophoresis Laboratory (NFGEL) evaluated the genetic diversity in *R. subumbellata*. The starch gel electrophoresis method was used to assess isozyme markers within and among *R. subumbellata* populations.

METHODS

Sample collection: In 1999, water levels receded to sufficient levels to allow for the collection of 140 individuals of *R. subumbellata* from 11 populations (Table 1, Map). All individuals of the small populations (<30 individuals) were sampled. The largest population, composed of approximately 80 individuals located at Upper Truckee-East, was sampled by collecting individuals from a distance of at least three to five feet between plants and sampling throughout its patchy distribution. Collections occurred on August 15, 1999 and September 1, 1999. Between one to two stems per plant were removed per individual and placed in zip-lock bags. Bags were kept cool in ice chests and transported to NFGEL in Camino, CA.

Sample preparation: Samples were prepared using standard NFGEL procedures (Anonymous 1995). An approximately 1cm long section of stem per individual (40 mm2), was clipped from a stem tip and submerged in 100 microliters of a Tris buffer pH. 7.5 (Gottlieb 1981). Tissue was frozen at -70C until electrophoresis. Two backup preparations of each individual were prepared and frozen. All sample tissue was green, healthy, and disease-free. There was ample tissue available for preparing samples for analysis. Tissue was prepared on 8/18/99 and 9/3/99.

Electrophoresis: Methods of electrophoresis followed the general methodology of Conkle et al. (1982) except that most enzyme stains are somewhat modified (Anonymous 1995). A lithium borate

electrode buffer (pH 8.3) was used with a tris citrate gel buffer (pH 8.3) (Conkle et al. 1982) to resolve the enzymes fluorescent esterase (FEST), leucine aminopeptidase (LAP), phosphoglucose isomerase (PGI), aconitase (ACO), phosphoglucomutase (PGM), and malic enzyme (ME). 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 uridine diphosphoglucose pyrophosphorylase (UGPP), glutamate-oxaloacetate transaminase (GOT), triosephosphate isomerase (TPI), glycerate-2-dehydrogenase (GLYDH), and catalase (CAT). A morpholine citrate electrode and gel buffer
(pH 6.1) (Conkle et al. 1982) was used to resolve malate dehydrogenase (MDH), diaphorase (DIA), isocitrate dehydrogenase (IDH), phosphogluconate dehydrogenasee (6PGD), and shikimic acid dehydrogenase (SKD). All enzymes were resolved on 11% starch gels. Enzyme stain recipes follow Anonymous (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, 7% of the individuals were run and scored twice.

Data Analysis: The genus *Rorippa* has a base number of n=8 as determined from chromosome counts of five species. Four of the species are diploid, with two of these species containing tetraploid populations. The fifth species is reported to be hexaploid (Darlington and Wylie 1955). There is no record of a chromosome count for *R. subumbullata*. There are also no known isozyme studies concerning *R. subumbullata*, therefore, genetic interpretations were inferred directly from isozyme phenotypes under the assumption that *R. subumbullata* is diploid.

Resulting genetic data was analyzed using Popgene version 1.31 (Yeh et al. 1999) and Biosys-1 version 1.7 (Swofford and Selander 1989). A locus was considered polymorphic if an alternate allele occurred once. Hierarchical structure was given to single populations and multiple populations. Statistics calculated included allele frequency, Shannon-Weaver Diversity Index (Shannon and Weaver 1949), unbiased genetic distances (Nei 1978), observed heterozygosity, expected heterozygosity (Nei 1978), F-statistics (Wier 1990), and effective allele number (Kimura and Crow 1964). A dendrogram was generated using Biosys-1 version 1.7 (Swofford and Selander 1989) using UPGMA and Nei's unbiased genetic similarity.

RESULTS

Eight of the eleven *Rorippa subumbullata* populations sampled were monomorphic for 23 enzyme loci (Table 2). Two of the populations have UGPP-1 locus variation, Taylor Creek and Upper Truckee-East, with Taylor Creek also exhibiting variation at the PGI-1 locus (Table 2). Tahoe Meadows showed variation at the DIA-1 locus only (Table 2). Among all the populations sampled, *R. subumbullata* has low enzyme variability (Table 3). The percentage of polymorphic loci at the species level was 13%, while at the population level was 1.6%. Within population diversity as measured by expected heterozygosity equaled 0.000 for all populations except Taylor Creek (0.0229, S.E=0.0790), Upper Truckee-East (.0050, S.E.=0.0241) and Tahoe Meadows (.0250, S.E.=0.1118) (Table 3). Tahoe Meadows was found to be the most diverse population via its unique variation in DIA-1. Fst (the proportion of the total variation measured found among populations within the taxon) equaled 0.2175. The dendrogram generated by Biosys shows that the most diverse population is Tahoe Meadows, followed by Taylor Creek and Upper Truckee-East (Figure 1).

DISCUSSION

Genetic Variation**:** Isozyme variation in *Rorippa subumbellata* was extremely limited. Eight of the eleven populations studied were completely monomorphic at the 23 loci assayed (Table 2). Therefore, no heterozygosity was observed, and all within population diversity values were equal to zero (Table 3). These data suggest that these eight populations consist of the same clone. The remaining three populations, Tahoe Meadows, Taylor Creek, and Upper Truckee-East, were slightly variable, and were also monomorphic for many of the same loci as the other eight populations (Table 2). The Upper Truckee-East population contained a small amount of variation at the UGPP-1 locus (two out of the 33 individuals were monomorphic for a second allele). Three of the eight individuals in the Tahoe Meadows population contained variation (they were monomorphic for a second allele) at the DIA-1 locus. The Taylor Creek population showed variation at two loci. Two out of the ten inviduals in the population were heterozygous at the PGI-1 locus, and also were monomorphic for an alternate allele at the UGPP-1 locus. The only heterozygosity observed in the study was in the Taylor Creek population at the PGI-1 locus. All other populations contained no heterozygosity.

R. subumbellata has much less isozyme variation than that observed in the average endemic taxon (Hamrick and Gott 1990). The average endemic species has 40% polymorphic loci, 1.80 alleles per locus, and 1.15 effective alleles per locus. Comparatively, R. subumbellata has 13.0% polymorphic loci, 1.13 alleles per locus, and 1.00 effective alleles per locus. However, other species with similar characteristics as *R. subumbellata* (restricted range and/or moist habitat), have also been found to be nearly to completely monomorphic. These species include *Sisyrinchium sarmentosum* (Wilson et al. 2000), *Iris lacustris* (Simonich and Morgan 1994), *Howellia aquatilis* (Lesica et al. 1988), *Limnanthes macounii* (Kesseli and Jain 1984), and *Lespedeza leptostachya* (Cole and Biesboer 1992).

Of the total variation measured by isozymes, 21.75% (Fst = 0.2175) is found among populations, indicating that, as a whole, populations are moderately differentiated. This slightly high among-population variation value is the result of three of the populations containing unique variation (Table 2). The Tahoe Meadows population contains an alternate DIA-1 allele at moderate frequency that exists in no other population sampled. The Taylor Creek population contains a unique low frequency alternate allele at the PGI-1 locus. Taylor Creek and Upper Truckee-East both contain an allele at the UGPP-1 locus that exists in no other population. This shared allele occurs at a frequency of 20% in Taylor Creek and 6% in Upper Truckee-East. Among the other eight populations that contain exactly the same isozymes, the among-population variation is, of course, 0% (they are monomorphic for all the same isozymes measured). Even though there are some genetic differences among a few of the populations, gene flow appears to be quite low. The presence of alleles unique to populations (allele 2 in DIA-1; allele 2 in PGI-1; allele 2 in UGPP-1) indicates that gene flow is limited, otherwise more populations would share these alleles. All the populations share above 99% genetic similarity (Table 4, Figure 1). The Tahoe Meadows population is the most dissimilar overall, followed by the Taylor Creek population. The Upper Truckee-East population, even though it does contain variation (a rare allele shared with Taylor Creek), is more similar to the monomorphic group of populations than to either Taylor Creek or Tahoe Meadows. Taylor Creek and Tahoe Meadows are the most genetically dissimilar pair of populations sampled (Table 4).

Gene Conservation: One goal of conservation biology is to preserve overall levels of genetic diversity. Isozyme electrophoresis is often used to estimate diversity in natural populations. Common measures of diversity include the percent of all loci that are polymorphic (P), the average number of alleles per locus (A), the effective number of alleles per locus (Ae), the observed frequency of heterozygotes (Ho), and the frequency of heterozygotes expected under Hardy-Weinberg equilibrium conditions (He). It is common to find low genetic diversity in populations of many rare or threatened species (Hamrick et al. 1991). However, individual endemic species vary widely in their genetic diversity (Gitzendanner and Soltis 2000). Although it is sometimes thought that low genetic diversity puts a species future at risk, a strong causal relationship between diversity and population viability has not been shown. Diversity is often reduced in rare species, likely because of bottlenecks associated with constrictions on population size (Falk and Holsinger 1991). It is important to point out that species that show low amounts of isozyme variation due to bottlenecks, may still contain ample variation at other gene loci, especially those loci involved in quantitative, adaptive traits. Also, if populations expand rapidly after experiencing a bottleneck, they can generate sufficient amounts of genetic variation to ensure future adaptive potential (Barrett and Kohn 1991).

There are several possibilities why the *R. subumbellata* populations are so invariant. First, low population sizes for several generations would reduce variation, regardless of whether the populations were initially variable (a genetic bottleneck). Second, the species could be reproducing largely vegetatively. Although vegetatively reproducing taxa are often variable (Ellstrand and Roose 1987), low variation could characterize an entirely vegetatively reproducing species. Third, low variation can result from many generations of selfing, or mating within the same clone (geitonogamous pollination). Selfing would produce a preponderance of homozygous individuals, which is observed in the *Rorippa* populations. Because the reproductive biology of *R. subumbellata* is unknown, it is difficult to distinguish between these possible causes of the observed lack of variation. The present level and structure of diversity is probably a result of a combination of all the above. When water levels were lower, *Rorippa* probably formed much larger and more extensive populations. At that time, the entire south shore may have been one slightly subdivided population. It is even possible that the plants around the entire lake comprised a single population.

CONCLUSIONS

The observed lack of genetic variation (as measured with isozymes) suggests that few restrictions need be placed on programs to increase the *R. subumbellata* populations through transplants. Moving plants (seeds or rhizomes) among populations may still be undesirable because it might lead to loss of rare genetic variations now restricted to single populations. However, as long as gene flow is as limited as it appears, establishment of new populations at sites not now occupied by *R. subumbellata* need be restrained only by the pragmatic test that populations can be established only where the plants can grow. This study suggests that preserving enzyme (and genetic) diversity in *R. subumbellata* requires preserving many populations because some, like Taylor Creek, Tahoe Meadows, and Upper Truckee-East, contain unique alleles. Also, large populations should be maintained at these areas, the only populations known to be variable. However, this study also suggests that management practices directed toward demographics, rather than genetics, can be effective in long-term preservation of *R. subumbellata* biodiversity. Demographic changes can be as good, or better indicators of the biological status of rare species than information about the level and structure of genetic variation (Schemske et al.

1994). This is especially true in a species such as *R. subumbellata* that has little genetic variability.

REFERENCES

Anonymous. 1995. National Forest Genetic Electrophoresis Laboratory
Standard Operating Procedures. NFGEL, USDA Forest Service, Camino, California

Barrett, S. C. H., and J. R. Kohn. 1991. Genetic and evolutionary
consequences of small population size in plants: implications for conservation. Pages 3-30 In Falk, D. A., and K. E. Holsinger(editors)

CSLC (California State Lands Commission). 1998. Tahoe Yellow Cress
Draft Biological Assessment (Administrative Draft), Sacramento CA 95825-8202.

Cole, C. T., and D. D. Biesboer. 1992. Monomorphism, reduced gene
flow, and cleistogamy in rare and common species of Lespedeza (Fabaceae). American Journal of Botany 79:567 575.

Conkle, M. T., P. D. Hodgskiss, L. B. Nunnally, and S. C. Hunter. 1982.
Starch Gel Electrophoresis of Conifer Seeds: a Laboratory Manual. Gen. Tech. Rep. PSW-64. Berkeley, CA: Pacific Southwest Forest and Range Experiment Station, Forest Service, U.S. Department of Agriculture. 18pp.

Crawford, D. J. 1990. Plant Molecular Systematics: Macromolecular
Approaches. John Wiley & Sons, New York, New York.

Darlington, C. D., and A. P. Wylie. 1955. Chromosome Atlas of
Flowering Plants. University Press, Aberdeen, Great Britain. 519 pp.

Ellstrand, N. C., and M. L. Roose. 1987. Patterns of genotypic diversity
in clonal plant species. American Journal of Botany 74:123-131.

Falk, D. A., and K. E. Holsinger (editors). 1991. Genetics and
Conservation of Rare Plants. Oxford University Press, Oxford, England.

Ferreira, J. E. 1987. The population status and phenological
characteristics of Rorippa subumbellata (Roll.) at Lake Tahoe, California and Nevada. M.A. Thesis, California State University, Sacramento, CA.

Gitzendanner, M. A., and P. S. Soltis. 2000. Patterns of genetic variation

in rare and widespread plant congeners. American Journal of Botany 87:783-792.

Gottlieb, L. D. 1981. Gene number in species of Asteraceae that have different chromosome numbers. Proceedings of the National Academy of Science, USA 78:3726-3729.

Hamrick, J. L., and M. J. W. Godt. 1990. Allozyme diversity in plant species. Pages 43-63 In Brown, A. H. D., M. T. Clegg, A. L. Kahler, and B. S. Weir (editors) Plant Population Genetics, Breeding, and Genetic Resources. Sinauer Associates, Inc., Sunderland, Maryland.

Hamrick, J. L., M. J. W. Godt, D. A. Murawski, and M. D. Loveless. 1991. Correlations between species traits and allozyme diversity: implications for conservation biology. Pages 75-86 In Falk, D. A., and K. E. Holsinger (editors) Genetics and Conservation of Rare Plants.Oxford University Press, Oxford, England.

Kesseli, R. V., and S. K. Jain. 1984. New variation and biosystematic patterns detected by allozyme and morphological comparisons in Limnanthes sect. Reflexae (Limnanthaceae). Plant Syst. Evol. 147:133-165.

Kimura, M., and J. F. Crow. 1964. The number of alleles that can be maintained in a finite population. Genetics 49:725-738.

Lesica, P., R. F. Leary, F. W. Allendorf, and D. E. Bilderback. 1988. Lack of genetic diversity within and among populations of an endangered plant, Howellia aquatilis. Conservation Biology 2: 275-282.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89: 583-590.

Schemske, D. W., B. C. Husband, M. H. Ruckelshaus, C. Goodwillie, I. M. Parker, and J. G.Bishop. 1994. Evaluating approaches to the conservation of rare and endangered plants. Ecology 75:584-606.

Shannon, C. E., and W. Weaver. 1949. The Mathematical Theory of Communication. Univ. of Illinois Press, Urbana, Illinois.

Simonich, M. T., and M. D. Morgan. 1994. Allozymic uniformity in Iris lacustris (dwarf lake iris) in Wisconsin. Canadian Journal of Botany 72:1720-1722.

Swofford, D. L., and R. B. Selander. 1989. BIOSYS-1, a computer

program for the analysis of allelic variation in population genetics and biochemical systematics, release 1.7 edition. Illinois Natural History Survey, Champaign, Ill.

Weir, B. S. 1990. Genetic data analysis. Sunderland, Massachusetts, Sinauer.

Wendel, J. F., and N. F. Weeden. 1989. Visualization and interpretation of plant isozymes. Pages 5-45 In Soltis, D. E., and P. S. Soltis (editors) Isozymes in Plant Biology. Dioscorides Press, Portland, Oregon.

Wilson, B. L., D. L. Doede, and V. D. Hipkins. 2000. Isozyme variation in Sisyrinchium sarmentosum (Iridaceae). Northwest Science 74: (in press).

Yeh, F. C., R.-C. Yang, and T. Boyle. 1997. Popgene Version 1.20. University of Alberta, Edmonton, Alberta.

Lake Tahoe map

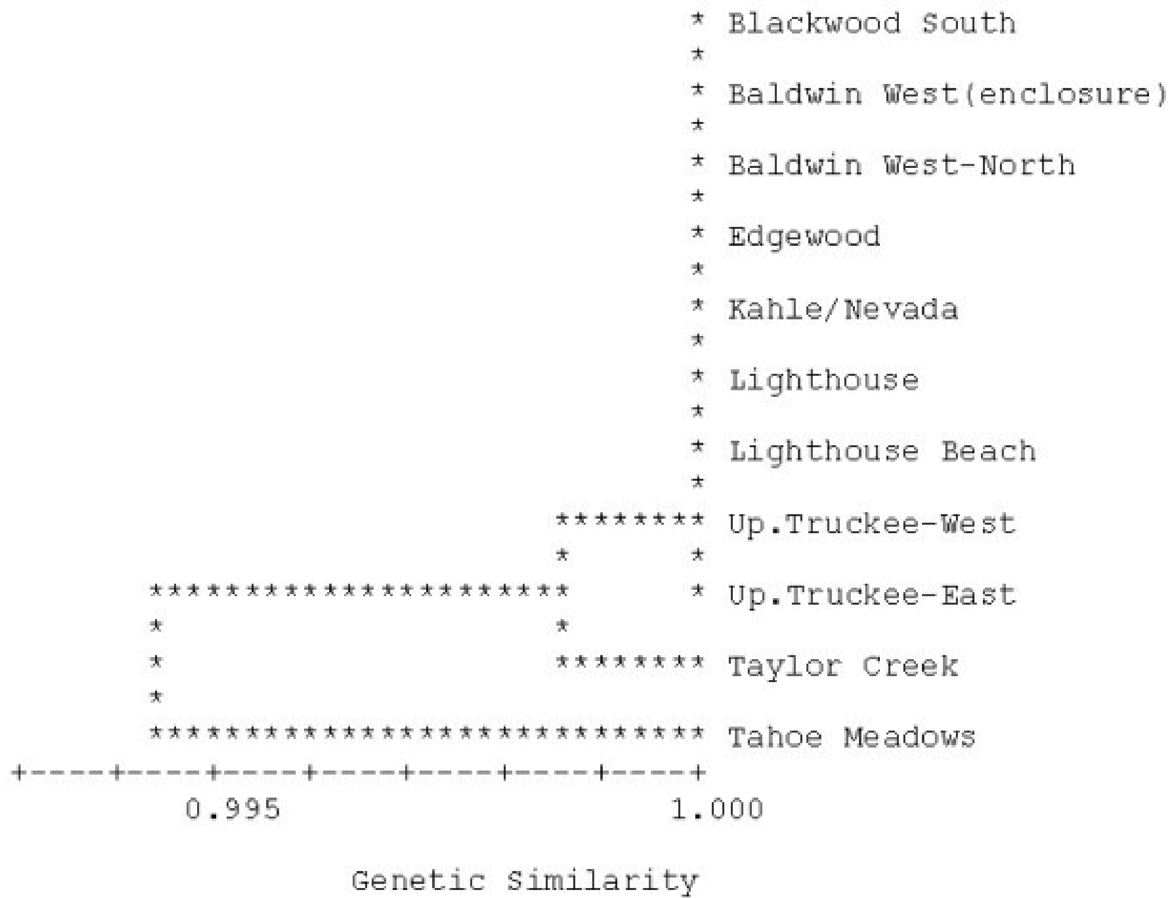

Figure 1. Dendrogram based on genetic similarity. Tahoe Meadows (0.99437) and Taylor Creek (0.99855) populations are shown as the most genetically dissimilar.

Table 1. Population name and number of *R. subumbellata* individuals sampled per occurrence.

Population Name	# Individuals Sampled	Collection Date
Upper Truckee East	33	8/15/99
Upper Truckee West	2	8/15/99
Edgewood	18	8/15/99
Taylor Creek (enclosure)	10	8/15/99
Baldwin West – N of parking lot	4	8/15/99
Baldwin West (enclosure)	13	8/15/99

Tahoe Meadows	8	9/01/99
Lighthouse Beach	7	9/01/99
Lighthouse	11	9/01/99
Kahle/Nevada	7	9/01/99
Blackwood South	27	9/01/99
TOTAL	**140**	

Table 2. Allele frequencies for 23 isozyme loci in *Rorippa subumbellata*. Migration distances are actual distances (mm) from the origin at which the enzyme band produced by this allele was observed, under the electrophoretic conditions used in this study. Alleles were numbered in the order in which they were observed, not in order of migration speed or frequency. Missing data is indicated with an asterisk.

Locus	Allele	Migration	Entire Study	Blackwood South	Baldwin West (enclosure)	Baldwin West-North	Edgewood	Kahle/ Nevada	Lighthouse	Lighthouse Beach	Tahoe Meadows	Taylor Creek	Upper Truckee-East	UpperTruckee-West
PGI-1	1	36	0.9929	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9000	1.0000	1.0000
PGI-1	2	40	0.0071									0.1000		
PGI-2	1	27	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
ME7	1	25	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
LAP-1	1	42	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
PGM-1	1	43	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
PGM-2	1	31	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
FEST-1	1	51	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
FEST-2	1	46	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
FEST-3	1	39	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
FEST-4	1	34	1.0000	1.0000	1.0000	1.0000	1.0000	*	1.0000	*	*	1.0000	1.0000	1.0000
ACO-1	1	41	1.0000	1.0000	1.0000	*	1.0000	*	1.0000	1.0000	*	1.0000	1.0000	*
UGPP-1	1	47	0.9714	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.8000	0.9394	1.0000
UGPP-1	2	40	0.0286									0.2000	0.0606	
GOT-1	1	48/45/42	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
TPI-1	1	55	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
TPI-2	1	43	1.0000	*	1.0000	1.0000	1.0000	*	1.0000	1.0000	*	1.0000	1.0000	1.0000
GLYDH	1	7	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
CAT-1	1	18	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
MDH-1	1	24	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
DIA-1	1	26	0.9786	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.6250	1.0000	1.0000	1.0000
DIA-1	2	23	0.0214								0.3750			
IDH-1	1	21/27	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
6PGD-1	1	28/25	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
6PGD-2	1	13	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
SKD-1	1	32	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000

Table 3. Summary of genetic variability in *Rorippa subumbellata* populations. N = mean number of individuals sampled per locus, per population; P = % of all loci that are polymorphic; A = average number of alleles per locus; Ae = effective number of alleles per locus; H_o = observed frequency of heterozygotes; H_e = frequency of heterozygotes expected under Hardy-Weinberg equilibrium conditions. S-W = Shannon-Weaver diversity index.

	N (S.E.)	P	A (S.D.)	Ae (S.D.)	H_o (S.E.)	H_e (S.E.)	S-W (S.D.)
Species level							
Entire Study	247	13.04%	1.1304 (0.3444)	1.0048 (0.0149)	0.0003 (0.0015)	0.0046 (0.0142)	0.0112 (0.0339)
Population level							
Entire study - Mean	11.2 (2.7)	1.58%	1.0164 (0.0300)	1.0068 (0.0144)	0.0004 (0.0004)	0.0048 (0.0029)	0.0067 (0.0127)
Blackwood South	22.2 (1.8)	0.00%	1.0000 (0.0000)	1.0000 (0.0000)	0.0000 (0.0000)	0.0000 (0.0000)	0.0000 (0.0000)
Baldwin West (enclosure)	13.2 (0.6)	0.00%	1.0000 (0.0000)	1.0000 (0.0000)	0.0000 (0.0000)	0.0000 (0.0000)	0.0000 (0.0000)
Baldwin West-North	2.9 (0.1)	0.00%	1.0000 (0.0000)	1.0000 (0.0000)	0.0000 (0.0000)	0.0000 (0.0000)	0.0000 (0.0000)
Edgewood	16.3 (0.8)	0.00%	1.0000 (0.0000)	1.0000 (0.0000)	0.0000 (0.0000)	0.0000 (0.0000)	0.0000 (0.0000)
Kahle/Nevada	5.9 (0.5)	0.00%	1.0000 (0.0000)	1.0000 (0.0000)	0.0000 (0.0000)	0.0000 (0.0000)	0.0000 (0.0000)
Lighthouse	8.9 (0.7)	0.00%	1.0000 (0.0000)	1.0000 (0.0000)	0.0000 (0.0000)	0.0000 (0.0000)	0.0000 (0.0000)
Lighthouse Beach	6.1 (0.4)	0.00%	1.0000 (0.0000)	1.0000 (0.0000)	0.0000 (0.0000)	0.0000 (0.0000)	0.0000 (0.0000)
Tahoe Meadows	6.5 (0.6)	4.35%	1.0500 (0.2236)	1.0441 (0.1973)	0.0000 (0.0000)	0.0250 (0.1118)	0.0331 (0.1479)
Taylor Creek (enclosure)	9.0 (0.4)	8.70%	1.0870 (0.2881)	1.0250 (0.0996)	0.0043 (0.0209)	0.0229 (0.0790)	0.0304 (0.1105)
Upper Truckee- East	30.9 (1.4)	4.35%	1.0435 (0.2085)	1.0056 (0.0268)	0.0000 (0.0000)	0.0050 (0.0241)	0.0099 (0.0477)
Upper Truckee-West	1.9 (0.1)	0.00%	1.0000 (0.0000)	1.0000 (0.0000)	0.0000 (0.0000)	0.0000 (0.0000)	0.0000 (0.0000)

Table 4. Genetic identities (above the diagonal) and distances (below the diagonal) (Nei's (1978) unbiased estimates) among populations of *Rorippa subumbellata*.

Population	Blackwood South	Baldwin West (enclosure)	Baldwin West-North	Edgewood	Kahle/ Nevada	Lighthouse	Lighthouse Beach	Tahoe Meadows	Taylor Creek	Upper Truckee East	Upper TruckeeWest
Blackwood South	****	1.000	1.000	1.000	1.000	1.000	1.000	0.994	0.998	1.000	1.000
Baldwin West (enclosure)	0.000	****	1.000	1.0000	1.000	1.000	1.000	0.994	0.998	1.000	1.000

Baldwin West-North	0.000	0.000	****	1.000	1.000	1.000	1.000	0.994	0.998	1.000	1.000
Edgewood	0.000	0.000	0.000	****	1.000	1.000	1.000	0.994	0.998	1.000	1.000
Kahle/Nevada	0.000	0.000	0.000	0.000	****	1.000	1.000	0.994	0.998	1.000	1.000
Lighthouse	0.000	0.000	0.000	0.000	0.000	****	1.000	0.994	0.998	1.000	1.000
Lighthouse Beach	0.000	0.000	0.000	0.000	0.000	0.000	****	0.994	0.998	1.000	1.000
Tahoe Meadows	0.006	0.006	0.006	0.006	0.006	0.006	0.006	****	0.992	0.994	0.994
Taylor Creek (enclosure)	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.009	****	0.999	0.998
Upper Truckee- East	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.006	0.001	****	1.000
Upper Truckee-West	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.006	0.002	0.000	****

Genetic variation and population structure of the rare California plant *Collomia rawsoniana* (Polemoniaceae)

ABSTRACT

Collomia rawsoniana is a rare, endemic plant that grows only in Madera County, California. It forms large, rhizomatous patches that might be interpreted as clones. This study tested the hypothesis that patches are single genets by isozyme analysis of 252 samples from 21 patches in six populations. A minimum of four multilocus genotypes were detected within each patch, and in some patches all samples had distinct genotypes. Only one of the 177 complete multilocus genotypes detected occurred in more than one patch. The 1.33 individuals/genotype suggests that much reproduction is sexual. The species was genetically variable for an endemic plant; 62% of the 24 loci examined were polymorphic, with an average of 1.83 alleles per locus. Most genetic diversity was partitioned among patches. Some within-patch genetic diversity may result from somatic mutation in ancient clones. However, individual patches included individuals that differed at several loci, indicating that many genets within patches originated from seed.

INTRODUCTION

Collomia rawsoniana E. Greene (Rawson's Flaming Trumpet) is a rare herbaceous perennial plant endemic to Madera County, California. Most individuals grow within the Sierra National Forest. Nearly all C. rawsoniana populations occur in the northwest portion of the upper San Joaquin River drainage (Taylor et al. 1985), although two population are known outside that drainage, at Nelder Creek and Mammoth Pool (Liskey 1993). The species is restricted to riparian habitats and meadows in conifer forests at elevations of 3000 to 7000 feet (= 900 to 2000 meters). Collomia rawsoniana grows in large patches that are produced at least in part by clonal spread through rhizomes. Patches vary from 0.1 to 32 m2, with a mean of 6.3 m2, and larger patches have a higher proportion of flowering stems (Taylor et al.

1985). Patches may be 200 m or more apart, especially at low elevations, but are often much closer (Taylor et al. 1985). Patches inhabit a variety of microhabitats where moisture stress is reduced by availability of groundwater or by shade (Liskey 1993). Collomia rawsoniana produces showy, red, tubular, hummingbird-pollinated flowers in June and July, and sets seed in September. The species is capable of producing seeds through self-pollination, but the sequence of floral development minimizes or prevents self-pollination within a single flower and rates of seed set from cross-pollination are much higher than for self-pollination (Hevron 1989). Flowers within a patch tend to bloom synchronously, but different patches bloom at different times (Hevron 1989). Fruits are explosively dehiscent, throwing seeds as much as 1 meter (Liskey 1993). Seeds germinate best on bare mineral soils. Seeds often germinate within the parental patch, where competition with the established mat of rhizomes is great and the seedlings usually die (Hevron 1989). Therefore, seedling establishment is rare (Hevron 1989). No information on the population genetics of this rare species is available. Most C. rawsoniana populations are located within grazing allotments in areas actively managed for timber harvest. Cattle grazing and trampling can have severe negative effects on C. rawsoniana (Liskey 1993). In particular, once an herbivore removes a flowering stalk, that stalk does not recover sufficiently to flower (Liskey 1993). Herbivores may remove most or all the flowering stalks in a patch, but grazing is not uniform among patches. The effect of grazing on the genetic diversity essential for the species' long term survival is difficult to assess because genetic individuals can not be recognized in the field. Are patches single genetic individuals (single clones)? Is the genetic variation in this species spread throughout the population, so that complete failure of seed set in some patches has little effect on the variation in the seeds that are formed? Or are patches genetically differentiated, so that failure of seed set prevents important components of genetic variation from being passed on to the next generation? This isozyme study of C. rawsoniana was initiated in order to answer some of these questions.

METHODS

In August 1999, leaf samples were collected from 21 patches collected at six locations in the Sierra National Forest (Table 1). Patch size and distance between patches varied. Five moderate-sized to large patches were sampled at each location. However, few patches of any size grew in three locations (CC, MP, and NC) and therefore all moderate-sized to large patches at those location were sampled. Each patch was divided into twelve sections, four near the center and eight around the outside (diagrammed in Fig. 2). Sampling was done on the same pattern no matter how big the patch was. Therefore, samples were further apart in large patches than in small ones. In most cases, one sample was collected in each of twelve portions of the patch, but only ten samples were collected from the patches at NC due to very uneven distribution of stems within the patches there. Samples were placed in plastic bags with wet paper towels and kept on ice in the field. Sample Preparation. Two medium sized (ca. 3 cm long) leaves were ground in liquid nitrogen. Then 250 µl of a Tris buffer pH 7.5 (Gottlieb 1981) was added to the powdered leaf and the slurry was transferred to microtiter plate wells and frozen at -70°C. On the morning of electrophoresis, the slurry was thawed and absorbed onto three 3-mm wide wicks prepared from Whatman 3mm chromatography paper. Electrophoresis. Methods of electrophoresis are outlined in Anonymous (1995), and follow the general methodology of Conkle et al. (1982) except that most enzyme stains are modified. The following enzymes were examined: aconitase (ACO), catalase (CAT), diaphorase (DIA), florescent esterase (FEST), glucose-6-phosphate dehydrogenase (G6PDH), glutamate-oxaloacetate transaminase (GOT), isocitrate dehydrogenase (IDH), leucine aminopeptidase (LAP), malate dehydrogenase (MDH), malic enzyme (ME), phosphoglucomutase (PGM), phosphogluconate dehydrogenase (6PGD), phosphoglucose isomerase (PGI), shikimic acid dehydrogenase (SKD), triosephosphate isomerase (TPI), and uridine diphosphoglucose

pyrophosphorylase (UGPP). A total of 24 loci resolved sufficiently well to use in genetic analysis. A lithium borate electrode buffer (pH 8.3) was used with a Tris citrate gel buffer (pH 8.3) (Conkle et al. 1982) to resolve ACO, ME7, PGI-1, PGI-2, PGM-1, and PGM-2. 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 CAT, GOT-1, GOT-2, G6PDH, TPI-1, TPI-2, TPI-3, and UGPP-1. A morpholine citrate electrode and gel buffer (pH 8.0) (Anon. 1985) was used to resolve DIA-1, DIA-2, FEST-3, IDH, MDH-1, MDH-2, MDH-3, MDH-4, 6PGD-1, 6PGD-2, 6PGD-3, and SKD. All enzymes were resolved on 11% starch gels. Enzyme stain recipes follow Anonymous (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. Most California Collomia are diploids with 2n = 16 (Wilken 1993), but we are not aware of a chromosome count for C. rawsoniana. We are not aware of any information about the genetics of isozymes in Collomia, although alleles of eight isozyme loci are known to be inherited in Mendelian fashion in the Ipomopsis aggregata (Pursh) V. Grant complex (Polemoniaceae) (Wolf et al. 1991). Therefore, genetic interpretations were based on the assumption of C. rawsoniana is diploid, plus knowledge of the generally conserved enzyme substructure, compartmentalization, and isozyme number in higher plants (Gottlieb 1981, 1982; Weeden and Wendel 1989). 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 heterozygosity (Nei 1973), expected number of alleles/locus (Kimura and Crow 1964), and gene flow (Nm = 0.25[1/Fs]/Fst; Slatkin and Barton 1989). A dendrogram was generated in Popgene using UPGMA and Nei's unbiased genetic distances. Hierarchical F statistics (Wright 1978) were generated using Biosys-1, version 1.7 (Swofford and Selander 1989), except that F statistics for the three-level hierarchy (Table 3) were generated using the method of Weir (1990) using Popgene (Yeh et al. 1997). Clonal structure of the population was measured by the "proportion detected" (Ellstrand and Roose 1987), for which 1 = all genotypes distinct and 0 = a monoclonal population. One multilocus genotype occurred in two patches. The probability of that genotype occurring twice by seedling establishment was estimated by the method of Parks and Werth (1993), using allele frequencies within the Whiskey Creek population (calculated using each genotype once per patch) and a sample size of 52 (the number of samples collected in Whiskey Creek, minus those in patch WC-P4, where that genotype was common).

RESULTS

Allele frequencies were calculated for populations and patches of C. rawsoniana. Three alleles were detected only in single populations (MDH-3 allele 4 in BM, and FEST-3 allele 3 and MDH-4 allele 2 in GM). No allele was confined to a single patch. Overall, sixteen (62%) of the 24 loci were polymorphic (Table 2), with an average of 36% of loci polymorphic in an individual patch (Table 3). In 16 (76%) of the 21 patches, the observed heterozygosity was greater than the expected heterozygosity, and therefore the fixation index (F) was negative (Table 3). The patches were highly differentiated genetically, but individual samples within a patch, and collection sites within the total species were much less differentiated (Table 4). Nei's unbiased genetic identities among patches averaged 0.8954 (SD = 0.3090), with a maximum of 0.9852 and a minimum of 0.7761. The patches most similar to a given patch were not always in the same collection site. Patches from one location usually clustered together in a dendrogram based on genetic identities, but that was not always true (Fig. 1). In particular, patches from collection sites BM and WC were intermingled on the tree (Fig. 1), and genetic identities among the patches in the CC collection site averaged lower than those between the CC patches and patches in other

collection sites (Table 5). A total of 177 complete multilocus genotypes were generated for 236 (94%) of the 252 samples. Therefore, there were an average of 1.33 individual samples per complete genotype. An additional nine genotypes were incomplete but unambiguously unique. If these are included, the C. rawsoniana patches had 1.32 individual samples per genotype, with a "proportion distinguished" (PD) (Ellstrand and Roose 1987) equal to 0.76, and 64% of the 245 samples with unambiguous genotypes were genetically unique. Each patch contained at least four different multilocus genotypes, and in three patches (BM-P5, GM-P2, and NC-P2), all sampled individuals were different. In most patches, the majority of samples were genetically distinct; the exceptions were patches MP-P1, NC-P1, and WC-P4. However, all individuals in some patches had the same heterozygous genotypes or unusual alleles at certain loci. In general, individuals with the same multilocus genotypes grew in adjacent sections of a patch (Fig. 2). Only one genotype was observed in two patches. Genotype number 59 occurred nine times in patch WC-P4 and once in patch WC-P3 (Fig. 2). The probability of second occurrence of this genotype within Whiskey by seedling establishment (Parks and Werth 1993) was 5.6 X 10-5. When each genotype was represented by a single sample, most statistics were similar to those produced when every sample were included in the analysis (data not shown). Genetic distances between patches averaged 0.9029 (SD = 0.0364), with a maximum of 0.9851 and a minimum of 0.7860.

DISCUSSION

Genetic diversity at the species level. Collomia rawsoniana exhibits high levels of isozyme diversity for an endemic plant (Hamrick and Godt 1990). Collomia rawsoniana is more variable than any of the species in the Ipomopsis aggregata (Pursh) V. Grant complex and other Ipomopsis species studied (Wolf et al. 1991). Like C. rawsoniana, the Ipomopsis species are outcrossing perennial plants pollinated by hummingbirds, are in the family Polemoniaceae, and live in mid-successional habitats. Most Ipomopsis species differ from C. rawsoniana in that they are short-lived and widespread, and they do not spread clonally. Widespread, sexually reproducing plants, like Ipomopsis species, usually have higher levels of isozyme diversity than endemic and vegetatively spreading plants, like Collomia rawsoniana (Hamrick and Godt 1990). Although C. rawsoniana had a higher level of genetic diversity than expected for a narrowly endemic plant, this level of diversity is not unique. For example, Hackelia venusta (Piper) St. John, known from only one large population, is very diverse (Wilson et al. MS). In the hierarchy of individuals within patches within populations (sites) within the species, the most genetically differentiated units are the patches (Table 4). The genetic identities among C. rawsoniana patches averaged 0.89; genetic identities among conspecific populations usually average somewhat above 0.90 (Crawford 1990). The C. rawsoniana patches do not appear to be inbred. The negative fixation indices in most patches (Table 3) and negative Fis in all populations (Table 2) support earlier observational and experimental evidence that self-pollination is rare (Hevron 1989). The lack of inbreeding may suggest a strong barrier to self-pollination. Collomia rawsoniana exhibits very low seed set when artificially self-pollinated (Hevron 1989). All the samples from Whiskey Creek were scored as heterozygous at the PGI-2 locus. If those scores resulted from a gene duplication, the fixation index (and evidence for outcrossing) for Whiskey Creek populations was artificially inflated. However, the PGI-2 locus had a much smaller effect in other populations. Origin of patches. The hypothesis that establishment of new C. rawsoniana patches occurs primarily vegetatively, from rhizomes fragmented during flooding (Taylor et al. 1985), is not supported by this study. Only one multilocus genotype was detected in more than one patch (Fig. 2). However, that one multilocus genotype may well have been transported between patches WC-P3 and WC-P4 as a fragmented rhizome, because its probability of second establishment by seed was very low (5.6 X 10-5). Genetic diversity within patches. The mat of rhizomes underlying each C. rawsoniana patch suggests that each patch is a single clone (Taylor et al. 1985, Hevron 1989), but that is

not true. The individuals in each patch apparently arise by both sexual and asexual reproduction. The plant's strongly rhizomatous growth form and the multilocus genotypes shared by many samples within patches (Fig. 2) provide obvious evidence for asexual reproduction within patches. The pattern of within-patch genetic variation provided more subtle evidence for asexual variation. For example, Some genotypes in a clone differed by only one allele. This evidence suggested somatic mutation within ancient clones. Examples included patch WC-P1, where genotypes 64 and 66 differed from genotype 65 at one locus each, and patch NC-P1, where genotypes 118 and 120 differed from genotype 119 at one allele each. The excess of heterozygotes in most patches may provide additional evidence for clonal spread. In some patches all samples were heterozygous for the same alleles at some loci. Shared heterozygous loci would be expected from vegetative spread of a heterozygous individual, from establishment of heterozygous full sibs near their mother plant, or from selection pressures that differ in the diverse microhabitats occupied by individual C. rawsoniana patches. It would not result from sexual reproduction in a randomly mating population. However, a technical problem may contribute to the reported excess of heterozygotes. The apparent heterozygosity at the PGI-2 locus in all individuals in the five patches in the Whiskey Creek population may result from an unrecognized gene duplication, with different alleles fixed at the two loci, although the pattern of variation in other sampled populations does not strongly support that hypothesis. Other evidence supports the hypothesis that the genetic individuals within a patch originated from sexual reproduction. First, the proportion of distinguishable genotypes in this study was 0.76, much higher than is typical of a clonally reproducing population (Ellstrand and Roose 1987). Also, some individuals within a patch have very different multilocus genotypes. For example, in the same patch NC-P1 referred to above, the genotype N6 differs from genotype 119 at eight alleles at six loci, more than could be accounted for by somatic mutation. Seeds have been observed to germinate within patches (Hevron 1989). The observed seedlings all died, but the presence of diverse genotypes in some patches indicates that seedlings do become established in patches, at least occasionally. Apparently sexual reproduction and within-patch seedling establishment contribute importantly to the genetic diversity within patches. If genets usually originate from seeds, what explains the evidence for somatic mutation? The genetic similarity of individuals in a patch may result from their being close relatives or from selection pressure in the patch microhabitat. Alternatively, some patches may be very old, and really may include some ancient clones that have undergone somatic mutation. Accumulation of somatic mutations in a clone is a slow process. If somatic mutation explains some of the differences among some of these genotypes, some C. rawsoniana patches may be ancient. That may plausible, for two reasons. First, plants that spread clonally are potentially immortal. For example, some aspen clones may be 10,000 years old (Tuskan et al. 1996). Second, the C. rawsoniana populations grow in an area that was never glaciated (Matthes 1960, Liskey 1993). Suitable habitat for them may have been available at that site for millennia. Management implications. Preserving Collomia rawsoniana genetic diversity means preserving many patches, because patches are the most genetically differentiated unit within the species. Collomia rawsoniana populations also differ, because the patches in them are genetically differentiated (Fig. 1), and therefore preserving patches of C. rawsoniana results in preserving many populations. Patches are not single clones. Therefore, destroying part of a patch may result in destruction of some genetic individuals (genets). The impact of destroying part of a patch on the genetic diversity of that patch can to some extent by estimated from maps of multilocus genotypes within patches (Fig. 2). The isozyme variation within patches of C. rawsoniana suggests that sexual reproduction is more important for this plant's survival and spread than had been recognized previously. Seedling establishment may be rare (Hevron 1989) and perhaps episodic, but it does occur. Management activities that interfere with seed set, such as grazing (Liskey 1993), do interfere with the normal reproduction and generation of genetic diversity in this rare plant.

ACKNOWLEDGEMENTS

This project was initiated by Joanna Clines (U.S.D.A. Forest Service, Sierra National Forest, 1600 Tollhouse Road, Clovis, California 93611) and funded by the U.S. Fish and Wildlife Service and the U.S.D.A. Forest Service (Sierra National Forest and Region 5 Genetic Resource Program). We thank Suellen Carroll, Patricia Guge, and Randy Meyer of the National Forest Genetic Electrophoresis Laboratory for technical support. We thank Ronald Cummings, Cheryl Herzer, and Elaine Yurch for collecting samples.

REFERENCES

Anonymous. 1995. National Forest Genetic Electrophoresis Laboratory Standard Operating Procedures. NFGEL, USDA Forest Service, Camino, California

Conkle, M. T., P. D. Hodgskiss, L. B. Nunnally, and S. C. Hunter. 1982. Starch Gel Electrophoresis of Conifer Seeds: a Laboratory Manual. Gen. Tech. Rep. PSW-64. Berkeley, CA: Pacific Southwest Forest and Range Experiment Station, Forest Service, U.S. Department of Agriculture. 18pp.

Crawford, D. J. 1990. Plant Molecular Systematics: Macromolecular Approaches. John Wiley & Sons, New York, New York.

Ellstrand, N. C., and M. L. Roose. 1987. Patterns of genotypic diversity in clonal plant species. American Journal of Botany 74:123-131.

Gottlieb, L. D. 1981. Gene number in species of Asteraceae that have different chromosome numbers. Proceedings of the National Academy of Science, USA 78:3726-3729.

Gottlieb, L. D. 1982. Conservation and duplication of isozymes in plants. Science 216: 373-380.

Hamrick, J. L., and M. J. W. Godt. 1990. Allozyme diversity in plant species. Pages 43-63 In Brown, A. H. D., M. T. Clegg, A. L. Kahler, and B. S. Weir (editors) Plant Population Genetics, Breeding, and Genetic Resources. Sinauer Associates, Inc., Sunderland, Maryland.

Hamrick, J. L., M. J. W. Godt, D. A. Murawski, and M. D. Loveless. 1991. Correlations between species traits and allozyme diversity: implications for conservation biology. Pages 75-86 In Falk, D. A., and K. E. Holsinger (editors) Genetics and Conservation of Rare Plants.Oxford University Press, Oxford, England.

Hevron, W. E. 1989. The reproductive ecology of Collomia rawsoniana.

Unpubl. M.A. thesis,School of Natural Sciences, California State University Fresno, Fresno, California.

Kimura, M., and J. F. Crow. 1964. The number of alleles that can be maintained in a finite population. Genetics 49:725-738.

Levene, H. 1949. On a matching problem in genetics. Ann. Math. Stat. 20: 91-94.

Liskey, E. N. 1993. Effects of Disturbance on Collomia rawsoniana. Unpubl. M.A. thesis, School of Natural Sciences, California State University Fresno, Fresno, California.

Matthes, F. 1960. Reconnaissance of the geomorphology and glacial geology of the San Joaquin basin, Sierra Nevada, California. Geological Survey Profession Paper 329. U.S. Government Printing Office, Washington, D.C.

Nei, M. 1973. Analysis of gene diversity in subdivided Populations. Proc. Nat. Acad. Sci. USA 70: 3321-3323

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89: 583-590.

Parks, J. C., and C. R. Werth. 1993. A study of spatial features of clones in a population of bracken fern, Pteridium aquilinum (Dennstaedtiaceae). Am. J. Bot. 80: 537-544.

Slatkin, M., and N. H. Barton. 1989. A comparison of three indirect methods for estimatingaverage levels of gene flow. Evolution 43: 1349-1368.

Swofford, D. L., and R. B. Selander. 1989. BIOSYS-1, a computer program for the analysis of allelic variation in population genetics and biochemical systematics, release 1.7 edition. Illinois Natural History Survey, Champaign, Ill.

Taylor, D. W., W. B. Davilla, and J. C. Stebbins. 1985. Distribution, Ecology, and Statusof Collomia rawsoniana within the Crane Valley Project Vicinity. Unpubl. Technical Report prepared for Pacific Gas and Electric Company, Department of Engineering Research, San Ramon, California.

Tuskan, G. A., K. E. Francis, S. L. Russ, W. H. Romme, and M. G. Turner. 1996. RAPDmarkers reveal diversity within and among clonal and seedling stands of aspen in Yellowstone National Park, U.S.A. Can. J. For. Res. 26: 2088-2098

Weeden, N. F. and J. F. Wendel. 1989. Genetics of plant isozymes. pp. 46-72 in Isozymes in plant biology, eds. D. E. Soltis and P. S. Soltis. Dioscorides Press, Portland, Oregon

Weir, B. S. 1990. Genetic Data Analysis. Sinauer Associates, Sunderland, MA.

Wendel, J. F., and N. F. Weeden. 1989. Visualization and interpretation

of plant isozymes. Pages 5-45 In Soltis, D. E., and P. S. Soltis (editors) Isozymes in Plant Biology. Dioscorides Press, Portland, Oregon.

Wilson, B. L, R. Harrod, V. Hipkins, and C. Aubry. MS. Isozyme variation in Hackelia venusta and H. diffusa (Boraginaceae).

Wolf, P. G., P. S. Soltis, and D. E. Soltis. 1991. Genetic relationships and patterns of allozymic divergence in the Ipomopsis aggregata complex and related species (Polemoniaceae). Am. J. Bot. 78: 515-526.

Wright, S. 1978. Evolution and the Genetics of Populations, Vol. 4. Variability within and among natural populations. University of Chicago Press, Chicago.

Yeh, F. C., R.-C. Yang, T. Boyle, Z-H. Ye, and J. X. Mao. 1997. Popgene, theuser-friendly shareware for population genetic analysis. Version 1.20. Molecular Biology and Biotechnology Centre, University of Alberta, Edmonton, Alberta

Table 1. Collection sites for *Collomia rawsoniana* samples used in this study. All sites were in Madera County, California, and all except patch #1 in CC were in the Sierra National Forest.

Location	Site code	Patches sampled	Latitude Longitude	Habitat	Elevation
Benedict Meadow	BM	5	37.25ºN 119.42ºW	Riparian forest within Mixed Conifer Forest.	5600 feet
Calvin Crest	CC	3	37.41ºN 119.60ºW	Riparian forest and montane meadow edge within Mixed Conifer Forest	4700 feet
Graham Mountain	GM	5	37.32ºN 119.50ºW	Jeffrey Pine plantation next to pond.	5280 feet
Mammoth Pool	MP	1	37.34ºN 119.33ºW	Riparian forest in ponderosa pine forest	3600 feet
Nelder Creek	NC	2	37.41ºN 119.59ºW	Riparian forest in Mixed Conifer Forest	4600 feet
Whiskey Creek	WC	5	37.24ºN 119.42ºW	Moist, seepy roadcut in Mixed Conifer Forest	5840 feet

Table 2. Summary of genetic diversity measures for populations (collection localities) of *Collomia rawsoniana*. All samples were used to calculate these statistics, whetherthey had the same multilocus genotype or not. All = overall statistics for the study.

Pop. = population name. N = number of individuals sampled per locus, per population.%P = percent of all loci that are polymorphic. A = average number of alleles per locus. Ae = Effective number of alleles (Kimura & Crow 1964). Ap = the average number of alleles per polymorphic locus. Ho = observed frequencyof 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 patches within that taxon. Mean = average of locations, for the five locations where at least two patches were sampled. SD = standard deviation.

Pop.	N	%P	A	Ae	Ap	Ho	He	Fis	Fit	Fst	Nm
ALL	251	62%	1.83	1.42	2.33	0.1630	0.2199	0.039	0.258	0.228	0.3662
Mean	48	52%	1.63	1.33	2.18	0.1673	0.1850	-0.280	0.074	0.276	0.6773
SD	19.2	3%	0.09	0.04	0.07	0.02	0.02	0.238	0.179	0.052	0.1600
Max	64	58%	1.75	1.37	2.28	0.1863	0.2075	-0.023	0.260	0.362	0.8742
Min	20	50%	1.54	1.27	2.08	0.1404	0.1611	-0.537	-0.143	0.223	0.4400
BM	60	53%	1.75	1.35	2.28	0.1404	0.1855	-0.034	0.236	0.261	0.7053
CC	36	50%	1.58	1.36	2.17	0.1863	0.2065	-0.435	0.085	0.362	0.4400
GM	60	58%	1.71	1.37	2.21	0.1520	0.2075	-0.023	0.260	0.277	0.6519
MP	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
NC	20	50%	1.54	1.27	2.08	0.1718	0.1648	-0.375	-0.069	0.223	0.8742
WC	64	50%	1.58	1.30	2.17	0.1860	0.1611	-0.537	-0.143	0.256	0.7151

Table 3. Summary of genetic diversity measures for patches of *Collomia rawsoniana*. Mean = average over the 21 patches. SD = standard deviation. Pop. = population name. 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. Ae = 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 (Levene 1949). F = the fixation index = (He-Ho)/He.

Pop.	Patch	N	%P	A	Ae	Ap	Ho	He	F
Mean		12	36%	1.40	1.23	2.10	0.1620	0.1364	-0.2045
SD		1.1	9%	0.09	0.04	0.10	0.0411	0.0282	0.2510
Max		16	0.5	1.58	1.32	2.4	0.2667	0.191	0.2084
Min		10	0.21	1.21	1.17	2	0.1139	0.0941	-0.6534

BM	1	12	38%	1.42	1.25	2.11	0.1208	0.1526	0.2084
BM	2	12	50%	1.58	1.31	2.17	0.1771	0.1910	0.0728
BM	3	12	42%	1.46	1.25				

Seed Zones for Eastern Sierra Grass Species

(NFGEL Project #89)

OBJECTIVES

The UC San Diego's White Mountain Research Station, the Bureau of Land Management, and the US Forest Service, Inyo NF have entered into a cooperative project to establish native grass seed zones and a seed bank system for the eastern Sierra and cis Great Basin (see the 9/30/98 project proposal submitted to the National Fish and Wildlife Foundation by Dr. Szewczak of the White Mt. Research Stn). Allozyme data will be used as part of this comprehensive program. This work was done in cooperation with Kathleen Nelson and Bob Westfall (USFS).

MATERIALS AND METHODS

Six grass species were submitted for genetic analysis: *Elymus elymoides, Achnatherum occidentalis, Acnatherum speciosum, Achnatherum thurberianum, Achnatherum pinetorum, and Achnatherum hymenoides*. Seedlots of 65 families planted in a common garden study, plus 27 seedlots from *Achnatherum hymenoides*, were sent to NFGEL. The goal was to obtain data from 5 seeds per family, but germination was a problem for many of the lots. Some families only produced one seedling, and all seedlings available were prepared and run for isozyme analysis.

Table. The numbers of families submitted (# Fam), the number of families germinated for isozyme analysis (# Fam Germ), the number of families genetoyped (# Fam Geno), the number of seedlings genotyped (# Seed Geno), and the number of families sown in a common garden study (excluding *Achnatherum hymenoides*) (# CG).

Species	# Fam	# Fam Germ	# Fam Geno	# Seed Geno	# CG
Elymus elymoides (ELEL)	25	25	20	85	25
Achnatherum occidentalis (ACOC)	32	32	1	1	29
Acnatherum speciosum (ACSP)	3	3	2	2	7
Achnatherum thurberianum (ACTH)	1	1	1	1	1
Achnatherum hymenoides (ACHY)	27	27	20	61	0
Achnatherum hymenoides (ACHY)	3	3	0	0	0

Seed was germinated using NFGEL Standard Operating Procedures. When seedlings appeared above the top of the germination paper, they were selected for sample prep. Using large acrylic plates and grinding rods, samples were ground in 450ul of fresh Melody/Neale extraction buffer and stored as 150ul in three replicate microtiter plates. Samples were stored at -70.

RESULTS

A total of 150 seedlings from 44 families were genotyped at 23 isozyme using three buffer systems.

Loci= LAP-1, PGM-1, PGM-2, ME7-1, ME7-2, IDH-1, UGP-1, TPI-1, TPI-2, GOT-1, GOT-2, GOT-3, GOT-4, PGI-4, 6PDG-1, 6PGD-2, MDH-1, MDH-2, MDH-4, MDH-5, SKD-1, SKD-2, SKD-3

Ac hy 1389 55 14 11 44 44 11 11 33 55 11 11 33 55 89 22 33 55 22 11 33 22 22 33

Ac hy 1389	55	11	44	44	44	11	11	33	55	11	11	33	55	89	22	33	55	22	11	33	22	22	33
Ac hy 1396	55	44	11	44	44	11	13	56	55	11	11	33	55	89	22	33	55	22	11	44	22	33	33
Ac hy 1396	55	44	44	55	44	11	13	56	55	11	11	33	55	89	22	33	55	22	11	44	22	33	33
Ac hy 1418	55	11	11	11	44	11	11	33	55	11	11	33	15	89	33	33	55	22	11	33	22	33	33
Ac hy 1418	55	11	11	11	44	11	12	33	55	11	11	33	15	89	33	33	55	22	11	33	22	33	33
Ac hy 1418	55	11	11	11	44	11	11	33	55	11	11	33	55	89	22	33	55	22	11	44	22	33	33
Ac hy 1418	55	11	44	11	44	11	11	33	55	11	11	33	55	68	22	33	55	22	11	33	22	33	00
Ac hy 1418	55	11	44	11	44	11	11	33	55	11	11	33	55	89	22	33	55	22	11	44	22	33	33
Ac hy 1422	55	00	00	44	44	11	13	33	55	11	11	00	00	68	22	34	55	22	11	33	22	22	33
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Ac oc 1471 55 44 44 44 44 11 11 55 55 11 11 33 46 66 22 33 55 22 11 44 22 33 22

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El el 1501 11 11 11 66 66 11 11 22 11 11 33 55 33 19 11 11 11 11 33 11 11 11 11

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El el 1501 11 11 11 66 66 11 11 25 11 11 33 55 33 19 11 11 11 11 33 11 11 11 22

El el 1501 11 11 11 66 66 11 11 25 11 11 33 55 33 19 11 11 11 11 33 11 11 22 11

El el 1505 11 11 11 66 66 11 11 22 11 11 33 55 33 19 11 11 11 11 33 11 11 11 11

El el 1519 11 11 11 66 66 11 11 22 11 11 33 55 33 19 11 11 11 11 33 11 11 11 11

El el 1519 11 11 11 66 66 11 11 22 11 11 33 55 33 19 11 11 11 11 33 11 11 11 11

El el 1519	11	11	11	66	66	11	11	22	11	11	33	55	33	19	11	11	11	11	33	11	11	11	11
El el 1519	11	11	11	66	66	11	11	22	11	11	33	55	33	19	11	11	11	11	33	11	11	22	11
El el 1531	11	11	11	66	66	11	11	11	11	11	33	55	33	19	11	11	44	11	33	11	11	11	22
El el 1531	11	11	11	66	66	11	11	11	11	11	33	55	33	19	11	11	44	11	33	11	11	11	22
El el 1531	11	11	11	66	66	11	11	11	11	11	33	55	33	19	11	11	44	11	33	11	11	11	22

FINAL PRODUCT

NFGEL provided the final data set and basic genetic statistics to Robert Westfall for further analysis. Genetic interpretations of isozyme patterns are subject to change. *Elymus elmoides* is a tetraploid. The *Achnatherum* species are also polyploid, likely either tetraploids or hexaploids. *A. thurberianum* is a high polyploid. Interpretations were made using the results of our prior work on the tetraploid grass *E. glaucus*. Some of the Inyo grass data was treated as overlapping loci (therefore creating as excess of homozygotes), other loci were scored as 'diploid' (creating as excess of heterozygotes). The addition of more samples would aid in the interpretation of the loci. We will leave the scores as is during the preliminary analysis of the data.

DNA Profiling and Identity Analysis of Ponderosa Pine Evidence Samples

INTRODUCTION

Although most forensic applications of DNA technology have focused on human identification, analysis of non-human DNA has also proven to be useful in the legal arena. DNA from wild and domesticated animals, microbes, insects, and plants have all been successfully used in prior court cases (Sensabaugh and Kaye 1998).

All living organisms contain DNA, from an animal to a plant to bacteria. The DNA in each of these organisms behaves the same way - it has the same function and structure. The technologies and statistical principles used to determine an individuals DNA profile is also the same (Herrmann and Hummel 1994; National Research Council 1996). In plants, DNA can be found at highest concentration in living tissues: leaves or needles, buds, seed, root tips. DNA also exists at much lower concentrations in woody tissues. In the stem, branches, and roots of woody plants, such as conifers, DNA is found in the cambial layer (between the xylem and phloem; a thin layer just inside the bark), and in certain cells within the wood (e.g. parenchyma cells). In most wood (xylem) cells, the DNA disintegrates in the final stages of cell maturation (via autolysis). The proportion of various cell types found in woody tissue is species dependent (Kramer and Kozlowski 1979).

I am unaware of any published work documenting the successful extraction of DNA from woody tissue. However, at our laboratory, we have extracted DNA from the wood (stump and branch) of *Eucalyptus* for use in another legal case. If DNA can reliably be extracted from wood, a number of forensic uses can be pursued including timber theft, fuelwood theft, christmas tree theft, fire investigation, and historic artifact authentication.

LABORATORY OBJECTIVE

The objective of this analysis was to obtain genetic profiles from each of six submitted wood samples of ponderosa pine (*Pinus ponderosa*) and determine if the genetic profiles were indistinguishable from each other (if they 'matched'). Any given two profiles could match because either (1) the two samples came from the same individual, or (2) the two samples came from different individuals and only matched coincidentally. A genetic profile is obtained in the lab by first extracting DNA (in sufficient quantity and quality) from a sample, in this case a piece of wood, and then amplifying the DNA to visualize a pattern of bands on a gel.

METHODS

Sample Collection

Six ponderosa pine wood samples, labeled 1, 4, 7, 10, 13, and 16, were received by NFGEL, Camino CA on March 9, 2000 for analysis. Each sample contained some cambial layer tissue. Samples were digitally photographed at NFGEL before lab analysis began, and then secured in the NFGEL office building.

DNA Extractions and Quantification

DNA extractions were carried out using three different standard techniques. The first technique used the FastPrep protocol (Bio101 Inc.), the second used the DNeasy protocol (Quigen Inc.), and the third technique used a modified CTAB protocol (Hipkins et al. 1995). Specific protocol steps can be provided upon request.

DNA was quantified (concentration of DNA determined in each sample with the unit of concentration equal to ng/ul) by specific fluorescence detection in a Hoeffer Scientific fluorometer. Based on quantification, samples were diluted to 3 ng/ul for PCR amplifications. On some samples, DNA was quantified using a DNA Dipstick (Invitrogen). The Dipstick measures low concentrations more accurately than the fluorometer. However, due to the high expense of the Dipsticks, these were used only on the more troublesome samples.

RAPD Analysis

The purpose of the RAPD (randomly amplified polymorphic DNA) analysis is to provide the genetic profiles. RAPD analysis is a lab technique that uses the polymerase chain reaction (PCR) to produce, or amplify, small DNA fragments that are visualized on gels as bands (Avise 1994). Bands are produced by using short, random 'primers' in a PCR reaction (a series of temperature controlled steps). Usually, 10 to 20 working primers are more than sufficient to yield a DNA profile for an individual. A working primer is one that produces bands in the PCR reaction. Many primers fail to yield any bands in a reaction. These primers are discarded until 10 to 20 'good' primers are found for use in the study. The RAPD program steps of denaturation, annealing, and extension, and well as primer sequences used in this work, are available upon request.

RESULTS

DNA Extractions and Quantification

DNA was extracted from all samples as indicated by concentration readings obtained by fluorometry (those samples extracted 3/10/00 through 6/13/00) or Dipstick (the extraction done on 7/7/00) (Table 1). To distinguish extractions done on different days, samples were coded. For example, sample #1 was extracted 3/10/00 by the FastPrep protocol, sample #1e was extracted on 7/7/00 by the CTAB method, and so on.

Table 1. DNA yields for all extractions (concentrations values are ng/ul).
FP = FastPrep protocol; DNeasy = DNeasy protocol; CTAB = CTAB protocol.

	Date	**3/10/00**	**3/10/00**	**5/4/00**	**6/7/00**	**6/13/00**	**7/7/00**
	Method	**FP**	**FP**	**FP**	**DNeasy** **[A, B]**	**CTAB**	**CTAB**
	Code		**a**	**b**	**c**	**d**	**e**
Sample #							
1		19	18	3			50
4		15	14	14	3, 2	15	
7		16	15	13			50
10		18	13	5	2, 1	7	
13		15	13	5			50
16		19	16	11			50

RAPD Analysis

All the RAPD reactions performed between 3/27/00 and 4/27/00 were done on the samples extracted on 3/10/00 (Table 2). A total of eleven primers were tested on these samples. Samples 4 and 10 were able to amplify using several different primers (W-09, W-06 and 241). The other samples did not amplify as well. This could be due to poor quality of DNA, or interfering compounds in the samples, among other things. We then performed amplifications on samples 4 and 10 extracted with the other protocols, in hopes that these other protocols would yield cleaner DNA, and thus 'better' amplifications (Table 3). Again, primers W-06 and 241 amplified, regardless of how sample 4 or 10 was extracted.

Table 2. RAPD results by date. Samples are coded by their extraction date and method (see Table 1). A blank cell indicates a reaction was not performed; a (-) indicated no bands were produced in the reaction; a (+) indicates bands were produced in the reaction.

	Date	3/27	3/28	3/28	3/30	4/3	4/4	4/20	4/20	4/24	4/24	4/25	4/26	4/26	4/27
	Primer	A-08	A-08	241	A-08	241	218	W-02	W-03	W-04	W-05	W-06	W-07	W-08	W-09
Sample															
1		-	-	-	-	-	-	-	-	-	-	-	-	-	-
1b															
4		+	-	-	-	+	-	-	-	+	-	+	-	-	+
4b															
4c															
4d															
7			-	-	-	-	-	-	-	-	-	-	-	-	-
7b															
10			-	-	-	+	+	-	-	-	+	+	-	-	+
10b															
10c															
10d															
13			-	-	-	-	-	-	-	-	-	+	-	-	+
13b															
16			-	-	-	+	-	-	-	-	-	-	-	-	-
16b															

Table 3. RAPD results by date. Samples are coded by their extraction date and method (see Table 1). A blank cell indicates a reaction was not performed; a (-) indicated no bands were produced in the reaction; a (+) indicates bands were produced in the reaction.

	Date	5/5	6/20	6/20	6/20	6/20	6/21	6/21	6/21	6/21
	Primer	W-06	W-06	W-09	W-09	241	W-08	W-05	W-06	A-08
Sample										
1										
1b		-								
4		+								+
4b		+								
4c			+		-	+	-	-	-	
4d			+	-	-	+	-	-	-	
7										
7b		-								

10		+								-
10b		-								
10c			-		-	+	-	-	-	
10d			+	-	-	+	-	-	-	
13		+								
13b		-								
16		-								
16b		-								

CONCLUSIONS

We were unable to obtain genetic profiles for the six submitted ponderosa pine samples, and therefore cannot provide DNA evidence regarding identity matches. However, even though we are unable to provide usable evidence for the court case at this time, we did overcome many technical barriers in the course of our work and were able to obtain genetic data for some of these wood samples.

According to our methods of quantification, we were able to extract DNA from all six ponderosa pine wood samples, using any of the three DNA extraction methods tested. Two of these samples, 4 and 10, were able to amplify in a RAPD analysis and produce bands using three different primers (out of eleven primers tested). Samples 1 and 7 did not amplify in any reaction. Samples 13 and 16 amplified sporadically (sample 13 using primer W-06 and W-09, and sample 16 using primer 241).

To be able to get a genetic profile, a given sample has to produce RAPD bands consistently every time the sample is amplified with the same primer. Also, more primers than the three we now have are needed to create an individuals genetic profile. Until each sample has a genetic profile, we cannot begin to look for 'matches' among the samples.

The following is what would still be needed to obtain genetic profiles for the samples.

> Sample 4 and 10. Need more working primers. We have three so far; would probably need five to ten more.
>
> Sample 13 and 16. Because they are not producing bands consistently, the DNA may have to be cleaned and the samples reamplified using the primers found to work on samples 4 and 10. The newest extractions from 7/7/00 could also be tested for their ability to amplify.
>
> Samples 1 and 7. Because they are not producing bands, the DNA could be cleaned and the samples reamplified using the working primers from above. Also the newest extractions from 7/7/00 could be tested for their ability to amplify.

REFERENCES

Avise, J.C. 1994. Molecular Markers, Natural History and Evolution. Chapman & Hall, New York.

Herrmann, B. and S. Hummel. 1994. Ancient DNA. Springer-Verlag, New York.

Hipkins, V.H., K.A. Marshall, D.B. Neale, W.H. Rottmann, and S.H. Strauss. 1995.

A mutation hotspot in the chloroplast genome of a conifer (Douglas-fir: *Pseudotsuga*) is caused by variability in the number of direct repeats derived from a partially duplicated tRNA gene. Curr. Genet. 27:572-579.

Kramer, P.J. and T.T. Kozlowski. 1979. Physiology of Woody Plants. Academic Press, Inc., Orlando, Florida

National Research Council. 1996. The Evaluation of Forensic DNA Evidence. National Academy Press, Washington DC.

Sensabaugh, G. and D.H. Kaye. 1998. Non-human DNA evidence. Jurimetrics J. 38:1-16.

Genetic Profiling of Black Walnut

A sample of Black Walnut was submitted to the laboratory for genetic analysis. This sample was a milled piece of lumber probably about 20 years old (from the time of milling). The goal was to extract DNA from the wood sample and obtain a RAPD profile. The purpose of this forensic work was to use DNA technology to determine the number or identity of individuals used to make antique wood furniture or other wooden objects.

To obtain a genetic profile from an individual (whether a plant or a human), DNA must be extracted from the sample, and then analyzed to visualize a pattern of bands on a gel. All living organisms contain DNA (plant, animal, bacteria, etc.), and the DNA in each of these behaves the same way -- it has the same function and structure. The technologies and statistical principles used to determine an individuals DNA profile is also the same. In plants, DNA can be found at highest concentration in living tissues: leaves or needles, buds, seed, root tips. DNA also exists at much lower concentrations in woody tissues. In the stem, branches, and roots of woody plants, DNA is found in the cambial layer (between the xylem and phloem), and in certain cells within the wood (e.g. parenchyma cells). In most wood (xylem) cells, the DNA disintegrates in the final stages of cell maturation (via autolysis). The proportion of various cell types found in woody tissue, and therefore the amount of DNA available for extraction, is dependent on the species (among other variables).

We were able to extract DNA from the black walnut sample. However, the yield (quantity measured in nanograms (ng)) was low, and the quality was also apparently poor since we were unable to obtain RAPD bands. We extracted DNA (see table) using three different methods, and a modification of one method. Two of the three methods were able to extract DNA as indicated by fluorometry.

Extraction Date	Extraction Method	Amount of Wood Tissue Used	DNA Yield	DNA Concentra-tion
5/4/00	FastPrep	100 mg	0 ng	0 ng
6/7/00	DNeasy	100 mg	200 ng	2 ng/ul
6/13/00	CTAB	1,500 mg	600 ng	6 ng/ul
7/13/00	CTAB	1,500 mg	1,500 ng	15 ng/ul

We have performed numerous RAPD reactions on these black walnut DNA samples. None of the reactions have actually produced a band. This could be because the DNA is poor quality, there are contaminants in the sample, or one of several other reasons. Work was halted on this sample and may be continued in the future.

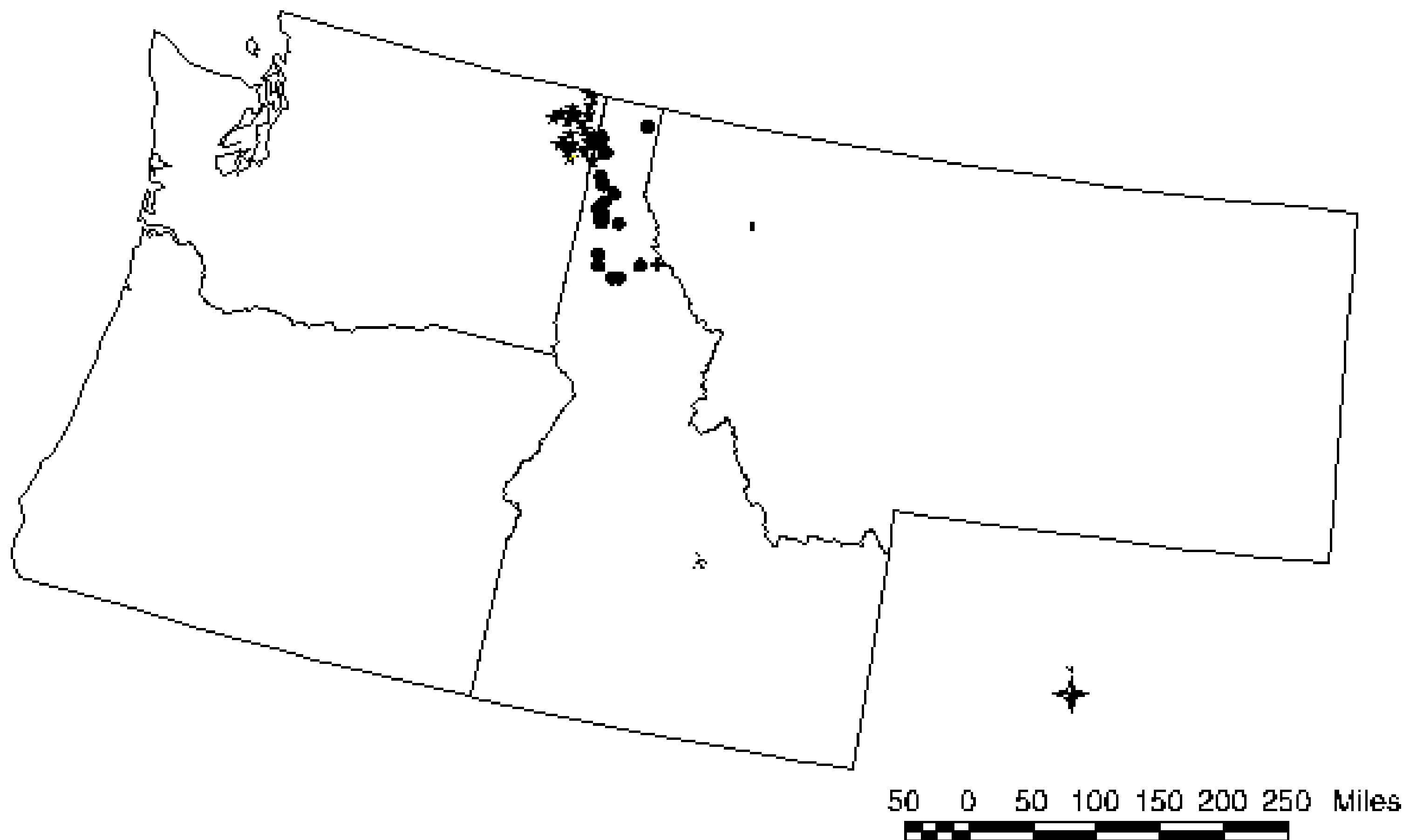
Table 1. Sources for Ponderosa Pine seed used in this study; Natural Stands.

NFGEL #	Source#	Source Name	State	Location	Latitude	Longitude	Habitat	Elev	Date
								feet	
3813	1	Avery SPA	ID	Idaho Panhandle NF; Avery RD	47.230	115.573		4300	
Natural stands:									
3877-3904	25	Avery Natural Stand	ID	Idaho Panhandle NF; Avery RD	47.23	115.57		3600	1999
3814	2	Bitterroot	MT	Bitterroot NF; Darby RD	47.9075	114.1676	312	5000	1985
3872	5	IPNF 7134	ID	Idaho Panhandle NF	47.21599	116.5563	520	2800	1997
3876	6	IPNF 7135	ID	Idaho Panhandle NF	47.57890	116.6204	520	3600	1997
3857	7	IPNF 7180	ID	Idaho Panhandle NF	47.99800	116.7306	310	2350	1993
3858	8	IPNF 7181	ID	Idaho Panhandle NF	48.08514	116.7952	320	2200	1993
3870	9	IPNF 7260	ID	Idaho Panhandle NF	48.5229	116.9248	530	2700	1997
3868	10	IPNF 7297	ID	Idaho Panhandle NF	47.60793	116.6418	506	2500	1995
3860	11	IPNF 7305	ID	Idaho Panhandle NF	47.72367	116.7307	506	3000	1994
3862	12	IPNF 7308	ID	Idaho Panhandle NF	48.50378	117.0540	506	3000	1995
3895	13	IPNF 7328	ID	Idaho Panhandle NF	47.08630	116.5120	524	4000	1994
3873	14	IPNF 7344	ID	Idaho Panhandle NF	48.36422	116.7947	260	2840	1997
3864	15	IPNF 7345	ID	Idaho Panhandle NF	48.45079	116.9248	260	2700	1995
3861	16	IPNF 7348	ID	Idaho Panhandle NF	47.92538	116.5152	261	4100	1994
3867	17	IPNF 7354	ID	Idaho Panhandle NF	47.66693	116.6217	260	2600	1995
3863	18	IPNF 7356	ID	Idaho Panhandle NF	48.36422	116.8164	506	2450	1995
3865	19	IPNF 7361	ID	Idaho Panhandle NF	47.80994	116.6445	506	2700	1995
3866	20	IPNF 7364	ID	Idaho Panhandle NF	48.75380	116.2089	260	3700	1995
3875	21	IPNF 7390	ID	Idaho Panhandle NF	47.60783	116.3425	506	3750	1997
3874	22	IPNF 7396	ID	Idaho Panhandle NF	46.98505	116.2381	---	3200	1997
3871	23	IPNF 7397	ID	Idaho Panhandle NF	46.99951	116.1327	530	3860	1997
3869	24	IPNF 7405	ID	Idaho Panhandle NF	47.18755	115.8377	520	4300	1997
Non-local sources:									
3816-3822	3	Black Hills	SD	Black Hills NF				4800-6300	1989 - 1998
3842 - 3851	4	Colville 1	WA	Colville NF; Colville RD	48.243 – 48.779	117.5517 - 117.8114	PSME/PHMA, PSME/SYAL, PIPO-PSME/AGSP, THPL/VAME	3450-4200	1995 - 1994
3823 3831	32	Colville 2	WA	Colville NF; Sullivan Lake RD	48.576 – 48.982	117.2201 - 117.4854	TSHE/PAMY, ABGR/PHMA	3205-4300	1985-1988
3832 - 3841	26	Colville 3	WA	Colville NF; Newport RD	48.228 – 48.503	117.1406 - 117.4868	THPL/CLUN, PSME/PHMA, ABGR/PHMA, ABGR/CLUN, TSHE/CLUN	3020-4200	1985-1994
3815	33	Wenatchee	WA	Wenatchee NF; Natches RD				< 4000	
Single-family tree improvement lots:									
3856	27	TI-1 (lot 666)	ID	St. Mary; 09N 21W 36	44.0653	113.9209		5200	1988
3853	28	TI-2 (lot 659)	ID	Silverthorn; 09N 21W 25	44.0798	113.9209		4700	1988

3852	29	TI-3 (lot 660)	ID	Silverthorn; 09N 21W 25	44.0798	113.9209		4700	1988
3854	30	T-I4 (lot 662)	ID	Smith Creek; 08N 21W 22	44.0084	113.9514		5000	1988
3855	31	TI-5 (lot 664)	ID	Smith Creek; 08N 21W 21	44.0084	113.9716		5300	1988

Figure 1.

Ponderosa Pine collection localities



Collection sites:

- + Avery SPA and Avery Natural Stand
- • Idaho Panhandle National Forest
- * Colville National Forest
- Tree Improvment Lots
- Bitterroot National Forest

Table 2. Allele frequencies in Ponderosa Pine sources, natural stands, and single-family tree improvement (TI) lots are available upon request.

Table 3. Summary of genetic diversity measures for populations of Ponderosa Pine. 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. Ae = Effective number of alleles (Kimura & Crow 1964). Ap = the average alleles per polymorphic locus. h = gene diversity (Nei 1973). * = 30 diploid samples, converted to haploid for analysis.

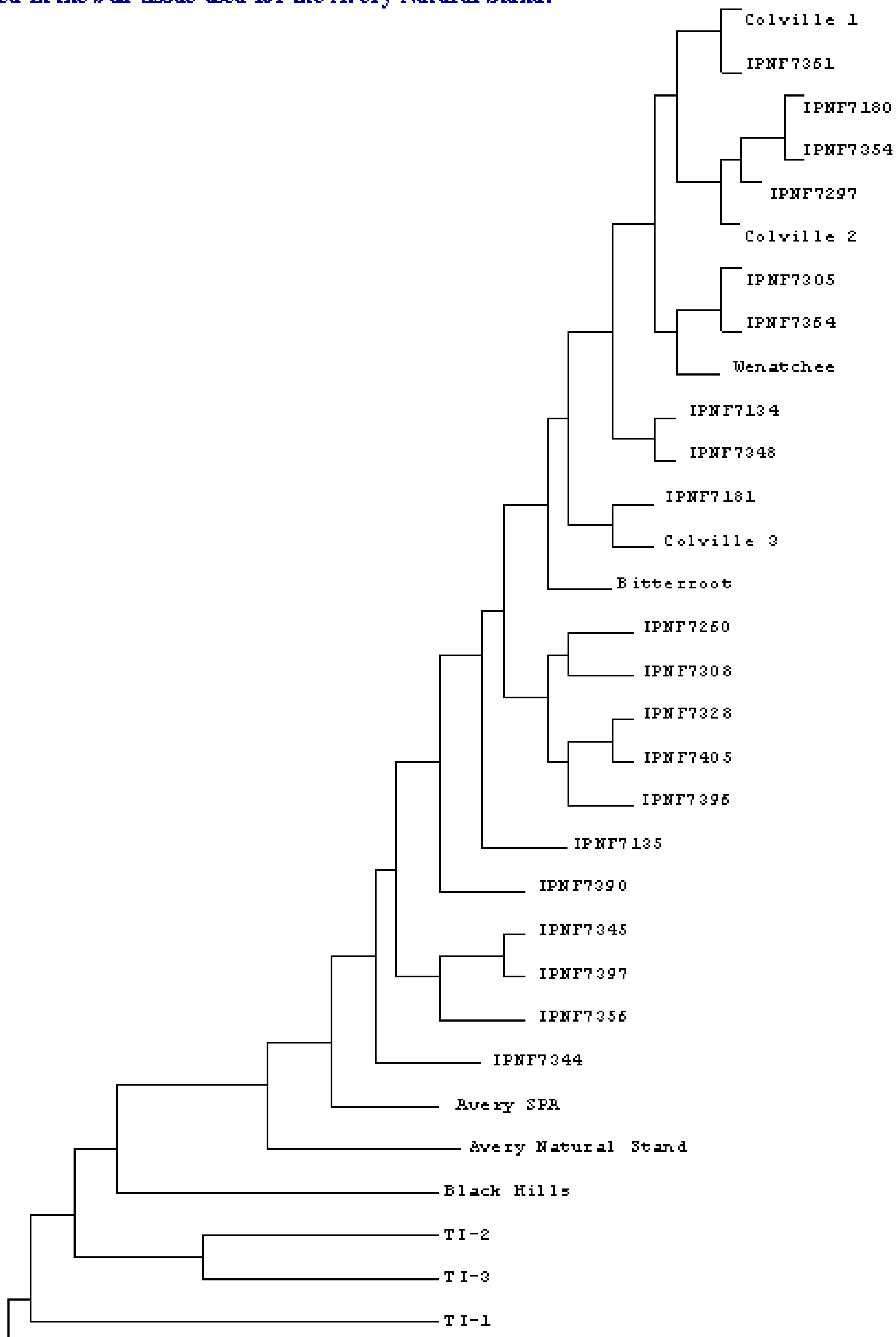
Population	N	%P	A	Ae	Ap	h
Avery SPA	40	62%	1.95	1.34	2.54	0.1884
Natural stands:						
Avery Natural Stand	60*	67%	2.10	1.41	2.58	0.2255
Bitterroot	40	66%	2.33	1.49	2.75	0.2303
IPNF 7134	39	62%	2.05	1.38	2.69	0.2078
IPNF 7135	40	57%	1.86	1.28	2.50	0.1654
IPNF 7180	20	62%	2.10	1.40	2.77	0.2153
IPNF 7181	48	67%	2.33	1.42	3.00	0.2231
IPNF 7260	32	62%	1.86	1.38	2.38	0.2120
IPNF 7297	40	71%	2.05	1.33	2.47	0.2322
IPNF 7305	39	77%	2.43	1.46	2.88	0.2322
IPNF 7308	39	71%	2.00	1.35	2.40	0.2022
IPNF 7328	32	62%	2.10	1.36	2.77	0.1969
IPNF 7344	39	67%	2.14	1.32	2.71	0.1722
IPNF 7345	40	71%	2.00	1.30	2.40	0.1716
IPNF 7348	39	62%	2.14	1.46	2.85	0.2458
IPNF 7354	40	72%	2.29	1.36	2.80	0.1980
IPNF 7356	40	57%	1.95	1.43	2.67	0.2184
IPNF 7361	44	76%	2.10	1.40	2.44	0.2109
IPNF 7364	40	76%	2.33	1.42	2.75	0.2313
IPNF 7390	40	62%	1.95	1.40	2.54	0.2177
IPNF 7396	39	67%	1.90	1.31	2.36	0.1783
IPNF 7397	40	47%	1.67	1.23	2.40	0.1396
IPNF 7405	42	62%	1.76	1.27	2.23	0.1623
Non-local sources:						
Black Hills	42	76%	2.71	1.35	3.25	0.1945
Colville 1	43	62%	2.00	1.35	2.62	0.2002
Colville 2	45	71%	2.10	1.36	2.53	0.1970
Colville 3	41	67%	2.14	1.31	2.71	0.1847
Wenatchee	40	81%	2.33	1.42	2.64	0.2383
Single-family tree improvement lots:						
TI-1 (666)	9	43%	1.48	1.32	2.11	0.1798
TI-2 (659)	30	38%	1.38	1.19	2.00	0.1125
TI-3 (660)	38	29%	1.43	1.10	2.50	0.0631
TI-4 (662)	40	43%	1.57	1.25	2.33	0.1368

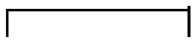
TI-5 (664)	23	43%	1.62	1.36	2.44	0.1752
Mean, Natural stands (standard deviation)	39 (5.39)	66% (7.3%)	2.07 (0.20)	1.37 (0.07)	2.61 (0.21)	0.2040 (0.0282)
Mean, Seed sources (standard deviation)	42 (1.92)	71% (7.4%)	2.26 (0.28)	1.36 (0.04)	2.75 (0.29)	0.2029 (0.0206)
Mean, Tree improvement (standard deviation)	28 (12.6)	39% (6.1%)	1.50 (0.10)	1.24 (0.10)	2.28 (0.21)	0.135 (0.0482)

Table 4. Summary of genetic diversity measures for groups of Ponderosa Pine populations. 21 loci. (ADH, CAT1, CAT2, IDH2, and ME removed because they did not resolve in the Avery Natural Stand.) All = overall statistics for the study. N = number of individuals sampled per locus, per population. %P = percent of all loci that are polymorphic. A = average number of alleles per locus. Ae = Effective number of alleles (Kimura & Crow 1964). Ap = the average number of alleles per polymorphic locus. Ht = gene diversity in the whole population. Hs = gene diversity in the subpopulations. Gst = proportion of variation found among populations within the total variation of that taxon. Nm = calculated gene flow among populations within that group (Nm = ½(1-Gst)/Gst; McDermott and McDonald 1993). IPNF = Idaho Panhandle National Forest. TI = Tree improvement lot.

	N	%P	A	Ae	Ap	Ht	Hs	Gst	Nm
ALL	1260	100%	4.19	1.40	4.19	0.2239	0.1915	0.1445	2.9562
Colville	129	76%	2.48	1.35	2.94	0.2015	0.1940	0.0376	12.8144
IPNF #'s	770	90%	3.48	1.38	3.74	0.2161	0.1997	0.0762	6.0609
TI's	140	81%	2.38	1.30	2.71	0.2150	0.1335	0.3791	0.8188

Figure 2. Similarity of Ponderosa Pine popualtions, based on genetic distances. Based on only those isozyme loci that resolved in the bud tissue used for the Avery Natural Stand.





TI-4

TI-5

Genetic distance 0.85 0.90 0.95 1.0

Table 5. Genetic similarities among Ponderosa Pine populations. Values involving the Colville, Idaho Panhandle National Forest (IPNF), and Tree Improvement (TI) populations are averages.

	Avery SPA	Avery Natural	Bitter-root	Colville	IPNF's	Wenatchee	Black Hills	TI's
Avery SPA	---------							
Avery Natural Stand	0.9643	---------						
Bitterroot	0.9764	0.9746	---------					
Colville	0.9797	0.9637	0.9815	0.9887				
IPNF's	0.9746	0.9622	0.9814	0.9861	0.9818			
Wenatchee	0.9738	0.9737	0.9878	0.9866	0.9832	---------		
Black Hills	0.9636	0.9104	0.9296	0.9438	0.9352	0.9207	---------	
TI's	0.9301	0.9315	0.9372	0.9268	0.9258	0.9318	0.8787	0.8781

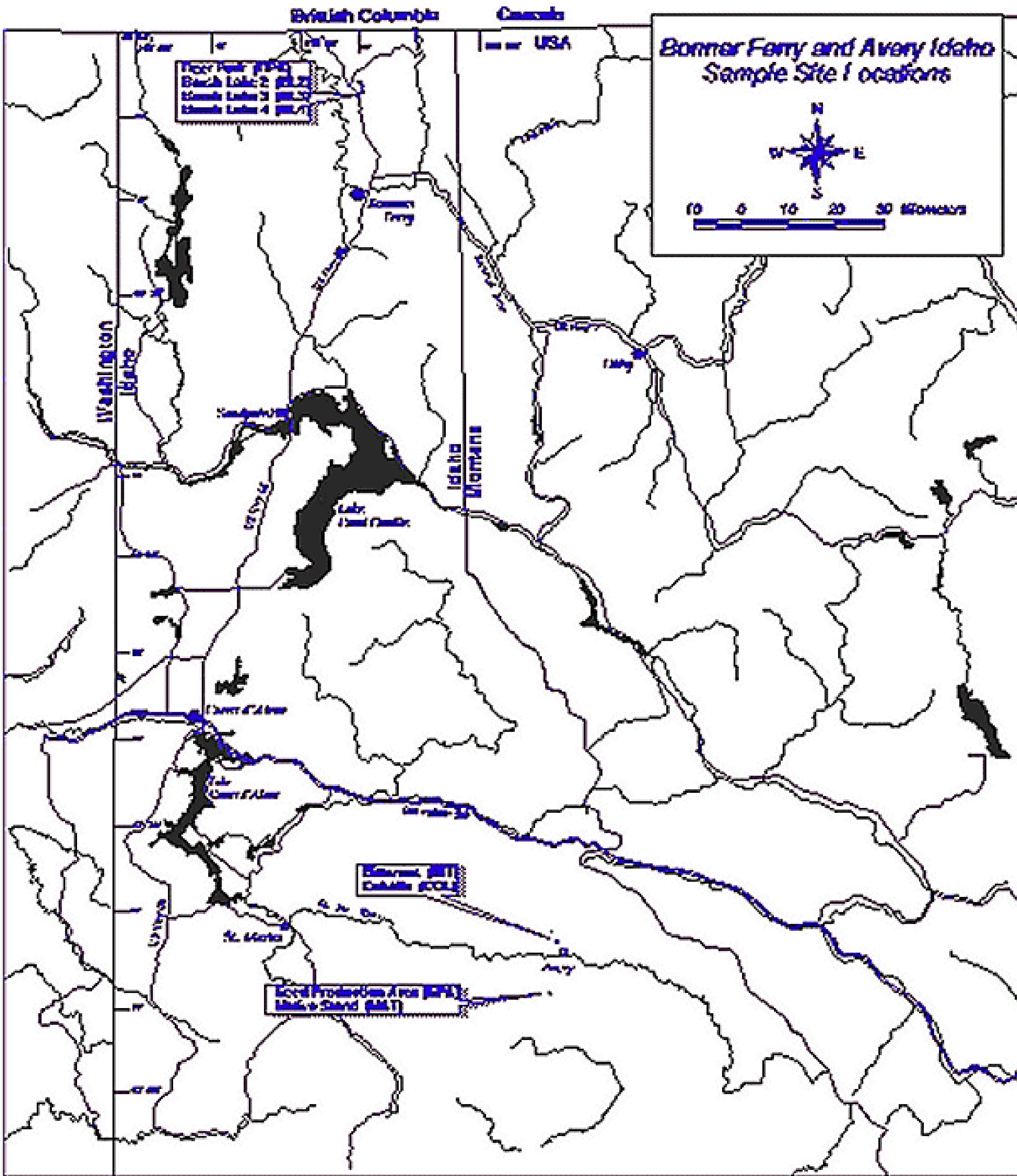

British Columbia
Canada
USA
Bonner Ferry and Avery Idaho
Sample Site Locations
N
S
E
Washington
Idaho
Idaho
Montana

Table 1. P values for exact G-tests for the null hypothesis that there is no haplotypic differentiation .

	SPA	MLO	BIT	COL	DPK	BL2	BL3
MLO	.589						
BIT	.775	.296					
COL	.188	.168	.058				
DPK	1.00	.353	.286	.212			
BL2	.613	.092	.057	.198	.831		
BL3	.750	.833	1.00	.360	.373	.092	
BL4	.258	.383	.279	.065	.075	.012	.414

STAFF ACTIVITIES

Meetings, Shortcourses, and Workshops

Presentations

- 1999. V. Hipkins. NFGEL's partnership with FS Research. USDA Forest Service, Research Genetics Review, Placerville, CA, October 19-20.
- 2000. V Hipkins. Genetic resource management in the United States Forest Service. Faculty of Arts and Sciences, Akdeniz University, Antalya Turkey, January 12.
- 2000. V. Hipkins. Genetic markers in Port-Orford Cedar and Alaska Yellow Cedar. Port-Orford Cedar Working Group. Redding, CA, May 23.
- 2000. V. Hipkins. TES Plant Studies in the Pacific Northwest. USDA Forest Service, National Forest System Forest Health and Genetics Review. Portland, OR, September 25.

Posters

- 2000. Vicky Erickson, Diane Shirley, B. Wilson, and V. Hipkins. Population genetics of isolated stands in northwest Oregon: implications for species conservation and management. Sustaining Aspen in Western Landscapes, Grand Junction, Colorado, June 13-15.
- 2000. R.C. Saich and V. Hipkins. The use of genetic information in monitoring rare and endangered plant populations. Rare and Endangered Plant Conference, Flagstaff AZ, September 25-29.

Shortcourses Presented

- 2000. V. Hipkins. Molecular and Biochemical Analysis in Genetic Resource Management. Faculty of Arts and Sciences, Akdeniz University, Antalya Turkey, January 10-21.

Attended

- 1999. Combating Tree Theft Using DNA Technology. Canadian Forest Service, Pacific Forestry Centre, Victoria, BC Canada, October 5-6. (V.Hipkins).
- 2000. Molecular Genetics in Fisheries, A Course for Geneticists. Joint University of Idaho and US Forest Service, Portland, OR, June 6.

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).
- Participated in the staffing of the US Forest Service Booth at the CA State Fair (R. Meyer).

Professional Activities

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

- Adjunct faculty member at Northern Arizona University, School of Forestry, Flagstaff, AZ (V.Hipkins).
- Western Forest Genetics Association Officer – Vice Chair (V.Hipkins).
- Wilson, BL, DL Doede, and VD Hipkins. 2000. Isozyme variation in *Sisyrinchium sarmentosum* (Iridaceae). Northwest Science 74(4):000-000 (in press).
- Schmidtling, R.C., V. Hipkins and E. Carroll. 2000. Pleistocene refugia for longleaf and loblolly pines. Journal of Sustainable Forestry 10(3/4):349-354.

Hosted

NFGEL continues to host a variety of visitors. Tours of the facility and operation were provided to (1) Forest Service employees representing three units of the Research branch, the Washington Office, and three Regions of the National Forest System, (2) members of the public, (3) private industry, (4) university faculty, and (5) employees from other state and federal government agencies. NFGEL hosted groups California State University San Luis Obispo and the USFS Genetics Review Team. The lab hosted a Northern Arizona University graduate student and a private industry employee. Our guests also included international visitors from Korea and Turkey.

Collaborations and Cooperations

NFGEL formed collaborations with FS Research Stations, Northern Arizona University (Flagstaff), California Department of Forestry and Fire Protection, Bureau of Land Management, US Fish and Wildlife Service, and private companies. 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 participating in teaching and extension workshops, both nationally and internationally.

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 FY00, 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 (10/99)	
Robert Saich	Lab Biotechnician	Temp (6/00– 9/00)	rcsaich@fs.fed.us
Barbara Wilson	Associate Director	Temp (1/00–7/00)	blwilson@fs.fed.us

NFGEL Projects (2000)

Project#	Collaborator	Species	Objective	Sample Type	Sample Size	Submission Dates	Preparation Dates	Electrophoresis Dates	Marker System	#Loci
79	Joanna Clines	*Collomia rawsonia* (Rawson's flaming trumpet)	(1) Distinguish genets from ramets (2) Characterize the degree of variability within and among populations	leaf	252 indiv.	8/10/99 -8/24/99	8/12/99 -8/27/99	11/2/99 -11/16/99	Isozymes (starch gel)	25
87	Rod Stevens	*Chamaecyparis lawsoniana* (Port-Orford Cedar), *Cupressus macrocarpa* (Monterey cypress), *C. nootkantensis* (Alaska Yellow Cedar)	Species identification	needles	53 indiv.	10/14/99	11/24/99	11/30/99, 12/2/99	Isozymes (starch gel)	19
90	Ron Schmidtling	*Pinus caribaea, P. palustris, P. taeda*, & hybrids	To investigate the natural genetic diversity in some Southern pines	bud	95 indiv.	12/1/99	12/3/99	12/7/99 -12/16/99	Isozymes (starch gel)	24
72	Dave Doede	*Lupinus latifolius*	Estimate levels of diversity within and among populations	seed	338 indiv.	3/16/99	4/8/98 -5/1/98	2/19/98 -3/9/00	Isozymes (starch gel)	25
94	Nick Wheeler	*Pseudotsuga menziesii* (Douglas-fir)	Assess pollen contamination	seed	8 indiv.	3/3/00	3/10/00 -3/15/00	3/15/00 -3/16/00	Isozymes (starch gel)	5
96	Bryan Schulz	*Pseudotsuga menziesii* (Douglas-fir)	Clonal identification	bud	10 indiv.	3/14/00	3/15/00/	3/16/00	Isozymes (starch gel)	18
93	Ron Schmidtling	*Pinus caribea, P. elliottii*, & hybrids	To investigate the natural genetic diversity in some Southern pines	bud	159 indiv.	2/24/00 -2/28/00	2/25/00 -3/3/00	3/14/00 -3/24/00	Isozymes (starch gel)	24
97	(Part 1&4) Richard Sniezko, (Parts 2&3) Rod Stevens	*Chamaecyparis lawsoniana* (Port-Orford Cedar), *C. obtusa*, *Cupressus macrocarpa* (Monterey cypress), *C. tortulsa, C. leylandi, C. nootkantensis*, and *C. Thyoides*, plus hybrids	(1)Verify ramets of each genotype are identical but that geneotype are unique (2)Ramet/hybrid identification (3&4) Hybrid identification	needle	(1)21indiv (2)4 indiv (3)27 indiv (4)18 indiv	(1) 3/15/00 (2) 3/30/00 (3) 5/10/00 (4) 5/11/00	(1) 3/20/00 (2) 3/30/00 (3&4) 5/12/00	4/4/2000 6/14/00 and 6/15/00	Isozymes (starch gel)	17
55	Jody Sawasaki	*Rorippa subumbellata* (Tahoe yellow cress)	Estimate levels of diversity within and among populations	leaf	140 indiv.	8/15/99 -9/1/99	8/11/99 -9/3/99	8/17/99 -4/26/00	Isozymes (starch gel)	23
89	Kathleen Nelson	*Elymus elymoides, Achnatherum occidentals, A .speciposum, A. thurberianum, A. pinetorum, and A .hymenoides*	Part of a project to establish native grass seed zones and a seed bank	seedlings	150 indiv.	11/19/99	3/3/00 -4/28/00	5/2/00 -5/19/00	Isozymes (starch gel)	23
84	Vicky Erickson	*Populus tremuloides* (Quaking aspen)	To investigate the patterns and levels of genetic diversity within and among populations in Oregon	leaf	270 indiv.	7/29/99 -9/15/99	8/3/99 -9/20/99	6/22/00 -7/25/00	Isozymes (starch gel)	19
78	Paul Berrang	*Perideridia eythrorhiza and P. oregana*	Characterize genetic diversity and make taxonomic determinations	seedlings	388 indiv.	8/28/98 -6/19/00	9/2/98 -6/20/00	8/8/00 -9/6/00	Isozymes (starch gel)	16

91	Zhong Chen	*Pseudotsuga menziesii* (Douglas-fir)	Geneotype trees that are susceptible or resistant to western spruce budworm	seed	36 indiv	12/9/99	12/15/99 -12/27/99	1/4/00 -1/7/00	Isozymes (starch gel)	25
88	Rod Stevens	*Chamaecyparis lawsoniana* (Port-Orford Cedar), *Cupressus macrocarpa* (Monterey cypress), *C. nootkantensis* (Alaska Yellow Cedar)	Species identification	needles	53 indiv.	10/14/99	12/13/99	12/14 - 12/21/99	Isozymes (IEF)	7
85	Dan Nichols	Eucalyptus	Forensics	leaves, wood	169 indiv.	9/15/99, 1/28, 2/24, 3/2/00	10/6/99 -3/4/00	--	DNA	--
86	Rod Stevens, Rich Sneizko	*Chamaecyparis lawsoniana* (Port-Orford Cedar), *Cupressus macrocarpa* (Monterey cypress), *C. nootkantensis* (Alaska Yellow Cedar)	Hybrid and species identification	leaves	53 indiv.	10/14/99	10/26/99 -6/13/00	5/18 - 12/11/00	DNA	--
95	Ron Pugh	*Pinus ponderosa* (ponderosa pine)	Forensics	wood	6 indiv.	3/9/00	3/10/00 -7/7/00	3/27 - 6/21/00	DNA	--
devel- oping	Joe Godla	Black Walnut	Forensics	wood	1 indiv.	5/1/00	5/4/00 -7/7/00	5/8 - 6/21/00	DNA	--

Workload by Region or Agency, FY00

(1) Isozymes (starch gel electrophoresis)

By Project

Region or Agency	Project #	Species	# gels	# days	# weeks
Weyerhaeuser	94	Douglas-fir	8	2	1.5
Pope Industries	96	Douglas-fir	6	1	1.0
BLM/FS-NFS-R6	97	Port Orford Cedar	13	1	2.0
	87	Port Orford Cedar	8	2	1.0
USFWS	55	Rorippa	19	3	3.0
N. Arizona University	91	Douglas-fir	18	4	1.0
FS-NFS-R5	79	*Collomia*	36	8	4.0
	89	Inyo grass	21	4	2.0
-R6	72	Lupine	55	16	10.
	84	Aspen	36	7	4.0
	78	*Perideridia*	57	12	6.0
FSR-SRS	90	Southern Pines	14	3	2.0
	93	Southern Pines	7	2	2.5
	92	Southern Pines	11	2	1.0
NFGEL	......	testing	32	7	3.0

By Agency

Region or Agency	# gels	# days	# weeks
Forest Service			
NFS			
R5	57	12	6.0
R6	148	35	20
Research			
SRS	32	7	5.5
Weyerhaeuser	8	2	1.5
Pope Industries	6	1	1.0
BLM/R6	21	3	3.0
USFWS	19	3	3.0
Northern Arizona University	18	4	1.0
NFGEL	32	7	3.0
TOTAL	341	74	44

(2) Isozymes (isoelectric focusing)

Region or Agency		# gels	# days	# weeks
BLM	Alaskan Yellow & Port Orford Cedars	9	4	2

(3) DNA (RAPDs)

Region or Agency	species	# extractions	# PCR reactions	# days	# weeks
CDF	Eucalyptus (PJ 85)	339	249	46	52
FS-NFS-R6/BLM	POC/AYC (PJ 86,97)	68	452	65	46
FS-NFS-R6	Ponderosa Pine (PJ 95)	30	136	30	20
Getty Museum	Black Walnut (devel)	8	22	12	8

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

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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