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Annual Report 2000-2001(FY01)

National Forest Genetic Electrophoresis Laboratory

USDA Forest Service -NFGEL, 2480 Carson Road, Placerville, CA 95667
530-622-1609 (office), 530-622-1225 (lab)
530-622-2633 (fax)

FY98

FY99

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INTRODUCTION

After residing for 13 years on the Eldorado National Forest, NFGEL has relocated to the Pacific Southwest Research Station, Institute of Forest Genetics, Placerville CA. Many thanks go to the NFGEL staff for their dedicated efforts in making this move happen. A move of this magnitude required both physical and mental energy - both of which they showed in full measure. It took only two weeks in our new home to be fully up-and-running, producing results of the high quality that are standard to our procedures. Thanks also go to the IFG scientists and staff who have been enormously welcoming and accommodating. It is also through their efforts that our transition has been so seamless. Everyone who has been involved in this move, at the local, Regional, Station, and Washington Office levels, deserves our sincere thanks.

NFGEL remains a National Forest System facility. With this move, we plan on strengthening our partnerships with the Forest Service Research branch, and improving our role of 'linking science to management'. We were able to add some new equipment this fiscal year that will enable us to extend the services we provide. Our most promising purchase was of an ABI-3100. We anticipate generating highly variable DNA markers and sequence data that can be used in the assessment of many of the plant species we study.

As can be seen in this report, the scope of our work continues to grow. At the end of this report period, we had accepted our 125th project. We look forward to continuing our work in support of genetic improvement and conservation efforts in our new home at the Institute of Forest Genetics.

Valerie Hipkins
NFGEL Director
October 2001

Overview

During this FY01 report year, we processed 6 projects utilizing isozyme markers obtained by starch gel electrophoresis, performed development work on 8 other isozyme projects, and initiated work on 11 additional projects using DNA markers. NFGEL projects were processed to meet a variety of management objectives. Nine reports, including results from 12 projects, follow.

Silviculture and Tree Improvement

1. Ramet/Genet Identification in Port-Orford Cedar (*Chamaecyparis lawsoniana*)
2. Genetic Variation and Hybridization in *Cupressus*, *Chamaecyparis*, and X *Cupressocyparis*
3. Efficacy Of Supplemental Mass Pollination Methods In A Douglas-Fir (*Pseudotsuga menziesii*) Seed Orchard
4. Effects Of Stand Density Reduction Treatments On The Genetic Variation Of Ponderosa Pine (*Pinus Ponderosa*) In Northern Arizona
5. Clonal Identification in Douglas-fir (*Pseudotsuga menziesii*)
6. Cross Verification Among *Chamaecyparis* Species

Conservation and Restoration

1. Genetic Diversity In *Perideridia erythrorhiza*: A Rare Plant In Southern Oregon
2. Genetic Differentiation In A Rare Plant With A Disjunct Range: *Lewisia kelloggii*
3. Genetic Diversity In Broadleaf Lupine (*Lupinus latifolius*) Accessions From The Mt. Hood National Forest

Ramet/Genet Identification in Port-Orford Cedar (*Chamaecyparis lawsoniana*)

Objective

The project objective was to verify that ramets of each clone are identical, but that clones are unique.

Materials

Twenty individuals of Port-Orford Cedar (Chamaecyparis lawsoniana) (POC), putatively labeled as eight different clones (one to six ramets per clone) were submitted for analysis.

Analysis

Individuals were prepared for both isozyme (starch gel electrophoresis) and RAPD (random amplified polymorphic DNA) analysis. Sample preparation for starch gel electrophoresis followed NFGEL Standard Operating Procedures. Genomic DNA was extracted by a modified Jorgenson CTAB protocol and yielded between 0.5 - 3ug of DNA per sample.

Starch Gel Electrophoresis. Individuals were genotyped at 22 isozyme loci (FEST-1, PGM-1, ME7, PGI-1, PGI-2, UGPP-1, TPI-1, G6PD, GOT-1, GOT-2, GDH, MDH-1, MDH-2, 6PGD-1, 6PGD-2, IDH, SKD-2, FDP-1, SOD-1, SOD-2, ACP, MNR). The multilocus genotype data show that all ramets of each genotype are identical. However, isozyme data did not distinguish all genotypes. Clone 510015 and clone CF01 share the same multilocus genotype. Clones CF02, CF03, and CF06 share an identical isozyme genotype. The remainder of the clones have unique genotypes. Overall, genetic variation among genotypes is low with 82.6% of the loci monomorphic. The loci that distinguish clones are FEST1, PGI2, TPI1 and GDH.

RAPD Analysis. In order to further distinguish groups 510015/CF01 and CF02/CF03/CF06, one individual per putative clone was characterized using RAPD markers. One individual per clone was used in the analysis based on the assumption that all ramets of each genotype are identical (as indicated by the isozyme analysis). Therefore, RAPD markers were generated for five individuals (510015, and one individual from each of CF01, CF02, CF03, and CF06) using 45 RAPD primers. Reaction samples contained 1.25mM dNTPs, 0.4uM primer, 1U Taq, and 3 ng DNA in a 25ul volume. Thermal steps consisted of forty cycles of one minute denaturation at 94o, one minute annealing at 40 o, and 2 minute extension at 72o. Entire reaction volumes were loaded onto 1.4% agarose gels in 1X TBE. Data was scored with arbitrary numbers representing the overall band pattern.

Generally, the RAPD patterns generated for 510015 and CF01 were similar to each other. Likewise, the pattern generated for CF02, CF03, and CF06 were similar to each other. The 510015/CF01 group varied distinctly from the CF02/CF03/CF06 group (Figure 1).

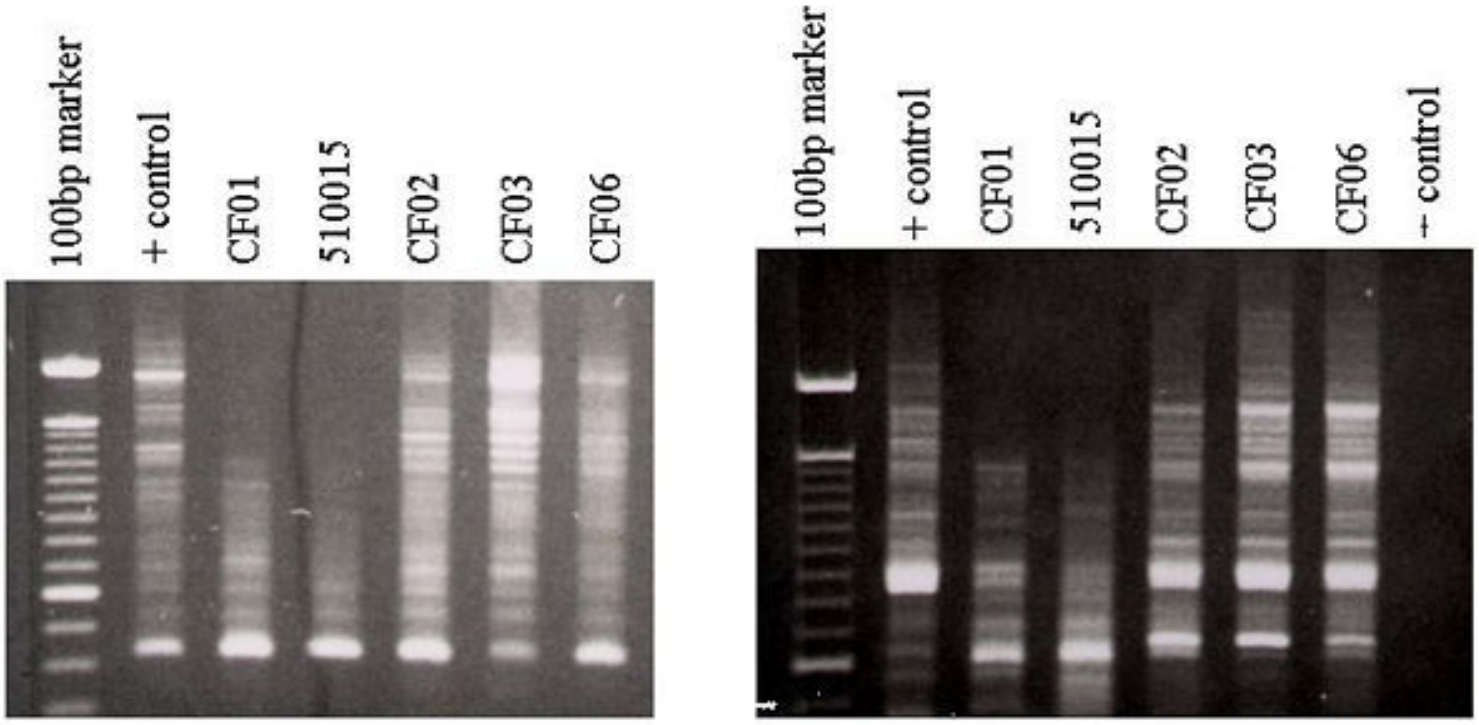


Figure 1. RAPD markers using primer OPK-06 on the left, and primer OPE-01 on the right

Although there were similarities within the groups, clones could be distinguished as unique. RAPD markers clearly distinguished clone 510015 from clone CF01 at 26 primers (see Figure 2 for example). RAPD markers distinguished clone CF03 from CF02/CF06 at 16 primers (see Figure 3 for example). Clones CF02 and CF06 were not easily distinguished and clearly differed at only one of the 45 primers tested (Figure 3). The bands obtained with the OPB-03 primer were reproducible. Four other primers (OPA-19, OPB-02, OPB-18, and OPH-11), also showed differences between CF02 and CF06, but these differences were not clear.

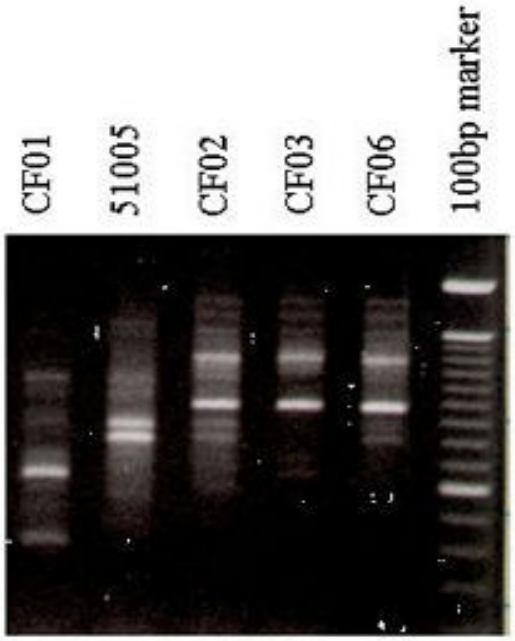


Figure 2. RAPD markers using primer OPE-12

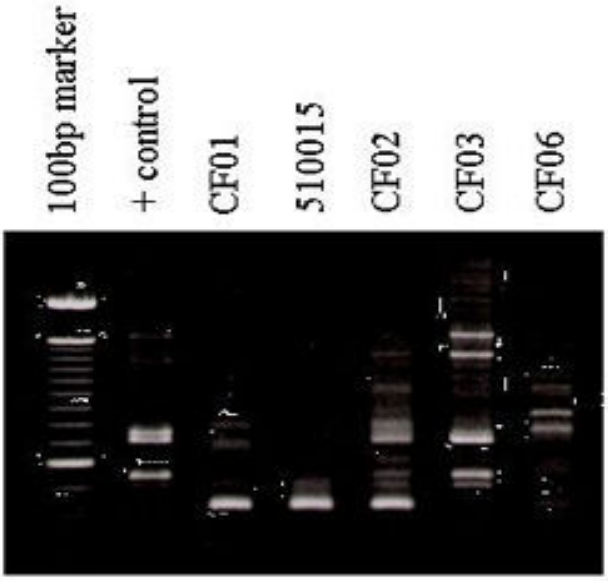


Figure 3. RAPD markers using primer OPB-03

Conclusion. All ramets of each submitted clone are identical. Clones 117490, 510015, CF01, CF03, CF08, and CF27 are unique. Although clones CF02 and CF06 could only clearly be distinguished with one RAPD primer, we conclude that they are also distinct clones.

This was part of NFGEL Project #97

Genetic Variation and Hybridization in *Cupressus*, *Chamaecyparis*, and X *Cupressocyparis*

INTRODUCTION

We used a variety of molecular techniques to test the hypothesis that several submitted individuals were hybrids between either:

1. *Cupressus macrocarpa* (Monterey cypress) X *Chamaecyparis lawsoniana* (Port-Orford cedar) (POC),
2. *Chamaecyparis lawsoniana* X *Chamaecyparis nootkatensis*(Alaska yellow cedar)(AYC), or
3. *Cupressus macrocarpa* X *Chamaecyparis nootkatensis*.

Levels of genetic variation within and among species was also assessed.

Molecular genetic data have been successfully used to address the question of interspecific hybridization in other species. As long as the parent species are sufficiently divergent genetically at the time of the hybridization event, the hybrid should show additivity of the genetic markers to the parent species. Unique bands can also be expected to be rare in the hybrid.

METHODS AND MATERIALS

A total of 38 individuals were used to assess intraspecific variation within POC. Collections were submitted by Rod Stevens, BLM (17 samples), and Rich Sniezko/Leslie Elliot, Dorena Genetic Resource Center, USDA Forest Service (21 samples). Dorena submissions included rangewide samples.

Twelve individuals, ten submitted by BLM and two submitted by Dorena, were used to assess variation in AYC. Thirty-five putative AYC X POC hybrids were included for testing (from BLM). A total of seven individuals of Monterey cypress were submitted from the BLM.

Also included for testing were four individuals of *Chamaecyparis obtusa* (two from BLM, two from Dorena), one individual of *Chamaecyparis thyoides* from Dorena, two individuals of Cupressus torulosa (from BLM), and five individuals of Leyland cypress (*Cupressus macrocarpa X Chamaecyparis nootkatensis*) (from BLM). Multiple collections of a putative Monterey cypress X POC hybrid were submitted.

Starch Gel Electrophoresis

A small section of needle tissue (~3 mm3) per tree was placed in a microtiter plate well containing 150ul of 'Melody/Neale' extraction buffer. The plate was frozen at -70C. On the morning of the electrophoretic run, the samples were thawed, macerated with a dremel tool, and the extract absorbed onto three, 3mm wicks. Sample wicks were loaded into 11% starch gels that accommodated 30 samples along the longitudinal axis.

The following enzymes were examined: fluorescent esterase (FEST), phosphoglucomutase (PGM),

phosphoglucose isomerase (PGI), malic enzyme (ME), phosphogluconate dehydrogenase (6PGD), triosephosphate isomerase (TPI), uridine diphosphoglucose pyrophosphorylase (UGPP), glucose-6-phosphate dehydrogenase (G6PDH), glutamate-oxaloacetate transaminase (GOT), glucose dehydrogenase (GDH), fructose-1,6-diphosphate (FDP), shikimic acid dehydrogenase (SKD), malate dehydrogenase (MDH), and isocitrate dehydrogenase (IDH). A lithium borate electrode buffer (pH 8.3) was used with a Tris citrate gel buffer (pH 8.3) to resolve PGM-1, PGI-1,2, ME7, and FEST-1. A sodium borate electrode buffer (pH 8.0) was used with a Tris citrate gel buffer (pH 8.8) to resolve TPI-1, UGPP-1, G6PD, GOT-1,2, and GDH. A morpholine citrate electrode and gel buffer (pH 8.0) was used to resolve MDH-1,2, 6PGD-1,2, SKD-2, FDP-1, and IDH. 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.

Isoelectric Focusing

Three hundred milligrams of needle tissue per tree was ground in a cold mortar containing 2ml of a glycine extraction buffer. Extracted samples, including liquid and solid materials, were stored in 5ml cryovials at -20C until electrophoresis.

On the day of electrophoresis, samples were thawed and loaded onto polyacrylamide gels, type FS5080 (pH 4-5). Gels were stained for EST, PER, PHI, MDH, DIA, ACP, and PGM.

Random Amplified Polymorphic DNA (RAPD)

DNA extractions were carried out using a modified FastPrep protocol (Bio101 Inc.). DNA was quantified 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).

PCR reaction mixtures (25ul) contained 0.5 uM of a 10-base primer (Operon Technologies Inc.), 3 ng of genomic DNA, and 1 unit *Taq* DNA polymerase. A DNA thermal cycler (MJ Research) was programmed for 2 min at 94C followed by 40 cycles of 1 min at 94C, 1 min at 40C, and 2 min at 72C to carry out the amplification reactions. A final step of 10 min at 72C was added at the end of the cycle. RAPD products were analyzed by electrophoresis on 1.4% agarose gels in 1X TBE and stained in ethidium bromide. Gels were photographed under transmitted UV light using a Polaroid camera. Scoring was performed directly from the photographs.

RESULTS AND DISCUSSION

VARIATION WITHIN SPECIES

The genetic variation found in POC (as measured by starch gel electrophoresis) is lower than the average gymnosperm, and only slightly lower than that of the average long-lived woody species with a regional distribution (see Table). Alaska yellow cedar contained greater levels of diversity than Port-orford cedar. The genetic variation detected in Monterey cypress is very low compared to the average gymnosperm. Other species analyzed had such small sample sizes that within species diversity level statistics are not meaningful.

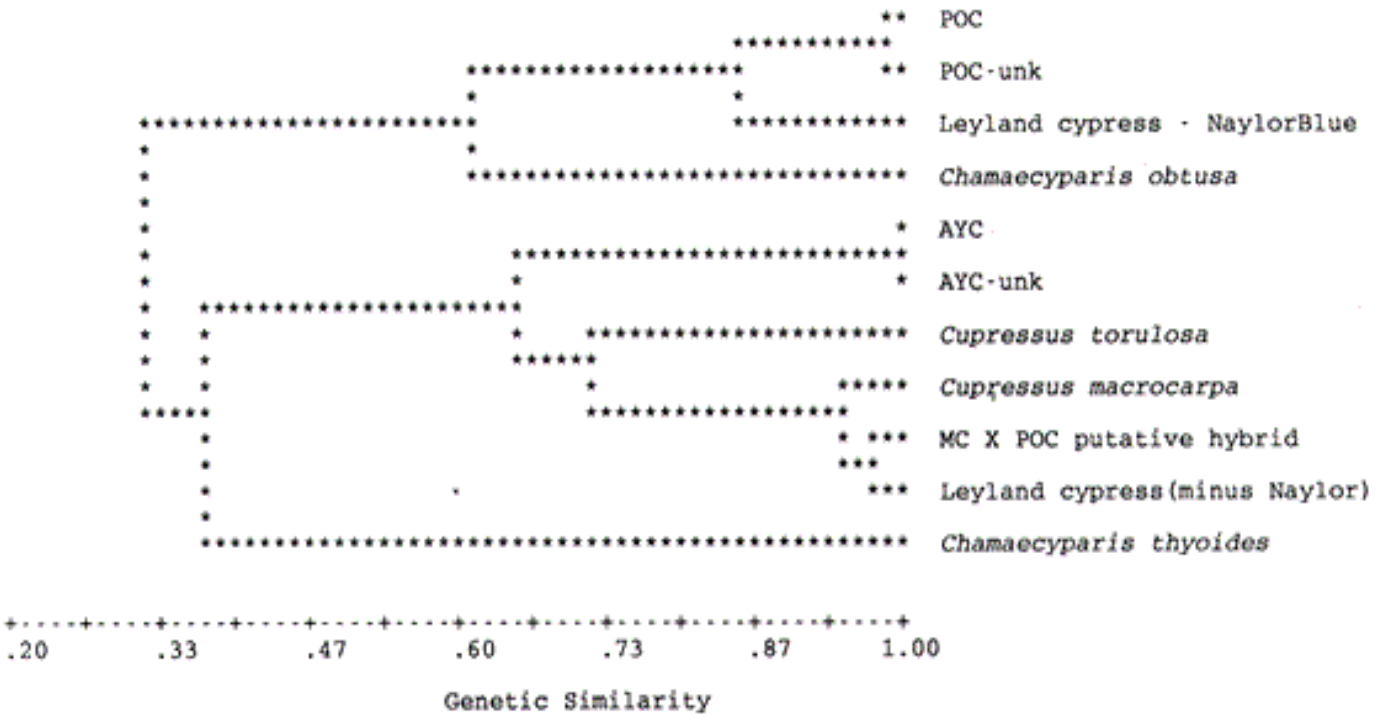
Of the seven Monterey cypress individuals analyzed, five of them had the same multilocus genotype. The 'Jam & Jelly - mature' sample had a unique genotype as did the 'Jam & Jelly - seedling' sample. The 'mature' and 'seedling' samples did not match each other. The five samples may have matching genotypes because (1) they are the same genetic individual, (2) there is little variation in Monterey cypress, or (3) there is little variation among the five samples that happened to have been chosen for analysis. A larger sampling of Monterey cypress would address this question. .

Table. Levels of genetic variation within taxa. Standard errors in parenthesis.

Taxon	Mean sample size per locus	Mean # of alleles per locus (A)	Percentage of polymorphic loci (P)	Expected heterozygosity (He)
Average gymnosperm	--	2.38 (0.1)	71.1 (2.6)	0.169 (0.008)
Ave. long-lived woody species with regional distribution	--	1.87 (0.1)	55.7 (2.3)	0.169 (0.008)
Port-Orford Cedar	11.8 (0.2)	1.8 (0.2)	55.6	0.131 (0.038)
Alaska yellow cedar	11.8 (0.2)	1.8 (0.2)	55.6	0.204 (0.054)
Monterey cypress	6.8 (0.2)	1.3 (0.1)	22.2	0.083 (0.044)

VARIATION AMONG SPECIES

There are many distinct genetic differences among the taxa studied. POC and AYC share only 33.7% genetic similarity. POC is only slightly more similar to Monterey cypress (37.8%) than it is to AYC. Monterey cypress is more similar to AYC (65.6% genetic similarity) than POC is to AYC (which is suprising given that Monterey cypress is in the genus *Cupressus*, while POC and AYC are in the genus *Chamaecyparis*). In the following dendrogram, the *Cupresses* species form a branch than includes AYC. *Ch. obtusa* is distinct from the other species, as is the *Ch. thyoides*. *Cupressus torulosa* shares over 74% genetic simarity to Monterey cypress, slightly greater than expected.



Six loci (out of 18 assessed) show fixed differences between POC and AYC. There are 35 allelic differences between the species (an allele that occurs in one species and not the other). There are five fixed differences (33 allelic differences) between POC and Monterey cypress. Comparing Monterey cypress to AYC, only two fixed differences and 26 allelic differences distinguish the species.

TESTING THE HYBRID HYPOTHESIS

POC X AYC

The putative POC X AYC hybrids appear to be either POC or AYC - not hybrids between the two species. All three molecular techniques used (starch gel electrophoresis, isoelectric focusing, and RAPDs) indicated that putative hybrids were either members of one species or the other. This was determined by starch gel electrophoresis at 18 isozyme loci, isoelectric focusing at three isozyme loci, and RAPD data at 171 band markers generated from 22 primers.

Individuals '11311B', '11312A', and 'number 01' appear to be AYC. This group of three individuals are referred to as 'AYC-unk'. All other submitted putative POC X AYC hybrids appear to be POC (this groups of 32 individuals is referred to as 'POC-unk'). POC and POC-unk share over 99% genetic similarity. AYC and AYC-unk share 99.8% similarity. The putative hybrids are not showing additivity of the genetic markers to the parent species, as would be expected in a hybrid. In fact, the AYC-unk group shares multiple markers with AYC that don't exist in POC. AYC-unk shares no markers with POC that don't also exist in AYC. This same pattern of non-additivity is true for the POC-unk group. This group shares many markers with POC that don't exist in AYC. POC-unk shares no marker with AYC that doesn't also exist in POC.

Leyland cypress (X Cupressocyparis leylandii)

Leyland cypress is a hybrid between Monterey cypress and Alaska yellow cedar. Five individuals of Leyland cypress were genetically analyzed: BLM#1, Castlewellan, Green, Naylor Blue, and Silver Dust. Naylor Blue showed no hybrid pattern between Monterey cypress and AYC. It instead showed a high degree of similarity to Port-orford cedar (around 85% genetic similarity). Naylor Blue showed

only 35% similarity to AYC, and 29% similarity to Monterey cypress. Because it appears that Naylor Blue is either a Port-orford cedar (see preceding dendogram), or at least a cross involving Port-orford cedar, it was removed from the Leyland cypress group for the remaining analysis.

Of the remaining four individuals of Leyland cypress, three of them have matching genotypes: BLM#1, Green, and Silver Dust. It is possible that these individuals are really ramets of the same clone, or they just happen to have matching genotypes. RAPD data was not generated on the Leyland cypress individuals. DNA was extracted and stored, so RAPDs could be generated if needed. Castlewellan has a unique genotype.

The Leyland cypress group is most genetically similar to the putative Monterey cypress X POC hybrid (97.7%), the Monterey cypress (91.1%), and the Alaska yellow cedar (70.7%). So even though the Leyland cypress is more similar to the Monterey cypress than the AYC, it does show additivity of the genetic markers between both parents. Leyland cypress shares four alleles with Monterey cypress that do not exist in AYC; it also shares four different alleles with AYC that do not exist in Monterey cypress. Its position on the dendogram also diagrams its relationship to both Monterey cypress and AYC.

Monterey cypress X Port-Orford cedar

Several individuals of a putative Monterey cypress X POC cross where submitted. These included samples 60886, 60887, 61326, and 'PO x *C. macrocarpa*'. In previous NFGEL results, we reported that all four samples had identical multilocus genotypes. We also stated that "It is likely that these are ramets of a single clone as opposed to separate individuals of the same controlled cross". The hybrid ('MC X POC') is very similar to that of Monterey cypress, and distinct from POC. 'MC X POC' shares 97.5% similarity to Monterey cypress, 70.9% similarity to Alaska yellow cedar, and only 34.7% similarity to POC. Interestingly, 'MC X POC' is most similar to Leyland cypress (excluding Naylor Blue). Leyland cypress and 'MC X POC' share 97.7% similarity. 'MC X POC' also shows some additivity of marker bands to Monterey cypress and AYC. Based on these genetic results, it is clear that the putative 'MC X POC' hybrid involves Monterey cypress. It either is a pure Monterey cypress or is the result of a cross with Monterey cypress. The data do suggest that the putative hybrid could be a cross between Montery cypress and AYC, not POC. This is supported by (1) the putative hybrid showing additivity of genetic markers to Monterey cypress and AYC, (2) the high genetic similarity (relative to other taxa) between the putative hybrid and both AYC and, especially, Monterey cypress, and (3) the placement of the putative hybrid with Leyland cypress in the dendrogram.

CONCLUSIONS

- Monterey cypress and Port-Orford cedar contain low levels of genetic diversity compared to the average gymnosperm.
- Alaska yellow cedar contains comparable (to slightly low) levels of genetic diversity compared to the average gymnosperm.
- The *Chamaecyparis* and *Cupressus* species studied are significantly divergent. They clearly show many genetic differences, both fixed differences and differences in marker frequencies. (It should be noted that several species were represented by only one to four individuals).
- All putative Port-Orford cedar X Alaska yellow cedar hybrids appear to be one species or the other, not hybrids between the species.
- Leyland cypress does appear to be a hybrid between Monterey cypress and Alaska yellow cedar (except for Leyland cypress-NaylorBlue which appears to be Port-Orford cedar, or from a cross involving Port-Orford cedar).

- We could not definitively identify the parents of the putative Monterey cypress X Port-Orford cedar hybrid. The hybrid clearly involves Monterey cypress as a parent (it is either pure Monterey cypress or is the result of a cross with Monterey cypress). If this material is a hybrid, the data suggest a higher probability that the other parent is Alaska yellow cedar instead of Port-Orford cedar. However, the data cannot rule out the possibility that the material is a Monterey cypress X Port-Orford cedar hybrid.
- It should be noted that molecular genetic evidence of hybridization can be misleading if it is used to assess advanced generation hybrids.

This was NFGEL Project #s 86, 87, 88 and part of 97.

Efficacy Of Supplemental Mass Pollination Methods In A Douglas-fir Seed Orchard

The goal of this project was to determine the efficacy of supplemental mass pollination methods in a privately-owned Douglas-fir seed orchard by genotyping megagametophyte and embryo (m/e) pairs from four controlled cross and two open-pollinated cross seedlots. Controlled crosses consisted of two different females each crossed with two different males. Open-pollinated seed was also collected from each female.

Seed was prepared and electrophoresed following NFGEL Standard Operating Procedures. We genotyped a total of 328 seed (m/e pairs) at 15 isozyme loci using three buffer systems via starch gel electrophoresis. Between 26 and 104 seed per cross was genotyped (sample size depended on seed availability - 100 seed per cross was the target).

Contamination levels in crosses using 'male #1' was low (four seed out of 104 (3.8%), and two seed out of 26 (7.7%) were detected contaminants). The crosses using 'male #2' showed high levels of contamination (67.3% and 30.9%). There seemed to be substantial pollen from an unknown male mixed with that from 'male #2'. The open-pollinated crosses were fairly diverse.

This is NFGEL Project #94.

Effects Of Stand Density Reduction Treatments On The Genetic Variation Of Ponderosa Pine In Northern Arizona

By: Kristin Kolanowski

The purpose of the research is to determine if the genetic variation of *Pinus ponderosa* var. *scopulorum* in Flagstaff's urban wildland interface (FUWI) has been affected by changes in composition, structure, and function that have taken place in the forests over the last 130 years. The research will also address the importance of including genetic considerations in resource management by quantifying the effects of different simulated thinning treatments, based on full restoration guidelines, on the genetic variation of ponderosa pine in FUWI.

The objectives of the research are to determine the genetic variation partitioning of clumps of trees that established prior to Euro-American settlement (1876), determine the genetic composition and structure of pre- and post-settlement trees, compare allozyme variation of the two age groups, and compare the genetic composition and structure of 5 stands prior to and after simulated random thinnings having 50, 25, and 10% post-settlement retaining percentages. The following hypotheses will be tested:

1. There is more within-clump variation than among-clump variation of pre-settlement trees.
2. The genetic variation of pre-settlement trees is different from that of post-settlement trees.
3. The genetic variation of spatially selected (replacement) post-settlement trees does not differ from that of randomly selected post-settlement trees.
4. A thinned stand will have less genetic variation (less heterozygosity, smaller % of polymorphic loci, mean # of alleles per locus, etc.) than that of the same stand that has not been thinned.

The goal and final step will be to suggest how to incorporate genetic guidelines resulting from the research into management of ponderosa pine ecosystems in northern Arizona.

A total of 465 ponderosa pine trees were genotyped at NFGEL using vegetative bud tissue. Data was obtained at 22 isozyme loci using three buffer systems. This information is part of a Master's Degree program by the author at Northern Arizona University in Flagstaff, Arizona. Analysis and reporting are in progress.

This is NFGEL Project #99.

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

The objective of this project was to perform clonal and parental identification in a Douglas-fir (*Pseudotsuga menziesii*) seed orchard operated by a private company out of Washington. Branch tips, including needles and expanding buds, from 44 individuals of Douglas-fir were submitted for analysis. Between two and five ramets were provided for 10 different clones. Eight open-pollinated progeny were provided from an eleventh clone, along with two possible mother trees (both labeled as ramets of the same clone).

Vegetative bud material from each tree was prepared for analysis following NFGEL Standard Operating Procedures. Material was electrophoresed on 11% starch gels and assayed at 17 isozyme loci using three buffer systems.

Isozyme data showed that all ramets of eight of the clones were correctly identified. Some ramet mislabeling was found in the other two clones. Progeny individuals were identified as offspring of one of the two submitted putative mother trees.

This was NFGEL Project #112.

Cross Verification Among *Chamaecyparis* Species

The project objective was to verify hybrid seedlings resulting from seven crosses between *Chamaecyparis lawsoniana* (Port-Orford Cedar, POC), *Chamaecyparis obtusa*(CHOB), *Chamaecyparis nootkatensis* (Alaskan Yellow Cedar, AYC), and *Chamaecyparis thyoides*(CHTH).

METHODS

Branch tips from 52 individuals were submitted for analysis. A small section of needle tissue (~3 mm3) per tree was placed in a microtiter plate well containing three drops of Gottlieb extraction buffer (NFGEL Standard Operating Procedures). One replicate plate was made of each set. Plates were frozen at -70C. On the morning of the run, samples were thawedd, macerated with a dremel tool, and the extract absorbed onto three, 3mm wicks. Samples were genotyped at 19 isozyme loci.

RESULTS

POC #1 x CHOB #2 Seedling '144-1' appears to be the product of a successful cross between these two parents. Seedlings '144-2' and '144-3' are not progeny from a cross between these two parents.

POC #2 x CHOB #2 Seedling '145-4' appears to be the product of a successful cross between these two parents. The remaining seedlings in this group are not progeny from a cross between these two parents.

POC #3 x CHOB #1 None of the seedlings could have been produced from a cross between these two parents. The seedlings are also not from a 117335 self cross (though the paternal parent for all is likely a Port-Orford Cedar(s)).

CHTH x self All seedlings appear to be the result of a successful self cross.

CHTH x POC #3 None of the seedlings could have been produced from a cross between these two parents. The seedlings could have been produced from a bluesport self cross. No Port-Orford Cedar appears to have served as a pollen parent for these seedlings.

CHOB #3 x POC #3 None of the seedlings could have been produced from a cross between these two parents. The seedlings could have been produced from a CHOB gracilis self cross. No Port-Orford Cedar appears to have served as a pollen parent for these seedlings.

POC #4 x AYC Because no parental material was sent for genotyping, verification of the cross is not possible. However, it is likely that the cross was not successful. Based on prior genotyping of Port-Orford Cedar and Alaska Yellow Cedar (AYC), these seedlings have none of the alleles typical of those found in AYC. This project was done in cooperation with Richard Sniezko, USDA Forest Service, Dorena Tree Improvement Center, Cottage Grove, OR 97424, and is NFGEL Project #102.

Genetic Diversity In *Perideridia Erythrorhiza*: A Rare Plant In Southern Oregon

Study Objectives and Summary

1a. Do the populations from the three general locations (Klamath Falls, Roseburg, and Cave Junction) differ on a genetic basis?

Yes, they do differ. However, the populations within the regions are more highly differentiated than the regions are. The Cave Junction region is the most genetically divergent of the general locations. Klamath and Roseburg locations are more similar to each other than either is to Cave Junction. The Klamath location shares 82.9% genetic similarity with Roseburg (mean genetic identities among conspecific populations are usually high (above 90%); among congeneric species are usually low (below 70%); and among infraspecific taxa are usually intermediate to the conspecific and congeneric values: although it should be noted that these are mean values, and individual situations vary greatly).

1b. If the populations/locations do differ on a genetic basis, do the differences warrant separation into different species?

The isozyme variation in itself does not warrant separation of the general locations into separate species. The great differentiation among populations might allow separation on the basis of other
(non-isozyme) evidence.

The Leather population from the Cave Junction location does not appear to be *Perideridia erythrorhiza*. It is either a mis-identified collection of another species, or a new species. The Leather population should be re-examined. That examination should include both the collection of herbarium voucher specimens and isozyme analysis of a second seed collection, to be sure the vouchers match the isozyme observations. If isozyme variation in *Perideridia erythrorhiza* is studied further, we recommend that at least one additional population from the Illinois River area be included.

1c. Are there genetic differences between the eastern and western populations (east or west of the Cascades)?

It has been hypothesized that variation in *P. erythrorhiza* can best be divided into two groups,

an eastern group (including populations Howard Prairie, Mud Flat, and Pelican Barn) and a western group (including populations Illinois River, Roseburg, and Umpqua). We did not find that division to best express the pattern of isozyme variation we observed. A more useful way to express the pattern of variation is to divide the populations into three regions; Roseburg (the Roseburg and Umpqua populations), Cave Junction (the Illinois River population), and Klamath Falls (the Howard Prairie, Mud Flat, and Pelican Barn populations).

2. Does the Pelican Barn population from the Winema National Forest differ from the population at Mud Flat?

The Pelican Barn and Mud Flat populations are not identical, but their genetic identity (90%) is the fourth highest observed between any pair of populations. The data indicate that there might be some inbreeding, selfing, or population substructuring in the Pelican Barn population that is not occurring in the Mud Flat population. Mud Flat shares 99.7% similarity with the Howard Prairie population.

3. Is the genetic diversity in the Pelican Barn population substantially smaller than the diversity in the Mud Flat population?

The Pelican Barn population was slightly less variable than the Mud Flat population in all diversity measures except for expected heterozygosity. If all diversity statistics are weighted equally, the Illinois River population is the most diverse, followed by, in descending order of diversity level, Mud Flat, Roseburg, Pelican Barn, Howard Prairie, and Umpqua.

INTRODUCTION

Perideridia erythrorhiza (Piper) Chuang & Constance is a rare perennial herb that occurs in southern Oregon in only three general locations: near Klamath Falls in Klamath and Jackson Counties, near Roseburg in Douglas County, and near Cave Junction in Josephine County. This plant is commonly known as red-rooted yampah and is related to parsnips and carrots in the family Apiaceae. This species is considered approximately tetraploid, with n = 19 (Chuang and Constance 1969). It is thought to be outcrossing, though capable of self-pollinating (Meinke 1998). The biology, history, and geographic range of the species are covered elsewhere (Chuang and Constance 1969, and Meinke 1998). *Perideridia erythrorhiza* is classified by the US Fish and Wildlife Service as a candidate threatened species, and by the USDA Forest Service Region 6 as a sensitive species.

Perideridia erythrorhiza occurs in two locations on the Klamath Ranger District of the Winema National Forest, USDA Forest Service (Klamath Fall area, Klamath County). These populations (Pelican Barn and Odessa sites) are small and not very robust. However, these populations are important for the conservation of the species because most other populations in this area are on private land or are threatened by development or recreational use. A third population located in the Klamath Falls area occurs in Jackson County and is located at Mud Flat on industrial land that is both grazed and logged. The Mud Flat population is the largest and most vigorous population known in the entire species. This population is an obvious choice of plants to augment the smaller populations on Forest Service land if mitigation became necessary. The Mud Flat site is separated from the Klamath County sites by 25 miles.

Figure 1. Locations of *Perideridia erythrorhiza* populations sampled for the genetic study.

The Forest Service is interested in obtaining genetic data to understand the taxonomic relationships among the general locations with the species (Figure 1), and also to determine the levels of diversity within and among the general locations and populations with a location. Specific objectives of the study include: (1) determine if populations from the three general locations (Klamath Falls, Roseburg, and Cave Junction) differ on a genetic basis, and if so, do the differences warrant separation into different species; (2) determine if the Pelican Barn population from the Winema National Forest differs from the population at Mud Flat; and (3) determine if the genetic diversity in the Pelican Barn population on the Winema NF is substantially smaller than the diversity in the Mud Flat population.

MATERIAL AND METHODS

Sample Collection. Seed was collected from between six and sixty-one individuals per population. Three to five seed per plant were stratified and germinated. The collection, stratification, and germination of seed was performed by Paul Berrang, USDA Forest Service. Once seedlings grew to where their shoots were approximately 5 - 10 cm in length, they were transferred to NFGEL for genetic analysis. In addition, a bulk seed sample from 75 - 100 plants was collected at the Illinois River population by Kim Roberts. Seedlings were grown and processed like those from the other populations, but results were omitted from most analysis; see Results and Discussion.

Sample Preparation. Seedlings from a total of 338 individuals were submitted for isozyme analysis using starch gel electrophoresis (excluding an Illinois River-bulk collection). One seedling per individual was prepared for analysis. A pilot study indicated that to obtain adequate isozyme activity, it was necessary to include the tuberous root in the preparation. Therefore, each seedling was gently dug out of the soil taking care not to break the shoot off of the tuberous root. Each seedling was washed with water and ground in a mortar using liquid nitrogen. Approximately 0.4 mls of a modified Pitel and Cheliak (1984) extraction buffer #7 was added to the ground powder (buffer modifications: 10% PVP-40, 5.0mM EDTA, 10mM DTT, 0.8mM NAD, 0.5mM NADP, buffer pH=8, excluded B-mercaptoethanol). Resulting slurry was transferred to microtiter plate wells and plates frozen until electrophoresis.

Electrophoresis. In preparation for electrophoresis, slurry was thawed and absorbed onto 3 mm wide wicks prepared from Whatman 3MM chromatography paper. Methods of electrophoresis followed the general methodology of Conkle et al. (1982) with some modifications (USDA Forest Service 2000). A

lithium borate electrode buffer (pH 8.3) with a Tris citrate gel buffer (pH 8.3) (Conkle et al. 1982) was used to resolve malic enzyme (ME), alcohol dehydrogenase (ADH), phosphoglucomutase (PGM), and phosphoglucose isomerase (PGI). A sodium borate electrode buffer (pH 8.0) was used with a Tris citrate gel buffer (pH 8.8) (Conkle et al. 1982) to resolve glutamate-oxaloacetate transaminase (GOT), glucose dehydrogenase (GDH), triosephosphate isomerase (TPI), and uridine diphosphoglucose pyrophosphorylase (UGPP). A morpholine citrate electrode and gel buffer (pH 8.0) (USDA Forest Service 2000) was used to resolve malate dehydrogenase (MDH), phosphogluconate dehydrogenase (6PGD), and fluorescent esterase (FEST). All enzymes were resolved on 11% starch gels. Stain recipes for enzymes follow USDA Forest Service (2000). 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 Interpretation. *Perideridia erythrorhiza* has a haploid chromosome number of n = 19 and is presumably a tetraploid (Chuang and Constance 1969). Although all loci of a tetraploid should theoretically contain four alleles, observed band patterns were not consistent with genetic expectations. We therefore treated the plants as if they were diploid and scored each set of homoeologous loci as one locus. See discussion for an explanation of this decision.

Genetic interpretations were inferred directly from isozyme phenotypes based on knowledge of the generally conserved enzyme substructure, compartmentalization, and isozyme number in higher plants (Gottlieb 1981, 1982; Weeden and Wendel 1989).

Data Analysis. Results were analyzed using Biosys-1, version 1.7 (Swofford and Selander 1989). A locus was considered polymorphic if an alternate allele occurred even once. We calculated unbiased genetic distances (Nei 1978), and expected heterozygosity (Nei 1973). F statistics for the hierarchy of populations within locations, populations within species, and locations with species were calculated by the method of Wright (1978). Dendrograms based on unbiased genetic distances (Nei 1978) were generated using UPGMA.

RESULTS AND DISCUSSION

Sampling. An Illinois River-bulk population was omitted from most analysis, for two reasons. First, it turned out to be a sample from the same population as the other Illinois River sample submitted. Second, its collection protocol was different from that used for all other populations. The Illinois River-bulk population was collected from 75-100 female parents and the seed was combined (an unknown number of seed per parent was collected). All other populations were sampled as individual female plant collections (seed from each plant was kept separate). Only one seed per plant was genetically analyzed from these populations. A total of 50 seed was analyzed from the Illinois River-bulk population (however, this could include multiple seeds from any one plant).

Genetic Interpretation. *Perideridia erythrorhiza* has a haploid chromosome number of n = 19 and is presumably a tetraploid (Chuang and Constance 1969). This necessitated considering several options for scoring isozyme bands. Polyploids often exhibit isozyme band patterns so complex that they defy genetic interpretation. Such patterns may be treated statistically as phenotypes (Chung et al. 1991, Strefeler et al. 1996). A genetic interpretation of isozyme band pattern data is preferred over a phenotypic analysis, because a genetic analysis provides more precise information about genetic variation (Gottlieb 1977) and because results can be compared directly with compilations of plant isozyme genetics (e.g. Hamrick and Godt 1990). The band patterns in *Perideridia erythrorhiza* were relatively simple, permitting genetic interpretation.

Once the decision to provide genetic analysis is made, further decisions are required about which loci to include, treatment of homoeologous loci, and the distribution of alleles among homoeologous loci. Studies of population genetics in polyploids usually employ one of three approaches to determining the number of loci scored for each enzyme. (1) Assume that a tetraploid has two homoeologous loci for each enzyme, and score both of them for every enzyme (Knapp and Rice 1996). This method maximizes the number of

monomorphic loci and minimizes the number of alleles per locus. Although this is a logical inference from polyploidy, this approach is rarely used. (2) Assume each enzyme is produced by one locus unless hypothesizing a second locus is necessary because of fixed heterozygosity or individuals with three or more alleles per apparent locus (Perez de la Vega 1994, Sanders and Hamrick 1980, Schierebeck et al. 1995). This method may be chosen because it does not require the inference of "invisible" loci (Nevo et al. 1982), and it may be the most common way of scoring isozymes in polyploids. It is used "by default" when the chromosome number is unknown. It is the most parsimonious approach for diploidized ancient tetraploids that exhibit gene duplications (Inoue and Kawahara 1990). (3) Treat the plants as if they were diploid and score each set of homoeologous loci as one locus. This approach is commonly employed for autopolyploid taxa exhibiting multiple chromosome numbers and polysomic inheritance (Bayer 1989, Cai et al. 1990, Ehrendorfer et al. 1996, Hamrick and Allard 1972, McArthur et al. 1986). Although most articles providing statistical analysis of isozyme genetic diversity in polyploids use one of these three approaches, other methods have been used (Petersen et al. 1993, Sun 1996).

Isozymes provided some evidence that *Perideridia erythrorhiza* might be tetraploid. First, two invariant UGPP bands not used in this analysis could be interpreted as a pair of homeologous loci exhibiting a pattern of fixed heterozygosity. This might suggest either gene duplication in a diploid or a lack of gene silencing in a tetraploid plant. Second, the PGM-1 locus exhibited variation in staining intensity consistent with the hypothesis that *P. erythrorhiza* is autotetraploid. We scored PGM-1 as one locus, ignoring the differences in staining intensity, but we suspect that the excess heterozygosity observed at this locus results from it being a pair of homeologous loci. Finally, excess heterozygosity observed at the GOT locus might also be attributed to the presence of homeologous loci. However, other than the monomorphic bands in UGPP, no fixed heterozygosity was noted at any locus used in this analysis

Because the chromosome number (38; base number in the complex is 9, 10, or 11) of *Perideridia erythrorhiza* is not divisible by four, some loss, amalgamation, or duplication of chromosomes has obviously occurred. Although all loci of a tetraploid should theoretically contain four alleles, observed band patterns were not consistent with genetic expectations (perhaps due to gene silencing or aneuploidy). We therefore treated the plants as if they were diploid and scored each set of homoeologous loci as one locus. Fourteen loci were scored, representing forms of eleven enzymes. All scored loci were polymorphic. What were probably two additional loci resolved in UGPP-2, and were invariant in all populations. Because the focus of this study was taxonomic, they were not included in analysis.

Genetic Variation. Isozyme variation was extremely high in *Perideridia erythrorhiza* at the species and population levels (Table 1). For example, the lowest percent polymorphic loci observed in a *P. erythrorhiza* population (P=50.0) was much higher than the percent polymorphic loci seen in the average plant species (34%; Hamrick and Godt 1990). This level of within-population variation would be unusual in a common, widespread species and is unexpected in such a rare taxon. The most variable population of *P. erythrorhiza* (not including the Leather site) is the Illinois River population. The other populations contain comparable levels of diversity. The Illinois River-bulk population contains very high levels of diversity (data not shown). Expected heterozygosity is 0.273, observed heterozygosity equals 0.173, percent polymorphic loci is 100%, and mean number of alleles per locus equals 2.5. The higher diversity levels observed in the bulked population may be at least a partial result of (1) high levels of within-family variation, and/or (2) sampling of the bulk lot over a larger geographic area compared to the other Illinois River collection.

Compared to the Mud Flat site, the Pelican Barn population contains slightly less alleles per locus (1.5 vs 1.9) and polymorphic loci (50.0 vs 57.1) (Table 1). However, Pelican Barn contains greater expected heterozygosity (0.169 vs 0.159). The low observed heterozygosity and high F value in Pelican Barn may be indicitive of some inbreeding or selfing occurring in the population.

Wright's F statistics were high for most populations and for the species as a whole (Table 1). High within-population values for F often result from inbreeding, self-pollination, or unrecognized subdivisions within the population.

Table 1. Summary of genetic variability in *Perideridia* populations. N = mean number of individuals sampled per locus, per population; P = % of all loci that are polymorphic; A = average number of alleles at all loci; H_o = observed frequency of heterozygotes; H_e = frequency of heterozygotes expected under Hardy-Weinberg equilibrium conditions; F = fixation index, = $(H_e-H_o)/H_e$.

	N	P	A	H_o	H_e	F
Species Level						
Perideridia erythrorhiza	283.9 (14.2)	100.0	3.4 (0.3)	0.109 (0.022)	0.352 (0.053)	0.690
Perideridia oregana	4.7 (0.4)	42.9	1.6 (0.2)	0.188 (0.080)	0.223 (0.080)	0.157
Location Level						
Cave Junction	69.5 (4.5)	92.9	3.0 (0.3)	0.218 (0.052)	0.435 (0.055)	0.499
Roseburg	81.8 (4.2)	78.6	2.2 (0.3)	0.049 (0.018)	0.204 (0.062)	0.760
Klamath Falls	132.6 (7.9)	85.7	2.4 (0.2)	0.102 (0.034)	0.176 (0.048)	0.420
Population Level						
Cave Junction-pop mean	*34.75*	*78.55*	*2.30*	*0.188*	*0.253*	*0.257*
Illinois River	34.7 (2.8)	85.7	2.4 (0.3)	0.169 (0.048)	0.248 (0.064)	0.319
Leather	34.8 (3.0)	71.4	2.2 (0.3)	0.208 (0.069)	0.258 (0.073)	0.194
Roseburg-pop mean	*40.90*	*60.70*	*1.85*	*0.047*	*0.165*	*0.715*
Umpqua River	37.1 (2.4)	64.3	1.8 (0.2)	0.047 (0.023)	0.155 (0.057)	0.697
Roseburg	44.7 (2.3)	57.1	1.9 (0.3)	0.048 (0.020)	0.176 (0.058)	0.727
Klamath Falls-pop mean	*44.23*	*54.73*	*1.73*	*0.097*	*0.146*	*0.336*
Pelican Barn	30.9 (2.5)	50.0	1.5 (0.1)	0.087 (0.047)	0.169 (0.057)	0.432
Mud Flat	53.4 (2.9)	57.1	1.9 (0.2)	0.115 (0.037)	0.159 (0.054)	0.277
Howard Prairie	48.4 (3.0)	57.1	1.8 (0.2)	0.089 (0.047)	0.110 (0.049)	0.191

Taxonomy. Isozyme variation does indicate that populations and Regions are highly differentiated, which is consistent with morphological, phenological, and physiological data (Meinke 1998). The isozyme data do not support dividing *Perideridia erythrorhiza* into two groups, east and west of the Cascade crest. Some populations of *P. erythrorhiza* had unique alleles, and some alleles were unique to regions.

The Leather population submitted as *Perideridia erythrorhiza* is probably not that species. It is highly genetically differentiated from the other populations (Fig. 2; Table 2). Usually, though not always, different species show fixed (completely consistent) differences at one or more isozyme loci. The distribution of alleles supported the hypothesis that all samples except Leather and *P. oregana* were samples from the one species. All Leather samples had the genotype '44' at UGPP-2; no other sample had the UGPP-2 '4' allele. In addition, the Leather samples consistently had faint, unscorable bands at MDH-2. Compare this with the P. oregana sample, which differed from all other samples because it had the '66' genotype at PGM-1; no other sample had the PGM-1 '6' allele. In addition, the *P. oregana* samples consistently had faint, unscorable bands at FEST-1. The differences between the Leather Site sample and *P. erythrorhiza*, and between the *P.*

oregana sample and *P. erythrorhiza* seem parallel, and the Leather sample is more genetically distinct from the *P. erythrorhiza* samples than is *P. oregana* (Table 2).

It is not possible with the isozyme data to taxonomically identify the Leather population. Perhaps it is a particularly odd population of *Perideridia erythrorhiza*, worthy of separate species status. Possibly it is *P. oregana*, although isozymes suggest that this is not the case. *Perideridia erythrorhiza* resembles *P. gairdneri* and *P. lemmonii*; could the Leather population be one of those species? Is it an unrecognized species? Without voucher specimens or seed to grow out, the identity of this population remains a mystery. This could be an interesting subject for further research.

Figure 2. Genetic similarity among *Perideridia* populations sampled.

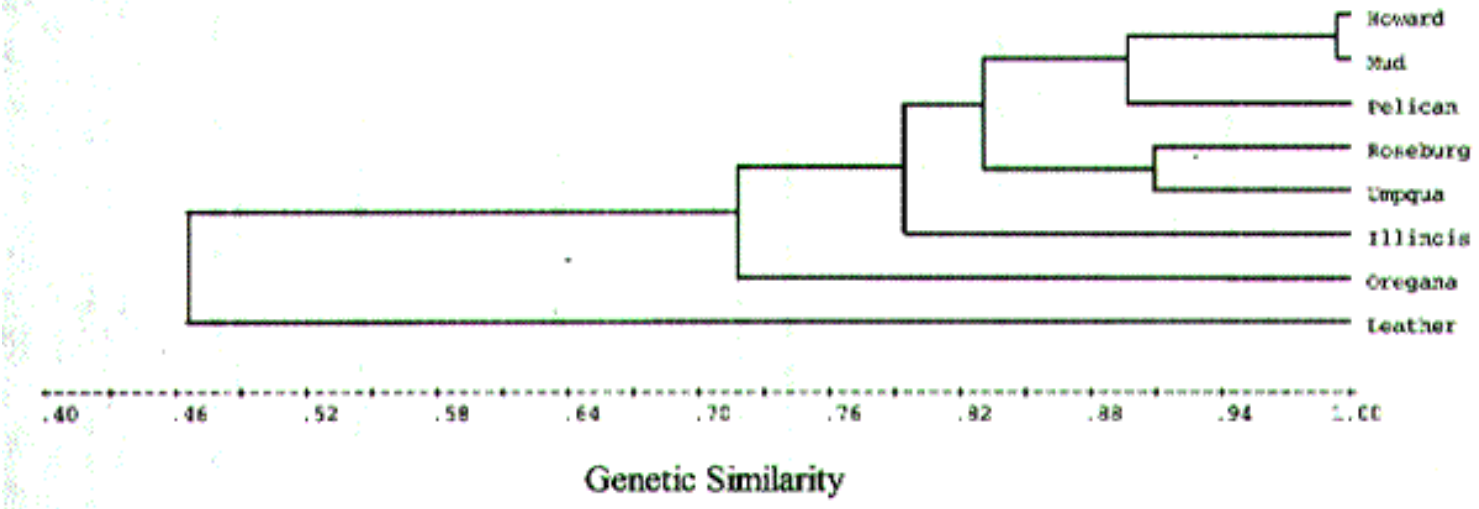


Table 2. Matrix of similarity/distance coefficients by Population. Below diagonal: Nei (1978) unbiased genetic distance; above diagonal: Nei (1978) unbiased genetic identity.

Population	1	2	3	4	5	6	7	8
1 Howard	*****	.766	.462	.997	.658	.904	.856	.785
2 Illinois	.266	*****	.552	.753	.753	.848	.801	.809
3 Leather	.772	.595	*****	.463	.410	.465	.442	.469
4 Mud	.003	.283	.769	*****	.654	.891	.857	.785
5 *P. oregana*	.418	.284	.891	.425	*****	.658	.812	.781
6 Pelican	.101	.164	.765	.115	.418	*****	.889	.803
7 Roseburg	.155	.221	.816	.154	.208	.117	*****	.911
8 Umpqua	.242	.212	.757	.242	.247	.220	.0930	*****

Regional Differentiation. As might be expected with such geographically isolated populations, the genetic identities among some *Perideridia erythrorhiza* populations were fairly low (excluding the Leather site) (Table 2). Genetic identities among *P. erythrorhiza* populations varied from 0.753 to 0.997. Genetic identities between the *P. oregana* sample and *P. erythrorhiza* populations varied from 0.654 to 0.812. Genetic identities between the Leather Site sample and *P. erythrorhiza* populations varied from 0.442 to 0.552, and its identity with the *P. oregana* sample was 0.410.

When these genetic identities are diagramed, the *Perideridia erythrorhiza* populations cluster together as would be expected geographically (Fig. 2). The three Klamath Falls populations form one cluster, the two

Roseburg populations form another, and the Illinois River population is sister group to this pair of clusters. Note that this dendrogram does not support division of *P. erythrorhiza* into just two groups, the eastern Klamath Falls and a western group including the Illinois Valley, Roseburg, and Umpqua populations.

As expected, the one *P. oregana* sample is distant from all *P. erythrorhiza*. The Leather population is more distant still, which would be highly unusual if it were *P. erythrorhiza*, but, as discussed previously, presumably it is not.

Wright's F-statistics provide another way to examine the pattern of variation among populations. *Perideridia erythrorhiza* populations are highly differentiated (Fpt = 0.388). It has been assumed that variation in *P. erythrorhiza* can best be divided into two groups, an eastern group (including populations Howard Prairie, Mud Flat, and Pelican Barn) and a western group (including populations Illinois River, Roseburg, and Umpqua). We did not find that division to best express the pattern of isozyme variation we observed. In a hierarchy of populations within east/west groups, populations are highly differentiated (Fpg = 0.422) and the east/west grouping does not contribute to the pattern of differentiation (Fgt = -0.058, which is approximately zero; the negative number is an error in the computer program).

A more useful way to express the pattern of variation is to divide the populations into three regions; Roseburg (the Roseburg and Umpqua populations), Cave Junction (the Illinois River population), and Klamath Falls (the Howard Prairie, Mud Flat, and Pelican Barn populations). Population differentiation is somewhat lower though still great (Fpr = 0.274) and regional differentiation is moderate (Frt = 0.157). Of course, this system should be tested using at least two populations in the Cave Junction region.

Genetic identities support this division of *Perideridia erythrorhiza* populations into three regions, rather than two. When the populations are divided into eastern and western groups, the genetic similarity of the eastern and western group is only slightly lower than the genetic similarity of the western populations among themselves. In fact, when the samples are divided into three regions, the Klamath and Roseburg regions are more similar to each other (0.829) than either is to the Cave Junction region (0.789 and 0.805, respectively).

REFERENCES

Bayer, R. J. 1989. Patterns of isozyme variation in western North American *Antennaria* (Asteraceae: Inuleae) II. Diploid an polyploid species of section Alpinae. Am. J. Bot. 76: 679-691.

Cai, Q., MacDonald, S. E., and Chinnappa, C. C. 1990. Studies on the *Stellaria longipes* complex (Caryophyllaceae): isozyme variability and the relationship between *Stellaria longipes* and *S. longifolia*. Pl. Syst. Evol. 173: 129-141.

Chuang, T-I., and Constance, L. 1969. A systematic study of *Perideridia* (Umbelliferae-Apioideae). University of California Publications in Botany, volume 55. 74 pages.

Chung, M. G., Hamrick, J. L. , Jones, S. B., and Derda, G. S. 1991. Isozyme variation within and among populations of *Hosta* (Liliaceae) in Korea. Systematic Botany 16: 667-684

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

Ehrendorfer, F., Samuel, R., and Pinsker, W. 1996. Enzyme analysis of

genetic variation and relationships in diploid and polyploid taxa of *Galium* (Rubiaceae). Pl. Syst. Evol. 202: 121-135.

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

Gottlieb, L. D. 1981. Electrophoretic evidence and plant populations. Prog. Phytochem. 7: 1-46.

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

Hamrick, J. L., and Allard, R. W. 1972. Microgeographical variation in allozyme frequencies in *Avena barbata*. Proc. Nat. Acad. Sci. USA 69: 2100-2104

Hamrick, J. L., and Godt, M. J. W. 1990. Allozyme diversity in plant species, pp. 43-63 in Brown, A. H. D., Clegg, M. T., Kahler, A. L., and Weir, B. S., eds., Plant Population Genetics, Breeding, and Genetic Resources. Sunderland, Massachusetts: Sinauer Associates

Inoue, K., and Kawahara, T. 1990. Allozyme differentiation and genetic structure in island and mainland Japanese populations of *Campanula punctata* (Campanulaceae). Am. J. Bot. 77: 1440-1448.

Knapp, E. E., and Rice, K. J. 1996. Genetic structure and gene flow in *Elymus glaucus* (blue wildrye): implications for native grassland restoration. Restoration Ecology 4: 1-10.

McArthur, E. D., Sanderson, S. C., and Freeman, D. C. 1986. Isozymes of an autopolyploid shurb, *Atriplex canescens* (Chenopodiaceae). Great Basin Naturalist 46: 157-160.

Meinke, R. J. 1998. Taxonomic evaluation of the rare species *Perideridia erythrorhiza* (Apiaceae). Internal report to the BLM, USDA Forest Service, and US Fish and Wildlife Service. 31 pages.

Nei, M. 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.

Nevo, E., Golenberg, E., and Beiles, A. 1982. Genetic diversity and environmental associations of wild wheat, *Triticum dicoccoides*, in Israel. Theor. Appl. Genet. 62: 241-254.

Pérez de la Vega, M., Sáenz-de-Miera, L. E., and Allard, R. W. 1994. Ecogeographical distribution and differential adaptedness of multilocus allelic associations in Spanish *Avena sativa* L. Theor. Appl. Genet. 88: 56-64.

Peterson, P. M., Duvall, M. R., and Christensen, A. H. 1993. Allozyme differentiation among *Bealia mexicana*, *Muhlenbergia argentea*, and *M. lucida* (Poaceae: Eragrostideae). Madroño 40: 148-160.

Pitel, J. A., and Cheliak, W. M. 1984. Effect of extraction buffers on characterization of isoenzymes from vegetative tissues of five conifer species: A user's manual. Information Report PI-X-34, Petawawa National Forestry Institute, Canadian Forestry Service.

Sanders, T. B., and Hamrick, J. L. 1980. Variation in the breeding system of Elymus canadensis. Evolution 34: 117-122.

Schierenbeck, K. A., Hamrick, J. L., and Mack, R. N. 1995. Comparison of allozyme variability in a native and an introduced species of *Lonicera*. Heredity 75: 1-9.

Strefeler, M. S., Darmo, E., Becker, R. L., and Katovich, E. J. 1996. Isozyme characterization of genetic diversity in Minnesota populations of purple loosestrife, *Lythrum salicaria* (Lythraceae). Am. J. Bot. 83: 265-273.

Sun, M. 1996. The allopolyploid origin of *Spiranthes hongkongensis* (Orchidaceae). Am. J. Bot. 83: 252-260.

Swofford, D. L., and Selander, R. B. 1989. Biosys-1, release 1.7. Illinois Natural History survey, Champaign, Illinois.

U.S.D.A. Forest Service. 2000. National Forest Genetic Electrophoresis Laboratory Standard Operating Procedures. NFGEL, Camino, California. 103pp.

Weeden, N. F., and Wendel, J. F. 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

Wright, S. 1978. Evolution and the Genetics of Populations, Vol. 4. Variability within and among natural populations. University of Chicago Press, Chicago.. Heredity 75: 1-9.

ACKNOWLEDGEMENTS

Project cooperator Paul Berrang developed the study proposal, carried out seed collection, germinated the seed, and transported the seedlings to NFGEL. Laboratory technical support was provided by Suellen Carroll, Randy Meyer, Pat Guge, and Robert Saich. Funding was provided by the USDA Forest Service, Region 6 and the Bureau of Land Management. This is a report of the findings of the National Forest Genetic Electrophoresis Laboratory's Project 78.

Genetic Differentiation In A Rare Plant With A Disjunct Range: *Lewisia Kelloggii*

ABSTRACT

Lewisia kelloggii isozyme variation was studied in six Idaho and seven California populations. Lewisia kelloggii is polyploid, and was treated in analysis as an allotetraploid. The species is highly variable genetically (85% of loci polymorphic, 3.3 alleles per locus) and populations are highly differentiated (Fst = 0.77). All California and the Greencreek Lake populations are diverse, but most sampled Idaho populations have relatively little within-population variation. Isozyme data support the hypothesis that the Idaho and California populations may be two different, though related, species. For example, genetic identities between the Idaho and California populations (0.47 - 0.70) are typical of cogeneric species rather than conspecific populations.

INTRODUCTION

Lewisia kelloggii K. Brandegee is a small rare plant found in open areas on excessively drained, coarse-textured granitic and volcanic soils on ridgelines. It has a disjunct range, occurring in the Sierra Nevada of California (from Plumas County south to Fresno County) and in Idaho (in Valley, Elmore, and Custer Counties).

Lewisia kelloggii has no special seed dispersal mechanisms that would allow dispersal across the 540 km separating the closest California and Idaho populations. The capsule splits open around the sides so that the top falls off, sometimes with a few seeds adhering. The small (2 mm) round seeds have no special mechanisms for dispersal. They may be shaken or blown out of the capsule (Matthew 1989), but often flowers are buried in blowing sand before seed maturation. In these cases, rodents digging to eat the fleshy taproot are important dispersal agents (Davidson 2000).

Its high elevation habitat might seem to protect this rare species from human disturbance, but that is not entirely true. *Lewisia kelloggii* is at risk in part because its open ridgetop habitats are often desirable sites for roads and for trails. It is vulnerable to damage by logging because its habitats are suitable for landings and for parking equipment. *Lewisia kelloggii* is probably tolerant of wildfire due to its geophytic growth form and open habitat, but it is vulnerable to damage during firefighting operations, when its ridgeline habitats may be preferred sites for establishing fire control lines and the plants may be killed by bulldozing or soil compaction. In addition, a few populations have suffered from overcollection for cultivation (K. van Zuuk, Forest Botanist, Tahoe National Forest, pers. comm.), although the diminutive, nearly stemless species is not as showy as most Lewisias and is difficult to maintain in cultivation (Matthew 1989).

The taxonomic history of *Lewisia kelloggii* has been relatively simple (Table 1). *Lewisia kelloggii* was published based on a specimen from Placer County, California (Brandegee 1894). Although it has been stated that the type specimen was lost in the fire that followed the San Francisco earthquake of 1906 (Davidson 2000), it is still at the California Academy of Sciences (B. Bartholomew, pers. comm.). Subsequently the plant was incorporated into another species (as *L. rediviva var. yosemitana*; Hall and Hall 1912) but that move was clearly an error, both because *L. kelloggii* differs in important ways from L. rediviva, and because the name *L. rediviva* var. *yosemitana* K. Brandegee is a synonym of a different small-statured species now called *L. disepala* Rydb. Later, the California species *L. yosemitana* Jeps. was described within *L. kelloggii* based on Jepson 4357, 6 July 1911, Summit of El Capitan, Yosemite (B. Ertter, pers. comm). *Lewisia yosemitana* was distinguished from *L. kelloggii* because *L. yosemitana* had 16 - 26 stamens; *L. kelloggii* had 10 - 15 stamens. That distinction did not win wide acceptance, and *L. kelloggii* as currently understood has a highly variable stamen number. The type specimen of *L. yosemitana* has been treated as the neotype of *L. kelloggii* (Davidson 2000), but that is unnecessary because the holotype still

exists. Recently, *L. kelloggii ssp. hutchisonii* L. T. Dempster was described from a single specimen collected on Saddleback Mountain in Sierra County, in the northern part of the species' California range (Dempster 1996). This subspecies is much larger than typical *L. kelloggii*; *L. kelloggii ssp. hutchisonii* has leaves 4 - 10 cm long and pteals 25 - 30 mm long, while *L. kelloggii ssp. kelloggii* has leaves 2 - 6 cm long and petals 10 mm long. To summarize, at this time the plants in Idaho and California are treated as one species, *L. kelloggii*. The only intraspecific taxon recognized (*L. kelloggii ssp. hutchisonii*), may have a very limited range in the northern Sierra Nevada.

Recently, small morphological differences between the Idaho and California populations have raised the possibility that they should be considered two separate species (D. Taylor, pers. comm. to T. Prendusi, Regional Botanist, Region 4, USDA Forest Service). This would have implications for *Lewisia kelloggii* nomenclature, conservation, and legal status. These concerns prompted this study of *L. kelloggii* genetic diversity using isozymes.

Table 1. Nomenclatural history of *Lewisia kelloggii*.

Name	Reference	Type specimen
Lewisia kelloggii K. Brandegee	Brandegee 1894	A. Kellogg s.n. 27 June 1870. California, Sierra Nevada, Placer County, Cisco ("Camp Yuba"). CAS
Lewisia kelloggii Curran	Curran 1894	type of *L. kelloggii*
Lewisia rediviva Pursh var. *yosemitana* H. M. Hall	Hall 1912	Unclear.
Lewisia yosemitana Jepson	Jepson 1923	Jepson 4357. California, Mariposa County, Yosemite, El Capitan. JEPS
Oreobroma kelloggii (K. Brandegee) Rydberg	Rydberg 1932	type of *L. kelloggii*
Oreobroma yosemitana (K. Brandegee) Rydberg	Rydberg 1932	type of *L. kelloggii*
Lewisia kelloggii K. Brandegee ssp. *hutchisonii* L. T. Dempster	Dempster 1996	P. C. Hutchison 8105, July 1982. California, Saddleback Mountain, ca. 9 miles north of Downieville.

METHODS

Two to three leaves per plant were collected from each of approximately 30 plants per population in seven California and six Idaho populations (Table 2). Three pairs of populations were collected very close together. The Burnt Creek and Noname Creek populations were within 0.6 mile on Red Mountain, Idaho. The two Soda Springs populations were collected within 2 miles. The two populations from the Plumas National Forest were a few hundred feet apart.

For further details of isozyme electrophoresis and data analysis, see Appendix 1.

Table 2. Collection locations for *Lewisia kelloggii* samples used in this study. N = sample size.

State	National Forest	Population	N	Latitude/Longitude	Collector	Date collected
CA	Eldorado	Brown Rock	32	38.6607N 120.2560W	Rob Saich	28-Jun-99
CA	Eldorado	Pack Saddle Pass	32	38.7552N 120.1694W	Rob Saich	28-Jun-99
CA	Plumas	Plumas – A	28	39.7541N 120.8600W	Molly Hunter	27-Jul-99
CA	Plumas	Plumas – B	18	39.7930N 120.8972W	Molly Hunter	27-Jul-99
CA	Sierra	Shuteye Peak	30	37.3527N 119.4226W	Rob Saich	28-Jun-00
CA	Tahoe	Soda Springs #1	30	39.3097N 120.4279W	Rob Saich	22-Jun-00
CA	Tahoe	Soda Springs #2	30	39.3097N 120.4093W	Rob Saich	22-Jun-00
ID	Boise	Burnt Creek	30	43.3145N 115.2802W	Amanda Dabbs	22-Jun-99
ID	Boise	Greencreek Lake	28	43.3446N 115.2032W	Amanda Dabbs	15-Jun-99
ID	Boise	Miller Mountain	30	44.0838N 115.3048W	Amanda Dabbs	07-Jul-99
ID	Boise	No Name Creek	30	43.3145N 115.2802W	Amanda Dabbs	22-Jun-99
ID	Boise	Road 409	30	44.3748N 115.4447W	Amanda Dabbs	17-Jun-99
ID	Boise	Whitehawk Summit	30	44.2298N 115.5253W	Amanda Dabbs	08-Jul-99

RESULTS

Lewisia kelloggii was obviously polyploid. For evidence, see Appendix 2.

Lewisia kelloggii was highly variable. Overall, 85% of the loci were polymorphic, with more than three alleles per locus (Table 3). In the California populations, 39% to 58% of the loci were polymorphic, but in Idaho, five of the six populations had less than 20% polymorphic loci.

Populations were highly differentiated genetically, with Fst of 0.40 or higher in each state and the study as a whole (Table 4). About half the genetic differentiation in L. kelloggii was variation between states (Table 4). Inferred gene flow among populations was therefore very low, especially within Idaho and between Idaho and California (Table 3). Each state had unique alleles. Eight of the unique California alleles and three of the unique Idaho alleles occurred at frequencies greater than 0.80. There were other consistent isozyme differences between the states.

Genetic identities among populations were highly variable (Table 5). When these genetic identities are expressed diagrammatically, the populations fall easily into two groups, one from California and one from Idaho (Figure 1). The mean geneic idenity between California and Idaho populations is 0.58 Table 6), extremely low for conspecific populations (Crawford 1989).

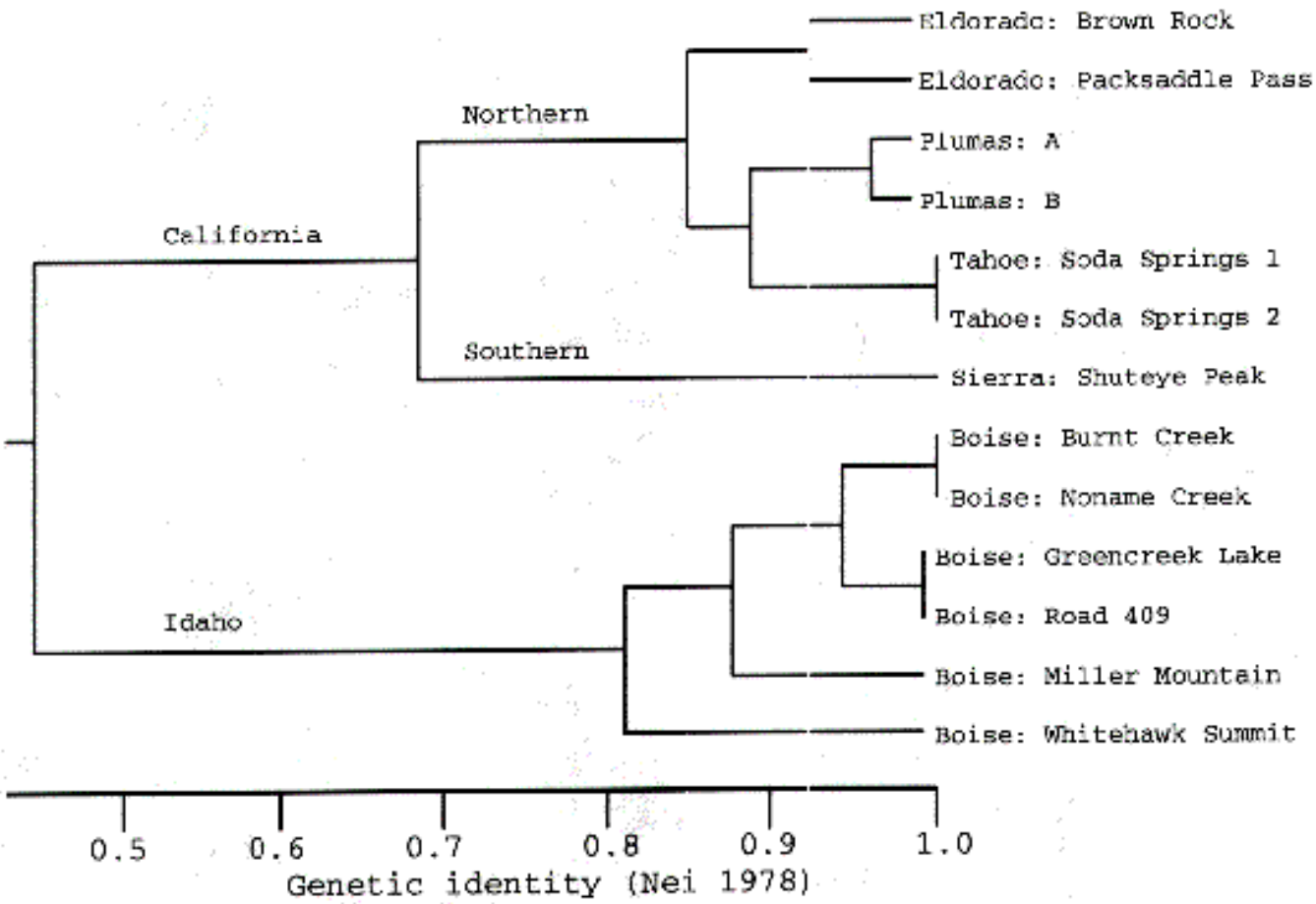


Figure 1. Similarities among *Lewisia kelloggii* populations, based on Nei's (1978) unbiased genetic distances.

Within states, about half the variation was within populations, and half was differentiation among populations (Table 4). Four California and three Idaho populations had unique alleles. Within California, populations on the same National Forest clustered together (Figure 1). Genetic identity among California populations varied from 0.69 to 0.99, and within Idaho varied from 0.76 to 0.99. Populations within 2 miles of each other were particularly similar (0.9649 for Plumas A and B, 0.9978 for Soda Springs 1 & 2, and 0.9916 for Burnt Creek and Noname Creek; Table 5).

Two Idaho populations had particularly interesting patterns of isozyme diversity. Whitehawk Summit population had no unique alleles, but every individual had certain alleles (PGI2 - 5, PGM2 - 3, and UGPP2 - 2) otherwise rare in Idaho and therefore its genetic identities with other Idaho populations were relatively low (averaging 0.82; Table 6). The Greencreek Lake population was by far the most diverse in Idaho, with 48% polymorphic loci (compared to less than 20% for each of the other Idaho populations; Tables 3 and 5).

The California population from Shuteye Peak was particularly distinct. Not only were genetic identities between the Shuteye Peak population and other California populations low (averaging 0.73; Table 6), but the population had four unique alleles and had other alleles (e.g. MDH2 allele 2) found elsewhere only in Idaho.

DISCUSSION

Genetic interpretation. Polyploidy complicated the analysis of *Lewisia kelloggii* isozyme variation. For details, see Appendix 2.

Taxonomy and genetic variation. As a species, *Lewisia kelloggii* showed great isozyme variation. Measures of isozyme diversity were much higher than average for plants with similar characteristics, and high even for widespread species (Hamrick and Godt 1990). Within each state, the variation was about as great as expected of widespread, insect-pollinated, herbaceous species. Of course, *L. kelloggii* is not widespread.

Within California, the Shuteye Peak population (Sierra National Forest) was particularly divergent. It was the southernmost population sampled in this study, and may represent a different subspecies or variety than the more northern populations. Genetic identities between Shuteye Peak and more northern California populations averaged 0.71 (range 0.66 to 0.74; Table 6), compared to expected identities of 0.90 or greater for members of the same species and subspecies. The Shuteye Peak population had some genes and band patterns that were unusual for California but were shared with the Idaho population (including the MDH1 pattern, and allele 2 of MDH2). Although earlier attempts to split off southern *L. kelloggii* as a separate taxon from the northern plants were unsuccessful (Hall and Hall 1912, Jepson 1923), perhaps the issue should be revisited.

Interestingly, for certain enzymes (e.g. MDH), some individuals in the two Eldorado National Forest have alleles that are typical of the northern populations and other individuals have the alleles seen in the southern Shuteye Peak population.

Lewisia kelloggii ssp. hutchisonii was recently described from northern California (Dempster 1996). The two populations sampled from the Plumas National Forest (Table 2) were identified as *L. kelloggii ssp. hutchisonii* (L. Janeway 1998, unpublished report to Plumas National Forest). In the absence of voucher specimens, we can not confirm that identification. If it is correct, and if the other northern specimens were *L. kelloggii ssp. kelloggii*, then *L. kelloggii ssp. hutchisonii* is so genetically similar to *L. kelloggii ssp. kelloggii* that the usefulness of this name is questionable. Alternatively, the name *L. kelloggii ssp. hutchisonii* may have been applied more broadly to these populations than is appropriate.

Three types of isozyme data show that the Idaho and California populations of *Lewisia kelloggii* probably should be considered two different species. First, about half the isozyme variation in the entire study was variation between the states (Table 4). Second, the genetic identities between Idaho and California populations averaged 0.58 (Table 6). In general, plant populations within the same species (and subspecies) have genetic identities greater than 0.90, and populations of different congeneric species have genetic identities averaging 0.68, though varying from 0.25 to 1.00 (Crawford 1989). Third, there were consistent differences between Idaho and California populations in several isozymes (Tables 5, 6 and 7). Consistent differences in isozymes are characteristic of pairs of closely related plant species. Obviously, the Idaho and California populations do not share the same gene pool. They are genetically isolated and have been isolated for a long time.

Outcrossing plants that are as genetically divergent as the Idaho and California populations of *Lewisia kelloggii* are usually treated as separate species. We hesitate to make such a recommendation based on isozyme variation alone, simply because species that have to be identified by electrophoresis have limited practical use. However, we strongly recommend that the present study be followed up with a morphological analysis of *Lewisia kelloggii*. If the recent suggestion that there are morphological differences between the plants growing in the two states
(D. Taylor, pers. comm. to T. Prendusi, Regional Botanist, Region 4, USDA Forest Service) is confirmed, the Idaho populations should be formally described as a separate species.

Conservation. *Lewisia kelloggii* populations in Idaho and California should be managed as distinct

entities. They are as distinct as two different species and perhaps should be treated taxonomically as species. Both of these genetically distinct units should be preserved.

Conserving the great isozyme variation seen in *Lewisia kelloggii* requires preserving the plants in each region (e.g. each National Forest) where it occurs. Individual populations are highly differentiated; about half the variation with states was variation among populations. However, groups of populations growing within two miles of each other can be managed as units because they are very similar.

In Idaho, two populations should be particularly high priorities for conservation. Most Idaho populations showed relatively little variation, but the Greencreek Lake population was as variable as California populations (Table 3). It had genes not observed in the other sampled Idaho populations. Some of these appear to be genes shared with California populations. The Whitehawk Summit population had very little variation, but some of its genes were unusual for Idaho, and it was the most genetically divergent of that state's populations.

In California, the National Forests were all differentiated, suggesting that it is important to conserve this rare plant in every National Forest where it occurs. The Shuteye Peak population (Sierra National Forest) was particularly divergent. We do not know if it is typical of southern populations because it was the only southern population sampled. At least until more is known about genetic diversity in southern *L. kelloggii*, the distinctive Shuteye Peak population should be a priority for conservation.

ACKNOWLEDGEMENTS

We thank Suellen Carroll, Patricia Guge, and Randy Meyer at the National Forest Genetic Electrophoresis Laboratory for technical support. We thank Bruce Bartholemew at CAS and Barbara Ertter at UC for information about Lewisia kelloggii type specimens. The project was initiated by Chris Frisbee, USDA Forest Service, and is NFGEL Project #74. This report was prepared by Barbara Wilson and Valerie Hipkins.

REFERENCES

Brandegee, K. 1894. Studies in Portulacaceae. Proc. Calif. Acad. Sci. Series 2, 4: 86-91.

Conkle, M. T., Hodgskiss, P. D., Nunnally, L. B., and Hunter, S. C. 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. 1989. Enzyme electrophoresis and plant systematics. pp. 146 - 164 in D. E. & P. S. Soltis, eds. Isozymes in Plant Biology. Dioscorides Press, Portland, Oregon.

Curran, in 1894. Brandegee, K. 1894. Studies in Portulacaceae. Proc. Calif. Acad. Sci. Series 2, 4: 86-92.

Davidson, B. L. 2000. Lewisias. Timber Press, Portland, Oregon.

Dempster, L. T. 1996. A new subspecies of *Lewisia* (Portulacaceae) in California Madroño 43: 415-416.

Gottlieb, L. D. 1981. Electrophoretic evidence and plant populations. Prog. Phytochem. 7: 1-46.

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

Hall, H. M. and C. C. Hall. 1912. A Yosemite Flora. Paul Elder & Co., San Francisco.

Hamrick, J. L., and Godt, M. J. W. 1990. Allozyme diversity in plant species, pp. 43-63 in Brown, A. H. D., Clegg, M. T., Kahler, A. L., and Weir, B. S., eds., Plant Population Genetics, Breeding, and Genetic Resources. Sunderland, Massachusetts: Sinauer Associates

Hartl, D. L, and Clark, A. G. 1989. Principles of Population Genetics, 2nd edition. Sinauer Associates, Sunderland, Massachusetts.

Hershkovitz, Mark A. 1990. Nomenclatural changes in Portulacaceae. Phytologia 68: 267-270.

Jepson, W. L. 1923. A Manual of the Flowering Plants of California. University of California, Berkeley, California. (p. 352)

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

Lewontin, R. C. 1972. The apportionment of human diversity. Evolutionary Biology 6: 381 - 398.

Matthew, B. 1989. The Genus *Lewisia*. Timber Press, Portland, Oregon.

Missouri Botanic Garden. (website accessed 30 August 2001). Index to Plant Chromosome Numbers (IPCN). http://mobot.mobot.org./W3T/search/ipcn.htm.

Moldenke, A. R. 1973. A contribution to a chormosome atlas of the California flora. Technial Report 73-22. University of Califoria, Santa Cruz, California.

Nei, M. 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.

Pitel, J. A., and W. M. Cheliak. 1984. Effects of extraction buffers . . . .

Rydberg. 1932. North American Flora 21: 326.

Soltis, D. E., and Rieseberg, L. H. 1986. Autopolyploidy in *Tolmiea menziesii* (Saxifragaceae): genetic insights from enzymes electrophoresis. American Journal of Botany 73: 310-318.

USDA Forest Service. 2000. National Forest Genetic Electrophoresis Laboratory Standard Operating Procedures. NFGEL, USDA Forest Service, Camino, California.

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

Wefald, M. (accessed August 2001) TRS2LL. Online. Available http://www.crl.com/~wefald/trs2ll.html.

Weir, B. S. 1990. Genetic Data Analysis. Sunderland, Massachusetts: Sinauer.

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

Table 3. Genetic variation in *Lewisia kelloggii* populations. n = average number of samples per locus. P = percent polymorphic loci. A = average number of alleles per locus. Ae = effective number of alleles per locus (Kimura and Crow 1964). Ho = observed heterozygosity. He = expected heterozygosity. SW = Shannon's information index (Lewontin 1972). Fst = fixation index = (He-Ho)/He. Nm = calculated gene flow.

Forest: Population	n	P	A	Ae	Ho	He	SW	Fst	Nm
Total	**370**	**85**	**3.364 (1.597)**	**1.724 (0.571)**	**0.057 (0.072)**	**0.352 (0.222)**	**0.608 (0.391)**	**0.6876**	**0.1136**
California	**196**	**82**	**2.848 (1.372)**	**1.563 (0.633)**	**0.088 (0.130)**	**0.276 (0.228)**	**0.488 (0.398)**	**0.4354**	**0.3242**
Eldorado: Brown Rock	32	52	1.758 (0.902)	1.392 (0.522)	0.107 (0.179)	0.203 (0.236)	0.322 (0.375)		
Eldorado: Pack Saddle Pass	32	58	1.939 (0.998)	1.450 (0.615)	0.127 (0.205)	0.216 (0.250)	0.354 (0.406)		
Plumas: A	27	39	1.636 (0.929)	1.267 (0.489)	0.036 (0.074)	0.136 (0.216)	0.228 (0.353)		
Plumas: B	18	42	1.576 (0.792)	1.276 (0.456)	0.077 (0.152)	0.150 (0.217)	0.238 (0.342)		
Sierra: Shuteye Peak	30	45	1.606 (0.748)	1.184 (0.296)	0.069 (0.134)	0.117 (0.169)	0.197 (0.272)		
Tahoe: Soda Springs #1	30	48	1.667 (0.816)	1.278 (0.489)	0.103 (0.194)	0.144 (0.214)	0.237 (0.343)		
Tahoe: Soda Springs #2	30	39	1.576 (0.830)	1.257 (0.454)	0.076 (0.161)	0.135 (0.212)	0.218 (0.337)		
Idaho	**174**	**58**	**1.879 (0.927)**	**1.258 (0.403)**	**0.024 (0.049)**	**0.146 (0.198)**	**0.241 (0.311)**	**0.6174**	**0.1549**
Burnt Creek	30	12	1.121 (0.331)	1.040 (0.139)	0.020 (0.075)	0.028 (0.091)	0.044 (0.138)		
Greencreek Lake	27	48	1.697 (0.847)	1.287 (0.531)	0.069 (0.138)	0.144 (0.216)	0.242 (0.345)		
Miller Mountain	30	18	1.182 (0.392)	1.072 (0.176)	0.005 (0.019)	0.049 (0.118)	0.077 (0.180)		
Noname Creek	30	15	1.152 (0.364)	1.082 (0.236)	0.021 (0.060)	0.049 (0.133)	0.073 (0.190)		
Road 409	29	18	1.212 (0.485)	1.089 (0.228)	0.029 (0.073)	0.057 (0.135)	0.089 (0.207)		
Whitehawk Summit	30	9	1.091 (0.292)	1.035 (0.149)	0.008 (0.041)	0.023 (0.086)	0.036 (0.127)		

Table 4. F-statistics (fixation indices) for *Lewisia kelloggii*, for a 3-level sampling hierarchy (individuals within populations within states within total).

Comparison			F	
individual	within	population	Fip =	0.4833
individual	within	state	Fis =	0.7549
individual	within	total	Fit =	0.8836
population	within	state	Fps =	0.5257
population	within	total	Fpt =	0.7747
state	within	total	Fst =	0.5250

Table 5. Nei's (1978) unbiased measures of genetic identity and genetic distance among *Lewisia kelloggii* populations. Genetic identify above diagonal and genetic distance below diagonal.

Pop ID	1	2	3	4	5	6	7	8	9	10	11	12	13
1 Brown Cr.	****	0.925	0.864	0.897	0.744	0.872	0.867	0.632	0.698	0.622	0.649	0.672	0.552
2 Packsaddle	0.078	****	0.835	0.845	0.745	0.833	0.827	0.601	0.675	0.600	0.619	0.649	0.574
3 Plumas A	0.146	0.181	****	0.965	0.739	0.873	0.874	0.579	0.636	0.533	0.595	0.635	0.577
4 Plumas B	0.109	0.168	0.036	****	0.745	0.909	0.907	0.608	0.661	0.558	0.626	0.665	0.564
5 Shuteye	0.296	0.294	0.302	0.295	****	0.690	0.695	0.567	0.632	0.535	0.580	0.607	0.587
6 Soda Spr.1	0.137	0.183	0.136	0.096	0.369	****	0.998	0.472	0.560	0.484	0.488	0.535	0.471
7 Soda Spr.2	0.143	0.190	0.134	0.097	0.364	0.002	****	0.478	0.557	0.484	0.492	0.534	0.470
8 Burnt Cr.	0.458	0.509	0.547	0.497	0.567	0.750	0.737	****	0.924	0.832	0.992	0.927	0.764
9 Greener. L.	0.360	0.393	0.452	0.413	0.458	0.580	0.585	0.079	****	0.907	0.947	0.979	0.860
10 Miller Mt.	0.474	0.511	0.630	0.583	0.626	0.726	0.726	0.183	0.098	****	0.866	0.896	0.826
11 Noname Cr.	0.432	0.480	0.519	0.469	0.544	0.718	0.709	0.008	0.055	0.144	****	0.940	0.788
12 Road 409	0.397	0.433	0.453	0.407	0.499	0.625	0.627	0.076	0.021	0.109	0.062	****	0.862
13 Whitehawk	0.595	0.555	0.550	0.573	0.532	0.753	0.756	0.269	0.151	0.191	0.238	0.149	****

Table 6. Summary of genetic identities among *Lewisia kelloggii* populations.

Comparison	Mean	Standard Deviation	Maximum	Minimum
Among California	0.8405	0.0853	0.9978	0.6915
Among California, except Shuteye	0.8860	0.0488	0.9978	0.8269
Between Shuteye and other California	0.7266	0.0260	0.7453	0.6915
Among Idaho	0.8874	0.0668	0.9916	0.7642
Among Idaho, except Whitehawk	0.9210	0.0484	0.9916	0.8324
Between Whitehawk and other Idaho	0.8202	0.0433	0.8617	0.7642
Between California and Idaho	0.5789	0.0637	0.6976	0.4694

APPENDIX 1: METHODS

Two to three leaves per plant were collected from each of approximately 30 plants per population in seven California and six Idaho populations (Table 1). Leaves from each individual were bagged separately from those of other individuals, and they were shipped to NFGEL on ice. Collection locations were reported in terms of TRS and converted to latitude/longitude using the program TRS2ll (Wefald 2001). One 7 mm

diameter leaf disk was submerged in each of three microtiter plate wells containing two drops (100 µl) of a 0.1 M Tris-HCl (pH 8.0) extraction buffer, with 10% (w/v) polyvinylpyrrolidone-40, 10% sucrose, 0.17% EDTA (Na2 salt), 0.15% dithiothreitol, 0.02% ascorbic acid, 0.10% bovine albumin, 0.05% NAD, 0.035% NADP, and 0.005% pyridoxal-5-phosphate (UDSA Forest Service 2000; Pitel & Cheliak 1984). Samples were frozen at -70ºC. On the day of electrophoresis, samples were thawed and ground and the extracts were absorbed onto 3 mm wide wicks prepared from Whatman 3MM chromatography paper.

Methods of sample preparation and electrophoresis follow the general methodology of Conkle et al. (1982), with some modifications (USDA Forest Service 2000). All enzymes were resolved on 11% starch gels. 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 alcohol dehydrogenase (ADH), fluorescent esterase (FEST), leucine aminopeptidase (LAP), phosphoglucomutase (PGM), and phosphoglucose isomerase (PGI). A sodium borate electrode buffer (pH 8.0) was used with a Tris citrate gel buffer (pH 8.8) (Conkle et al. 1982) to resolve glucose-6-phosphate dehydrogenase (G6PDH), glutamate-oxaloacetate transaminase (GOT), glucose-6-phosphate dehydrogenase (G6PDH), triosephosphate isomerase (TPI), and uridine diphosphoglucose pyrophosphorylase (UGPP). A morpholine citrate electrode and gel buffer (pH 6.1) (USDA Forest Service 2000) was used to resolve isocitrate dehydrogenase (IDH), phosphogluconate dehydrogenase (6PGD), and malate dehydrogenase (MDH). Enzyme stain recipes follow USDA Forest Service (2000).

Initially, two people independently scored each gel, assuming that *Lewisia kelloggii* was diploid, like species examined in a previous study (NFGEL project 36; *Lewisia cantelovii*, *L. condonii*, and *L. serrata*). When they disagreed, a third person resolved the conflict. For further quality control, 10% of the individuals were run and scored twice. In addition, gels were photographed for future reference. Genetic interpretations were inferred directly from isozyme phenotypes based on knowledge of the generally conserved enzyme substructure, compartmentalization, and isozyme number in higher plants (Gottlieb 1981, 1982; Weeden and Wendel 1989), and following a previous study of *Lewisia isozymes* (NFGEL project 36). However, complex patterns consisting of four or more bands demonstrated that *Lewisia kelloggii* must be polyploid. The samples were rescored as tetraploids by one of us (BLW), using photographs of the gels plus the initial scores, and at least some of the samples in eleven of the populations were run and scored again.

We are not aware of any published chromosome count for *Lewisia kelloggii*. The data were analyzed as 33 diploid loci. One locus each was scored for ADH, FEST, and PGM1. Two loci were scored for G6PDH, GOT2, IDH1, LAP2, MDH2, PGI2, PGM2, TPI1, TPI2, UGPP1, UGPP2, 6PGD1, and 6PGD2. Four loci were scored for MDH1. (See Discussion for explanation). For enzymes scored as pairs of loci, an isozyme band pattern consisting of a single band was considered to consist of two homeologous loci. All alleles detected in this study were assigned to only one of the two homoeologous loci, unless the observed band combinations or intensities suggested that two alleles belonged to different loci. That is, we assumed one locus of each pair of homeologous loci was invariant, unless band patterns indicated this was not the case. In addition, one haploid locus was scored for PGI1 (which is encoded in the chloroplast) but it was not included in analysis.

Results were analyzed using Popgene, version 1.21 (Yeh et al. 1997). A locus was considered polymorphic if an alternate allele occurred even once. We calculated unbiased genetic distances (Nei 1978), expected heterozygosity (Nei 1973), and gene flow [Nm (the effective number of migrants per year) = 0.25(1-Fst)/Fst; Slatkin and Barton 1989]. The fixation indices for populations (F) were calculated in Popgene (Yeh et al. 1997) following Hartl and Clark (1989), but F statistics for the hierarchy of regions within the species (Fpt), populations within the species (Fst), populations within regions (Fsp), and individuals within the species (Fit), regions (Fip), and populations (Fis) were calculated by the method of Weir (1990); the two methods produced slightly different values for F. Dendrograms based on unbiased genetic distances (Nei 1978) were generated using UPGMA.

APPENDIX 2: POLYPLOIDY

Interpreting isozyme band patterns as genotypes requires some knowledge of the plant's diploid or polyploid nature. We are not aware of a published chromosome number for *Lewisia kelloggii*. Certainly none is listed in Moldenke (1973), Matthew (1989) or in the Index of Plant Chromosome Numbers (Missouri Botanic Garden 2001). This lack of information may result from technical difficulties. Root tip squashes are unclear in *Lewisia*, making accurate counts difficult (Matthew 1989). Anthers from very immature flower buds are preferred, but collecting young enough buds is difficult in a short-stemmed plant like *L. kelloggii* even if plants are readily available, which they are not; *Lewisia kelloggii* is very difficult to maintain in cultivation (Matthew 1989).

We began our interpretation with the assumption that *Lewisia kelloggii* is diploid, an assumption that proved feasible in an earlier study of other *Lewisia* species (NFGEL project 36, unpublished; *Lewisia cantelovii*, *L. condonii*, and *L. serrata*). However, isozymes demonstrated that *L. kelloggii* must be polyploid. Evidence for the polyploid nature of *L. kelloggii* includes complicated band patterns with four or more bands and populations showing fixed or nearly fixed heterozygosity. We assumed that the plant was tetraploid and scored two loci (four alleles) for each enzyme (each region of activity on the gel). However, a tetraploid interpretation seemed inappropriate for ADH, FEST, and PGM1 because in some populations most individuals were homozygous for one of two alleles, heterozygotes were rare, and the heterozygotes all appeared balanced. Therefore, these enzymes were scored as diploid. The isozyme patterns in MDH1 involved 5 to 6 bands in some populations and could only be explained by the presence of four interacting loci.

Inferring variable numbers of loci for regions of activity on isozyme gels is unsatisfying, but may be consistent with chromosome evolution in *Lewisia*. Published chromosome numbers in the genus include 20, 24, 28, 30, 56, and 66 (Table 7). (The species considered most closely related to *L. kelloggii* is *L. brachycalyx* Engelmann ex A. Gray with 2n = 20.) Apparently *Lewisia* evolution has featured the loss, duplication, or fusion of chromosomes, as well as simple polyploidy.

Although *L. kelloggii* is clearly polyploid, we are not certain that it is tetraploid. Evidence for possible higher polyploidy included consistent uneven band staining with one homodimer much darker than either the heterodimer or the other homodimer (GOT, MDH1, PGI2, and 6PGD1) and certain unexpectedly common multibanded genotypes, such as the one scored as 1136 in PGI2. However, this evidence was unclear, and we chose to treat *L. kelloggii* was tetraploid.

We are not certain that the Idaho and California populations have the same chromosome number. The kind of isozyme band pattern that result from three or more alleles occurred in only one Idaho population, and fixed heterozygosity was less common in Idaho than in California. These data suggest that Idaho plants may have a lower chromosome number than California plants, although they may be an artifact of the lower genetic variation in Idaho populations (Table 3).

Evidence about the mode of inheritance in homeologous loci was mixed. Some enzymes in some populations exhibited fixed or nearly fixed heterozygosity expected of allotetraploids with disomic inheritance. On particularly clear gels, such as those for TPI2, unequal staining intensities suggested that the allele combinations 11 11, 11 14, and 11 44 occurred. If inheritance were autotetraploid or allotetraploid but tetrasomic, the combinations 1444 and 4444 would have been observed, but they were not. On the other hand, some enzymes in some populations gave evidence for tetrasomic inheritance (Soltis and Rieseberg 1986). For example, individuals in some populations showed alternate homozygous conditions. In three cases (ADH, FEST, and PGM1), the alternate homozygous states plus balanced staining in heterozygotes led us to interpret the enzymes as diploid, but in the other cases we scored the enzyme as tetraploid because of unequal staining intensities in heterozygotes, fixed heterozygosity in some populations, and/or the occurrence of some individuals with more bands than could be explained in a diploid. We hypothesized that *L. kelloggii* was an allotetraploid with disomic inheritance.

In order to perform a genetic analysis of *Lewisia kelloggii* isozymes, we had to make assumptions about polyploidy and mode of inheritance. Although each assumption is a reasonable hypothesis based on evidence, it may be wrong; *L. kelloggii* may not be a tetraploid with disomic inheritance. Fortunately, this study's implications for taxonomy and conservation genetics are clear and are robust to errors in our hypotheses.

Table 7. Published chromosome numbers of Lewisia. See Matthew (1989) for references. * = recently moved to a different genus: Cistanthe tweedyi (A. Gray) Hershkovitz 1990.

Chromosome number	Species
2n = 20	*L.brachycalyx* G. Engelmann ex A. Gray
2n = about 24	*L. congdonii* (Rydberg) S. Clay
2n = 28	*L. cantelovii* J. T. Howell *L. cotyledon* (S. Watson) B. L. Robinson *L. leeana* (T. C. Porter) B. L. Robinson *L. rediviva* Pursh
2n = 30	*L. columbiana*(T. J. Howell ex A. Gray) B. L. Robinson
2n = about 56	*L. nevadensis* (A. Gray) B. L. Robinson
2n = about 66	*L. pygmaea* (A. Gray) B. L. Robinson
2n = 92	*L. tweedyi* (A. Gray) B. L. Robinson*

Genetic diversity in Broadleaf Lupine (*Lupinus latifolius*) accessions from the Mt. Hood National Forest

MANAGEMENT SUMMARY

OBJECTIVES

1. Estimate levels of genetic diversity within and among populations of broadleaf lupine. Information from this will be used to answer questions about sampling wild populations for seed collections. Do we need to sample just a few or many different populations to provide a broad genetic base for this species?

   Presumed diploid and presumed tetraploid populations of *Lupinus latifolius* all are highly variable. Individuals are differentiated, but (within each ploidy level) populations are little differentiated. Therefore, collecting seed from many individuals from relatively few populations would be sufficient to provide a broad genetic basis for a cultivated seed source.

2. Verify there are no consistent major differences which might imply different taxa or hybrids.

   Isozyme data do not readily support the hypothesis that the study includes more than one species. Within each ploidy level, populations are little differentiated. The presumed tetraploid populations (large populations 1, 7, and 8, and small sample 18) are strongly differentiated from the remaining populations (although genetic distances between these populations and the presumed diploid populations are within the range expected of conspecific plants. Populations 1, 7, 8, 18, and 19 differ from other populations in allele frequencies, not because they have alternate alleles (fixed differences). All alleles that were unique to the presumed diploid or presumed tetraploid populations were rare. Polyploidy and its immediate consequences do not imply that the presumed tetraploid populations should be recognized taxonomically. If observed isozyme differentiation coincides with morphological differentiation, some sort of formal taxonomic separation may be warrented, most likely at an intraspecific level.

INTRODUCTION

Guidelines for transferring seed of Broadleaf Lupine (*Lupinus latifolius* J. Agardh; Fabaceae) in restoration projects will be based in part on its genetic variation, as assessed using both morphological

and isozyme markers. In 1995 and 1996, seeds were collected from 83 locations on the Mt. Hood National Forest, Oregon. These seeds were used to establish common gardens with two replications at each of two facilities, one in Corvallis, Oregon, and one in Carson, Washington. An analysis of morphological results has been published (Doede et al.1998).

Seed from a subset of the sampled populations was sent to the National Forest Genetic Electrophoresis Laboratory (NFGEL) for isozyme analysis. Isozymes were used to evaluate how many wild populations should be sampled to provide an adequate representation of natural genetic diversity.

Identity of the plants was verified by Caitlin Cray, who found that all samples conformed to the descriptions in Hitchcock et al. 1961. However, taxa in the genus *Lupinus* are variable, prone to hybridization, difficult to identify, and often controversial. Therefore, isozymes were also used to verify that all samples were from one taxon.

METHODS

Sample collection. Seed was collected in the Mt. Hood National Forest in 1995 and 1996. Seeds collected from each maternal plant were kept separate. One to five seeds were collected from each of 4 to 32 individuals per population. Seed submitted for this study came from a total of 339 individuals from 19 populations (Table 1).

Sample Preparation. One seed from each parent plant was nicked with a scalpel and soaked in water for two hours. The seed coat was then removed and each seed was placed in a microtiter plate well containing 150 µl of a Tris buffer (pH 7.5; Gottlieb 1981). The plates were frozen at -70°C. On the morning of electrophoresis, the samples were thawed and ground, and the slurry was absorbed onto five 3-mm wide wicks prepared from Whatman 3MM chromatography paper. Two of the five wicks were returned to the freezer to serve as backup samples.

Electrophoresis. Methods of electrophoresis are outlined in USDA Forest Service (2000), and follow the general methodology of Conkle et al. (1982) except that most enzyme stains are modified. The following enzymes were examined: aconitase (ACO), alcohol dehydrogenase (ADH), acid phosphatase (ACP), catalase (CAT), fluorescent esterase (FEST), glyceraldehyde-2-dehydrogenase (GLY), 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), and triosephosphate isomerase (TPI). All scored enzymes migrated anodally, except that ACP-2 migrated cathodally. A total of 19 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, ADH, FEST-1, FEST-3, LAP, ME, and PGM. 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, GLY, TPI-1, and TPI-2. A morpholine citrate electrode and gel buffer (pH 6.0) (USDA Forest Service 2000) was used to resolve ACP-2, IDH, 6PGD, and SKD. All enzymes were resolved on 11% starch gels. Enzyme stain recipes follow USDA Forest Service (2000). 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 isozyme variants are inherited in a Mendelian fashion in legumes. Therefore, genetic interpretations were

inferred directly from isozyme phenotypes, based on knowledge of the generally conserved enzyme substructure, compartmentalization, and isozyme number in higher plants (Gottlieb 1981, 1982; Weeden and Wendel 1989).

Lupinus latifolius can be diploid or tetraploid (Hitchcock et al. 1961). Populations 1, 7, and 8 were treated as tetraploid because many individuals in each were unambiguously tetraploid, as determined by the presence of three-banded patterns in monomeric enzymes or four-banded patterns in dimeric enzymes. Other populations were treated as diploid. There are no standardized approaches for assessing genetic diversity in population that includes both diploids and autotetraploids. Three different datasets wre constructed and used for quantifying genetic diversity. (1) In the 25-locus dataset, GLYDH, GOT2, LAP1, PGI2, PGM1, and SKD were each represented by two loci each. In tetraploid populations, individuals scored as having only two alleles were scored as having missing data at the second homoeologous locus, and in diploid populations the second locus was scored as homozygous for a null allele. (2) In the 19-locus dataset, GLYDH, GOT2, LAP1, PGI2, PGM1, and SKD were represented by one locus each. For those individuals with three or four alleles at one of these loci, that locus was scored as having missing data. (3) In the 13-locus dataset, GLYDH, GOT2, LAP1, PGI2, PGM1, and SKD were entirely removed.

Results were analyzed using Popgene version 1.21 (Yeh et al. 1997) and Biosys-1, version 1.7 (Swofford and Selander 1989). A locus was considered polymorphic if an alternate allele occurred even once. Statistics calculated included unbiased genetic distances (Nei 1978), expected heterozygosity (Levene 1949), expected number of alleles/locus (Kimura and Crow 1970), 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 4) were generated using the method of Weir (1990) using Popgene (Yeh et al. 1997).

RESULTS

Unbalanced heterozygous genotypes or evidence of three or four alleles per locus were observed in 37 individuals, most in populations 1, 7, and 8. Unambiguously tetraploid genotypes occurred in six loci, GLYDH, GOT2, LAP1, PGI2, PGM1, and SKD. The other 13 loci examined could be scored as diploid in all individuals. For analysis we treated populations 1, 7, and 8 as presumed tetraploids and populations 2, 3, 4, 5, 6, 9, and 10 as presumed diploids. Possible polyploidy could not be evaluated in the small populations (populations 11 through 19) due to sample size of four plants each, except that one tetraploid was detected in population 18.

To determine the mode of inheritance, genotype frequencies were calculated for all 19 loci (or sets of homoeologous loci) in the 37 individuals that were unambiguously tetraploid. Twelve of these loci provided evidence for tetrasomic inheritance. None had fixed heterozygosity, which would have provided evidence for disomic inheritance.

In order to generate genetic diversity statistics and the cluster diagrams based on them using software designed for diploid plants, three datasets were produced. (1) In the 25-locus dataset, GLYDH, GOT2, LAP1, PGI2, PGM1, and SKD were each represented by two loci each. In tetraploid populations, individuals scored as having only two alleles were scored as having missing data at the second homoeologous locus, and in diploid populations the second locus was scored as homozygous for a null allele. (2) In the 19-locus dataset, GLYDH, GOT2, LAP1, PGI2, PGM1, and SKD were represented by one locus each. For those individuals with three or four alleles at one of these loci, that locus was scored as having missing data. (3) In the 13-locus dataset, GLYDH, GOT2, LAP1, PGI2, PGM1, and SKD were entirely removed. Allele frequencies were calculated from these datasets under the assumption that the

plants were diploid.

Both diploid and tetraploid populations were variable, with 89% of loci polymorphic loci and more than three alleles per locus (Tables 1 - 4). Most alleles occurred in both large diploid and large tetraploid populations. The ten alleles observed in only diploids or tetraploids were rare (frequency <0.05). Five of these alleles also occurred in small populations.

Genetic identities within a species are usually greater than 0.9 (Crawford 1989). Genetic identities among the ten large samples of Lupinus latifolius were greater than 0.9 (Tables 5 - 7; summarized in Table 8), except that genetic identities between tetraploid Population 1 and diploid populations varied from 0.88 and 0.901 (using the 19 locus dataset; Table 6). Population 19, represented by only four samples, differed greatly from other populations (Table 6). Within each ploidy level, populations were little differentiated (Fpt < 0.03; Table 9), but the two ploidy levels were greatly differentiated (Fpt > 0.15; Table 10).

No matter which dataset was used, the similarities among populations followed the same pattern (Figures 1 - 3). Large presumed diploid populations clustered with each other and with most small population. The three large presumed tetraploid populations clustered together. Small population 18, which had one clearly tetraploid individual, clustered more or less near the tetraploids. Small population 19 was divergent due to its unusual combination of allele frequencies, not rare alleles.

DISCUSSION

Polyploidy. Species presenting the most difficult problems for scoring isozymes are those that have two or more ploidy levels, tetrasomic inheritance, and dosage regulated gene expression. Unfortunately, Lupinus latifolius is such a species.

Some individuals of Lupinus latifolius are diploid (2n = 2X =24), and others are tetraploid (2n = 4X = 48; Hitchcock et al. 1961). In this study, L. latifolius inheritance appeared tetrasomic; unbalanced heterozygotes and alternate homozygous states were observed. Polyploids with tetrasomic inheritance have more possible genotypes than tetraploids with disomic inheritance. Many of these genotypes are difficult to distinguish, particularly the balanced heterozygotes (AABB) and unbalanced heterozygotes (AAAB and ABBB).

Dosage regulation of gene expression contributed to scoring difficulty in Lupinus latifolius. If protein production is dosage regulated, each set of homologous or homoeologous loci will produce the same total amount of protein, no matter how many loci contribute to that total. On gels, the intensity of a band is roughly proportional to the amount of protein. Production of the proteins we studied appeared to be dosage regulated in Lupinus latifolius because the bands produced by homozygous genotypes were equally dark in diploid and polyploid plants. Each band in a heterozygote is less intense than the single band of a homozygote, and bands are not equally dark. In a polyploid with three or more alleles at a locus, some bands are 1/8 to 1/16 as dark as the single band of a homozygote. These faint bands may be difficult or impossible to see. Our understanding of polyploid isozyme patterns helps us compensate for this missing information, but scoring gels with faint or missing bands is a slow, difficult process.

Despite these difficulties, NFGEL personnel inferred alleles from the Lupinus latifolius isozyme patterns and produced a data set of allele occurrences. Unfortunately, no software is available to provide genetic diversity or genetic distance statistics for tetraploids with tetrasomic inheritance. There is no consensus on how to deal with taxa which have both diploid and tetraploid individuals or populations. The most common treatment is to force all scores to a diploid format and use standard genetics software. We did this with the L. latifolius data set, as detailed in results. We provided separate analyses of

presumed diploid and tetraploid populations, as well as a combined analysis.

Isozymes patterns of 38 individuals in one small and four large populations exhibited characteristics of tetraploids; uneven band staining associated with unbalanced heterozygosity or more bands than could be produced by a diploid. Most individuals and most populations in this study appeared diploid. However, or any given locus, a tetraploid individual may have an isozyme pattern that could be scored as diploid. Some tetraploids may have diploid-like patterns at every observed locus. Therefore, we cannot be sure that each sampled population has a consistent chromosome number.

We chose to treat populations 1, 7, and 8 as tetraploid because eight to fifteen individuals had isozyme patterns typical of tetraploids, including patterns demonstrating three or more alleles per locus. We treated all the others as diploid, although three individuals in population 6 had isozyme patterns that we interpreted as unbalanced heterozygotes.

Lupinus latifolius appears to be an autopolyploid. Its isozyme patterns show that inheritance is tetrasomic.

We recommend determining chromosome numbers of these Lupinus latifolius individuals before any further molecular work, whether using DNA or isozymes. Chromosome numbers of individual plants can be determined efficiently through flow cytometry. Live leaf tissue is assayed. The Seed Lab at Oregon State University, Corvallis, Oregon, performs this laboratory technique commercially. Normally, a standard of known chromosome number is used for comparison, but in this case that would not be necessary, as long as the tested sample included both diploids and tetraploids.

Genetic diversity and population differentiation. Both diploid and tetraploid populations were highly variable, with 90% polymorphic loci and an average of more than three alleles per locus.

In all populations, observed heterozygosity was substantially lower expected and thus the inbreeding coefficient F was greater than zero (Tables 1 - 4). The values seen in diploid populations suggestthat considerable selfing or other inbreeding occurred. Inbreeding coefficients were lower for tetraploid populations (Tables 1 - 4). That does not imply that they had less inbreeding than the diploid populations. Inbreeding coefficients are calculated by comparing observed and expected heterozygosities. The expected heterozygosities used here (Tables 1 - 4) are based on the assumption that the populations are diploid (Levene 1949). Heterozygosity is expected to be much greater in autotetraploids than in diploids. The lower inbreeding coefficients for tetraploid Lupinus latifolius populations show that in tetraploids, heterozygosity was closer to that expected for diploids than it was in diploids. That is more likely the result of polyploidy itself, rather than breeding system. More accurately calculating inbreeding rates for autotetraploids is complicated.

When diploid populations were compared to other diploids, or when tetraploids were compared with tetraploids, population differentiation was low (Fpt = 0.03). However, diploid populations were greatly differentiated from tetraploids (Fpt = 0.1653).

Taxonomy. Isozyme data do not readily support the hypothesis that more than one taxon was included in this study. The most divergent population (population 19) had the same alleles as other populations, differing only in allele frequencies. This population was represented by only four individuals and therefore deviation from expected allele frequencies may be due to chance.

Larger Lupinus latifolius populations formed two groups, which seemed to correspond with chromosome numbers. Polyploids usually have characteristic morphological differences from diploids (Lewis 1980). The morphological variation observed in the Lupinus latifolius common garden study may result directly from polyploidy.

If Lupinus latifolius morphological variation is due to polyploidy, should the variants be given a formal taxonomic name? Autopolyploids are often not separated from diploids taxonomically, though the decision is made on a case by case basis (Lewis 1980). If two series of populations differing in chromosome number fail to interbreed, if they also differ consistently in morphology, habitat, and range,

and if each arose only once, these series of populations should be recognized as two different species. Not every species meets all of these standards, of course. The diploid and tetraploid populations of Lupinus latifolius may meet few of them. There are several possible problems.

First, tetraploid Lupinus latifolius may have arisen more than once from its diploid ancestors. Therefore, a particular tetraploid population's closest relatives may be diploids, rather than other tetraploid populations. This does not preclude considering diploid and tetraploid L. latifolius as two species; recurring polyploids can be named. However, it is a problem. The hypothesis that L. latifolius tetraploids have originated repeatedly can be tested by isozyme analysis of both diploid and tetraploid populations occurring at distant locations.

Second, irregularities in meiosis and fertilization may facilitate gene flow between diploids and tetraploids. Diploids may produce unreduced (diploid) pollen or ovules which (in mixed populations) may fertilize or be fertilized by the gametes of tetraploids. If so, diploids produce some tetraploid offspring. Also, the normal haploid ovules of tetraploids are functionally diploid. If they are pollinated in such a way that endosperm is produced but the ovule is not fertilized, the unfertilized diploid ovule may develop into a diploid seedling.

There is evidence that reproductive barriers between diploid and tetraploid Lupinus latifolius exist but may be porous. Diploids and tetraploids are genetically differentiated (Fpt > 0.15; Table 10). In all analyses based on genetic distances, populations 1, 7, and 8, each with several tetraploid individuals, cluster with each other, not with diploids (Figures 1 - 3). However, population 6, which clustered with diploids, had three individuals which appeared to have the unbalanced heterozygous genotypes characteristic of tetraploids. Diploid and tetraploid populations share most alleles, and the alleles unique to one group are rare. If two groups of populations represent two different species, fixed (consistent) isozyme differences between them are usually observed, but there were no fixed differences between the diploid and tetraploid L. latifolius populations observed in this study. Genetic distances among populations within a species are usually greater than 0.9 (Crawford 1989), and genetic distances among sampled L. latifolius populations vary from 0.88 to 0.995 (Table 8).

A literature search might well reveal direct experimental evidence of the success or failure of cross pollination between diploid and tetraploid legumes. In addition, the question could be addressed with Lupinus latifolius now under cultivation.

LITERATURE CITED

Conkle, M. Thompson, 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. 1989. Enzyme electrophoresis and plant systematics.

pp. 146 - 164 in D. E. & P. S. Soltis, eds. Isozymes in Plant Biology. Dioscorides Press, Portland, Oregon.

Doede, David L., Caitlin Cray, Joan Trindle, and Dale Darris. 1998.

Adaptive genetic variation of broadleaf lupine (*Lupinus latifolius*) and implications for seed transfer. pp. 12 - 17 in Rose, R. and D.L. Haase, editors. Symposium Proceedings: Native Plants, Propagating and Planting, Dec. 9-10, 1998, Corvallis, Oregon. Nursery Technology Cooperative, Oregon State University.

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.

Hitchcock, C. L., A. Cronquist, M. Ownbey, and J. W. Thompson. 1961. Vascular Plants of the Pacific Northwest. III. Saxifragaceae to Ericaceae. University of Washington Press, Seattle, Washington.

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.

Lewis, W. 1980. Polyploidy in species populations. pp. 103 - 144 *in* Lewis, W., editor. Polyploidy: Biological Relevance. Proceedings of the International Conference on Polyploidy, Biological Relevance Held at Washington University, St. Louis, Missouri, May 24 - 27, 1979. New Yrok. Plenum Press.

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.

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. 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.

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

Swofford, D. L. and R. B. Selander. 1989. Biosys-1, release 1.7. . Illinois Natural History survey, Champaign, Illinois.

USDA Forest Service. 2000. National Forest Genetic Electrophoresis Laboratory Standard Operating Procedures. NFGEL, USDA Forest Service, Camino, California.

Weeden, N. F. and J. F. Wendel. 1990. 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. pp. 5-45 in D. E. Soltis and P. S. Soltis, eds. Isozymes in Plant Biology. Dioscorides Press, Portland, Oregon.

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, the user-friendly shareware for population genetic analysis. Version 1.20. Molecular Biology and Biotechnology Centre, University of Alberta, Edmonton, Alberta

ACKNOWLEDGEMENTS

We thank Suellen Carroll, Patricia Guge, and Randy Meyer of the National Forest Genetic Electrophoresis Laboratory for technical support.

This report was prepared by Barbara Wilson and Valerie Hipkins, and are the results of NFGEL Project #72.

Table 1. Summary of genetic variability in *Lupinus latifolius* populations, based on the 25-locus dataset (see Results). 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. F = (He – Ho)/He.

	N (S.E.)	P	A (S.D.)	H_o (S.E.)	H_e (S.E.)	F
Species level						
Entire Study	337.3 (0.5)	92.0%	3.5 (0.3)	0.177 (0.035)	0.248 (0.045)	0.2863
Population level						
Entire study – Mean	17.76	56.84%	1.98	0.1680	0.2163	0.228
Diploid mean	29.6 (4.3)	63.4% (2.8)	2.1 (0.08)	0.135 (0.016)	0.185 (0.018)	0.252 (0.074)
Population 2	29.9 (0.1)	60%	2.2 (0.2)	0.128 (0.029)	0.189 (0.042)	0.234
Population 3	32.0 (0.0)	60%	2.0 (0.2)	0.142 (0.034)	0.163 (0.039)	0.129
Population 4	31.8 (0.1)	64%	2.1 (0.2)	0.119 (0.032)	0.180 (0.043)	0.339
Population 5	31.9 (0.1)	68%	2.2 (0.2)	0.130 (0.035)	0.196 (0.043)	0.337
Population 6	29.7 (0.1)	64%	2.2 (0.2)	0.132 (0.033)	0.183 (0.040)	0.279
Population 9	31.9 (0.1)	64%	2.1 (0.2)	0.129 (0.033)	0.166 (0.039)	0.223
Population 10	20.0 (0.0)	64%	2.1 (0.2)	0.168 (0.042)	0.216 (0.049)	0.222
Tetraploid mean	31.4 (0.7)	84.0% (4.0)	2.8 (0.1)	0.281 (0.008)	0.318 (0.005)	0.118 (0.026)
Population 1	31.8 (0.1)	84%	2.9 (0.2)	0.281 (0.051)	0.324 (0.047)	0.133
Population 7	31.8 (0.1)	80%	2.7 (0.2)	0.289 (0.052)	0.317 (0.048)	0.088
Population 8	30.6 (0.1)	88%	2.8 (0.2)	0.272 (0.049)	0.314 (0.046)	0.134
Small population mean	4.0 (0.0)	42.% (4.9)	1.59 (0.09)	0.156 (0.038)	0.207 (0.034)	0.247 (0.125)
Population 11	4.0 (0.0)	40%	1.5 (0.1)	0.110 (0.038)	0.189 (0.051)	0.418
Population 12	4.0 (0.0)	36%	1.5 (0.2)	0.170 (0.057)	0.181 (0.053)	0.061
Population 13	4.0 (0.0)	48%	1.6 (0.2)	0.170 (0.051)	0.217 (0.053)	0.217
Population 14	4.0 (0.0)	44%	1.6 (0.1)	0.150 (0.043)	0.171 (0.046)	0.123
Population 15	4.0 (0.0)	40%	1.5 (0.2)	0.120 (0.050)	0.196 (0.053)	0.388
Population 16	4.0 (0.0)	40%	1.6 (0.2)	0.150 (0.046)	0.220 (0.058)	0.318
Population 17	4.0 (0.0)	44%	1.6 (0.2)	0.140 (0.048)	0.213 (0.055)	0.343
Population 18	4.0 (0.0)	40%	1.6 (0.2)	0.150 (0.046)	0.189 (0.052)	0.206
Population 19	4.0 (0.0)	52%	1.8 (0.2)	0.243 (0.060)	0.285 (0.059)	0.1474

Table 2. Summary of genetic variability in the larger *Lupinus latifolius* populations, based on the 19-locus dataset (see Results). 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. F = (He – Ho)/He.

	N	P	A (S.D.)	Ae (S.D.)	H_o (S.E.)	H_e (S.E.)	S-W (S.D.)	F
Species level								
All diploids	207	89.5	3.37 (1.50)	1.45 (0.50)	0.176 (0.160)	0.248 (0.203)	0.452 (0.344)	0.290
All tetraploids	92	89.5	3.37 (1.42)	1.80 (0.60)	0.357 (0.223)	0.384 (0.213)	0.675 (0.367)	0.071
Population level								
Mean for diploids	29.6	83.3	2.46	1.44	0.178	0.243	0.418	0.267
Population 2	30	79	2.53 (1.22)	1.45 (0.51)	0.169 (0.145)	0.249 (0.205)	0.429 (0.337)	0.321
Population 3	32	79	2.26 (0.87)	1.36 (0.41)	0.188 (0.175)	0.215 (0.194)	0.364 (0.305)	0.126
Population 4	31.5	84	2.42 (1.07)	1.45 (0.59)	0.156 (0.167)	0.237 (0.221)	0.415 (0.378)	0.342
Population 5	32	89	2.58 (1.07)	1.46 (0.47)	0.171 (0.184)	0.258 (0.211)	0.429 (0.339)	0.337
Population 6	29.5	84	2.58 (0.96)	1.42 (0.45)	0.174 (0.169)	0.241 (0.200)	0.420 (0.314)	0.278
Population 9	32	84	2.47 (0.90)	1.37 (0.40)	0.170 (0.168)	0.219 (0.196)	0.382 (0.310)	0.224
Population 10	20	84	2.42 (1.17)	1.59 (0.71)	0.221 (0.217)	0.285 (0.241)	0.486 (0.424)	0.225
Mean for tetraploids	30.7 (0.6)	89.5	3.03 (0.12)	1.78 (0.00)	0.356 (0.010)	0.381 (0.003)	0.654 (0.014)	0.065
Population 1	31	89.5	3.10 (1.20)	1.78 (0.58)	0.358 (0.243)	0.381 (0.221)	0.667 (0.379)	0.062
Population 7	31	89.5	2.89 (1.10)	1.78 (0.58)	0.365 (0.243)	0.378 (0.224)	0.639 (0.372)	0.033
Population 8	30	89.5	3.10 (1.15)	1.78 (0.60)	0.346 (0.223)	0.385 (0.206)	0.657 (0.351)	0.100

Table 3. Summary of genetic variability in the small *Lupinus latifolius* samples, based on the 19-locus dataset (see Results). 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. F = (He – Ho)/He.

	N (S.E.)	P	A (S.D.)	Ae (S.D.)	H_o (S.E.)	H_e (S.E.)	S-W (S.D.)	F
Species level								
All small populations	36	84	2.89 (1.24)	1.63 (0.68)	0.204 (0.194)	0.308 (0.233)	0.539 (0.391)	0.336
Population level								
Mean	4	56	1.78 (0.14)	1.49 (0.10)	0.205 (0.050)	0.272 (0.044)	0.381 (0.066)	0.247 (0.124)
Population 11	4	53	1.63 (0.68)	1.42 (0.52)	0.145 (0.209)	0.248 (0.266)	0.335 (0.362)	0.415
Population 12	4	47	1.63 (0.83)	1.43 (0.58)	0.224 (0.311)	0.239 (0.282)	0.325 (0.395)	0.063
Population 13	4	63	1.84 (0.83)	1.50 (0.60)	0.224 (0.275)	0.286 (0.268)	0.404 (0.388)	0.217
Population 14	4	58	1.74 (0.73)	1.36 (0.47)	0.197 (0.229)	0.226 (0.237)	0.326 (0.339)	0.128
Population 15	4	53	1.68 (0.82)	1.46 (0.57)	0.158 (0.279)	0.258 (0.280)	0.350 (0387)	0.388
Population 16	4	53	1.84 (0.90)	1.56 (0.67)	0.197 (0.244)	0.289 (0.304)	0.412 (0.437)	0.318
Population 17	4	58	1.79 (0.86)	1.52 (0.68)	0.184 (0.261)	0.280 (0.285)	0.392 (0.410)	0.343
Population 18	4	53	1.74 (0.81)	1.43 (0.54)	0.197 (0.244)	0.248 (0.272)	0.351 (0.384)	0.206
Population 19	4	68	2.10 (0.94)	1.70 (0.66)	0.320 (0.310)	0.375 (0.284)	0.532 (0.480)	0.147

Table 4. Summary of genetic variability in the larger presumed diploid *Lupinus latifolius* populations, based on the 13-locus dataset (see Results). 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. F = (He – Ho)/He.

	N	P	A (S.D.)	Ae (S.D.)	H_o (S.E.)	H_e (S.E.)	S-W (S.D.)	F
Species level								
All diploids	212	84.5	2.69 (1.03)	1.32 (0.35)	0.142 (0.157)	0.199 (0.176)	0.353 (0.275)	0.284
All tetraploids	94	84.5	2.77 (1.24)	1.57 (0.46)	0.263 (0.183)	0.309 (0.207)	0.531 (0.337)	0.148
Population level								
Mean for diploids	29.6 (4.34)	77 (0.06)	2.12 (0.11)	1.17 (0.39)	0.145 (0.027)	0.197 (0.030)	0.331 (0.044)	0.259 (0.114)
Population 2	30	69	2.15 (1.07)	1.33 (0.34)	0.139 (0.143)	0.210 (0.184)	0.346 (0.279)	0.338
Population 3	32	69	1.92 (0.76)	1.26 (0.35)	0.151 (0.176)	0.163 (0.181)	0.274 (0.271)	0.072
Population 4	31.5	77	2.08 (0.86)	1.29 (0.36)	0.111 (0.140)	0.181 (0.181)	0.318 (0.303)	0.386
Population 5	32	84.5	2.23 (0.93)	1.34 (0.38)	0.130 (0.176)	0.210 (0.193)	0.362 (0.298)	0.381
Population 6	29.5	77	2.23 (0.93)	1.35 (0.40)	0.168 (0.196)	0.211 (0.199)	0.358 (0.303)	0.206
Population 9	32	77	2.15 (0.90)	1.25 (0.36)	0.125 (0.163)	0.159 (0.181)	0.274 (0.275)	0.213
Population 10	20	84.5	2.08 (0.76)	1.40 (0.39)	0.189 (0.081)	0.242 (0.196)	0.387 (0.295)	0.219
Mean for tetraploids	31.2 (0.58)	84.5 (0.00)	2.51 (0.12)	1.55 (0.03)	0.263 (0.024)	0.307 (0.009)	0.515 (0.005)	0.143 (0.064)
Population 1	31.5	84.5	2.62 (1.04)	1.53 (0.46)	0.251 (0.187)	0.296 (0.213)	0.515 (0.357)	0.152
Population 7	31.5	84.5	2.38 (0.87)	1.59 (0.49)	0.291 (0.229)	0.315 (0.221)	0.511 (0.342)	0.076
Population 8	30.5	84.5	2.54 (0.97)	1.54 (0.43)	0.246 (0.164)	0.309 (0.197)	0.521 (0.317)	0.202

Table 5. Nei's (1978) unbiased genetic identities (above the diagonal) and distances (below the diagonal) among populations of *Lupinus latifolius*, based on the 25-locus dataset (see Results).

1	7	8	2	3	4	5	6	9	10	11	12	13	14	15	16	17
****	0.992	0.998	0.926	0.918	0.928	0.927	0.925	0.928	0.932	0.942	0.937	0.940	0.924	0.910	0.935	0.977
0.008	****	0.994	0.948	0.939	0.946	0.946	0.946	0.945	0.937	0.957	0.947	0.960	0.942	0.948	0.961	0.974
0.002	0.006	****	0.942	0.938	0.945	0.945	0.938	0.945	0.948	0.950	0.954	0.954	0.941	0.936	0.944	0.984
0.077	0.053	0.06	****	0.995	0.993	0.998	0.985	0.996	0.995	0.994	0.984	0.999	0.994	0.992	0.988	0.985
0.085	0.063	0.064	0.005	****	0.990	0.998	0.99	0.997	0.992	1.000	0.984	1.000	0.995	0.993	0.982	0.980
0.074	0.055	0.056	0.007	0.010	****	0.992	0.991	0.996	0.987	0.981	0.980	0.996	0.999	0.972	0.980	0.990
0.076	0.056	0.056	0.002	0.002	0.008	****	0.989	0.998	0.997	0.999	0.990	1.000	0.991	0.994	0.979	0.987
0.078	0.056	0.064	0.015	0.010	0.009	0.011	****	0.993	0.985	0.993	0.964	0.998	0.991	0.971	0.974	0.973
0.075	0.056	0.057	0.004	0.003	0.004	0.002	0.007	****	0.989	0.993	0.983	0.998	0.998	0.980	0.978	0.989
0.070	0.044	0.054	0.005	0.008	0.013	0.005	0.015	0.011	****	1.000	0.979	1.000	0.976	0.997	0.986	0.990
0.060	0.044	0.051	0.006	0.000	0.020	0.001	0.007	0.008	0.000	****	0.988	1.000	0.986	0.993	0.991	0.991
0.066	0.055	0.047	0.016	0.017	0.020	0.010	0.037	0.017	0.021	0.012	****	1.000	0.993	0.987	0.964	0.983
0.061	0.041	0.047	0.001	0.000	0.004	0.000	0.002	0.002	0.000	0.000	0.000	****	1.000	1.000	1.000	0.987
0.079	0.060	0.061	0.006	0.005	0.001	0.009	0.009	0.002	0.025	0.014	0.007	0.000	****	0.982	0.990	0.976
0.094	0.058	0.066	0.008	0.007	0.028	0.006	0.030	0.020	0.003	0.007	0.013	0.000	0.018	****	0.992	0.962
0.068	0.040	0.058	0.012	0.018	0.020	0.021	0.027	0.022	0.014	0.009	0.037	0.000	0.010	0.008	****	0.971
0.024	0.026	0.016	0.015	0.020	0.010	0.013	0.027	0.011	0.010	0.009	0.017	0.013	0.024	0.038	0.030	****
0.065	0.067	0.062	0.065	0.056	0.048	0.046	0.034	0.055	0.041	0.039	0.072	0.034	0.076	0.083	0.085	0.029
0.123	0.094	0.091	0.102	0.111	0.087	0.095	0.109	0.101	0.095	0.139	0.085	0.074	0.087	0.078	0.094	0.102

Table 6. Nei's (1978) unbiased genetic identities (above the diagonal) and distances (below the diagonal) among populations of *Lupinus latifolius*, based on the 19-locus dataset (see Results).

	7	8	2	3	4	5	6	9	10	11	12	13	14	15	16	17	
*	0.9837	0.9832	0.8940	0.8843	0.8973	0.8957	0.8932	0.8973	0.9012	0.9092	0.9014	0.9038	0.8833	0.8631	0.8951	0.9545	0.9
64	****	0.9884	0.9212	0.9098	0.9188	0.9183	0.9197	0.9190	0.9321	0.9258	0.9146	0.9302	0.9065	0.9077	0.9286	0.9498	0.9
68	0.0117	****	0.9132	0.9085	0.9178	0.9178	0.9085	0.9181	0.9193	0.9173	0.9230	0.9191	0.9041	0.8952	0.9040	0.9634	0.9
21	0.0820	0.0908	****	0.9901	0.9875	0.9949	0.9764	0.9919	0.9893	0.9789	0.9655	0.9838	0.9805	0.9762	0.9687	0.9645	0.9
30	0.0946	0.0959	0.0099	****	0.9831	0.9944	0.9843	0.9943	0.9848	0.9878	0.9658	0.9859	0.9830	0.9774	0.9608	0.9592	0.9
84	0.0847	0.0858	0.0125	0.0171	****	0.9862	0.9847	0.9918	0.9784	0.9609	0.9604	0.9796	0.9872	0.9486	0.9575	0.9716	0.9
01	0.0852	0.0858	0.0051	0.0056	0.0139	****	0.9824	0.9953	0.9917	0.9859	0.9744	0.9903	0.9762	0.9786	0.9557	0.9683	0.9
29	0.0837	0.0960	0.0239	0.0159	0.0155	0.0178	****	0.9681	0.9754	0.9784	0.9382	0.9835	0.9771	0.9473	0.9494	0.9486	0.9
83	0.0845	0.0854	0.0081	0.0057	0.0083	0.0047	0.0120	****	0.9809	0.9777	0.9649	0.9840	0.9860	0.9598	0.9550	0.9714	0.9
40	0.0703	0.0842	0.0108	0.0153	0.0219	0.0083	0.0249	0.0193	****	0.9865	0.9582	0.9867	0.9538	0.9820	0.9647	0.9710	0.9
52	0.0771	0.0863	0.0214	0.0123	0.0399	0.0142	0.0219	0.0225	0.0136	****	0.9620	0.9838	0.9608	0.9687	0.9631	0.9639	0.9
38	0.0893	0.0802	0.0351	0.0348	0.0404	0.0259	0.0638	0.0357	0.0427	0.0388	****	0.9771	0.9701	0.9600	0.9260	0.9541	0.9
11	0.0724	0.0843	0.0164	0.0142	0.0206	0.0097	0.0166	0.0161	0.0134	0.0164	0.0232	****	0.9857	0.9872	0.9746	0.9565	0.9
41	0.0982	0.1008	0.0197	0.0171	0.0129	0.0241	0.0232	0.0141	0.0473	0.0400	0.0304	0.0144	****	0.9547	0.9642	0.9445	0.9
72	0.0969	0.1107	0.0241	0.0228	0.0525	0.0216	0.0542	0.0410	0.0181	0.0318	0.0408	0.0129	0.0464	****	0.9642	0.9233	0.9
08	0.0741	0.1009	0.0318	0.0399	0.0435	0.0453	0.0520	0.0461	0.0359	0.0376	0.0769	0.0257	0.0364	0.0365	****	0.933	0.9
66	0.0515	0.0373	0.0361	0.0417	0.0288	0.0322	0.0528	0.0290	0.0294	0.0368	0.0470	0.0445	0.0571	0.0798	0.0693	****	0.9
51	0.1148	0.1260	0.1134	0.0989	0.0895	0.0837	0.0686	0.0970	0.0798	0.0805	0.1255	0.0794	0.1377	0.1479	0.1577	0.0728	*
07	0.1573	0.1608	0.1730	0.1839	0.1494	0.1635	0.1827	0.1698	0.1651	0.2396	0.1553	0.1440	0.1565	0.1457	0.1752	0.1864	0.1

Table 7. Nei's (1978) unbiased genetic identities (above the diagonal) and distances (below the diagonal) among populations of *Lupinus latifolius*, based on the 13-locus dataset (see Results).

pop ID	1	7	8	2	3	4	5	6	9	10
1	****	0.9895	0.9961	0.9359	0.9328	0.9337	0.9354	0.9351	0.9378	0.9324
7	0.0106	****	0.9918	0.9420	0.9353	0.9285	0.9410	0.9392	0.9367	0.9429
8	0.0039	0.0083	****	0.9356	0.9344	0.9342	0.9363	0.9359	0.9381	0.9317
2	0.0663	0.0597	0.0666	****	0.9945	0.9891	0.9965	0.9864	0.9938	0.9906
3	0.0695	0.0669	0.0679	0.0056	****	0.9833	0.9967	0.9880	0.9936	0.9836
4	0.0686	0.0742	0.0680	0.0110	0.0168	****	0.9905	0.9909	0.9946	0.9750
5	0.0668	0.0608	0.0659	0.0035	0.0033	0.0095	****	0.9950	0.9970	0.9857
6	0.0671	0.0627	0.0663	0.0137	0.0121	0.0092	0.0050	****	0.9939	0.9869
9	0.0642	0.0654	0.0639	0.0062	0.0064	0.0054	0.0030	0.0061	****	0.9837
10	0.0700	0.0588	0.0707	0.0094	0.0064	0.0253	0.0043	0.0132	0.0165	****

Table 8. Summary of genetic distances among large *Lupinus latifolius* samples (populations 1 to 10).

Comparison	Average	Maximum	Minimum
Using 13 diploid loci:			
Diploid to diploid	0.9909	0.9970	0.9750
Tetraploid to tetraploid	0.9925	0.9961	0.9895
Diploid to tetraploid	0.9359	0.9429	0.9285
Using 19 diploid loci:			
Diploid to diploid	0.9869	0.9953	0.9754
Tetraploid to tetraploid	0.9884	0.9932	0.9837
Diploid to tetraploid	0.9098	0.9321	0.8843
Using 25 diploid loci:			
Diploid to diploid	0.993	0.998	0.985
Tetraploid to tetraploid	0.995	0.998	0.992
Diploid to tetraploid	0.939	0.957	0.918

Table 9. Heirarchical F-statistics for large populations of diploid and tetraploid *Lupinus latifolius*.

Comparison			F	
13 loci; diploids				
individual	within	population	Fip =	0.2492
individual	within	ploidy level	Fit =	0.2788
population	within	ploidy level	Fpt =	0.0394
13 loci; tetraploids				
individual	within	population	Fip =	0.1290
individual	within	ploidy level	Fit =	0.1436
population	within	ploidy level	Fpt =	0.0167
19 loci; diploids				
individual	within	population	Fip =	0.2534
individual	within	ploidy level	Fit =	0.2846
population	within	ploidy level	Fpt =	0.0417
19 loci; tetraploids				
individual	within	population	Fip =	0.0497
individual	within	ploidy level	Fit =	0.0668
population	within	ploidy level	Fpt =	0.0180

Table 10. F-statistics (fixation indices) for *Lupinus latifolius*, for a 3-level sampling hierarchy (individuals within populations within ploidy level within total).

Comparison			F	
13 loci				
individual	within	population	Fis =	0.1885
individual	within	ploidy level	Fip =	0.2044
individual	within	total	Fit =	0.3359
population	within	ploidy level	Fsp =	0.0195
population	within	total	Fst =	0.1816
ploidy level	within	total	Fpt =	0.1653
19 loci				
individual	within	population	Fis =	0.1973
individual	within	ploidy level	Fip =	0.2212
individual	within	total	Fit =	0.3168
population	within	ploidy level	Fsp =	0.0297
population	within	total	Fst =	0.1488
ploidy level	within	total	Fpt =	0.1228

Figure 1. Cluster diagram based on Nei's (1978) genetic identities among *Lupinus latifolius* populations. Calculated using the 25-locus dataset (see results).

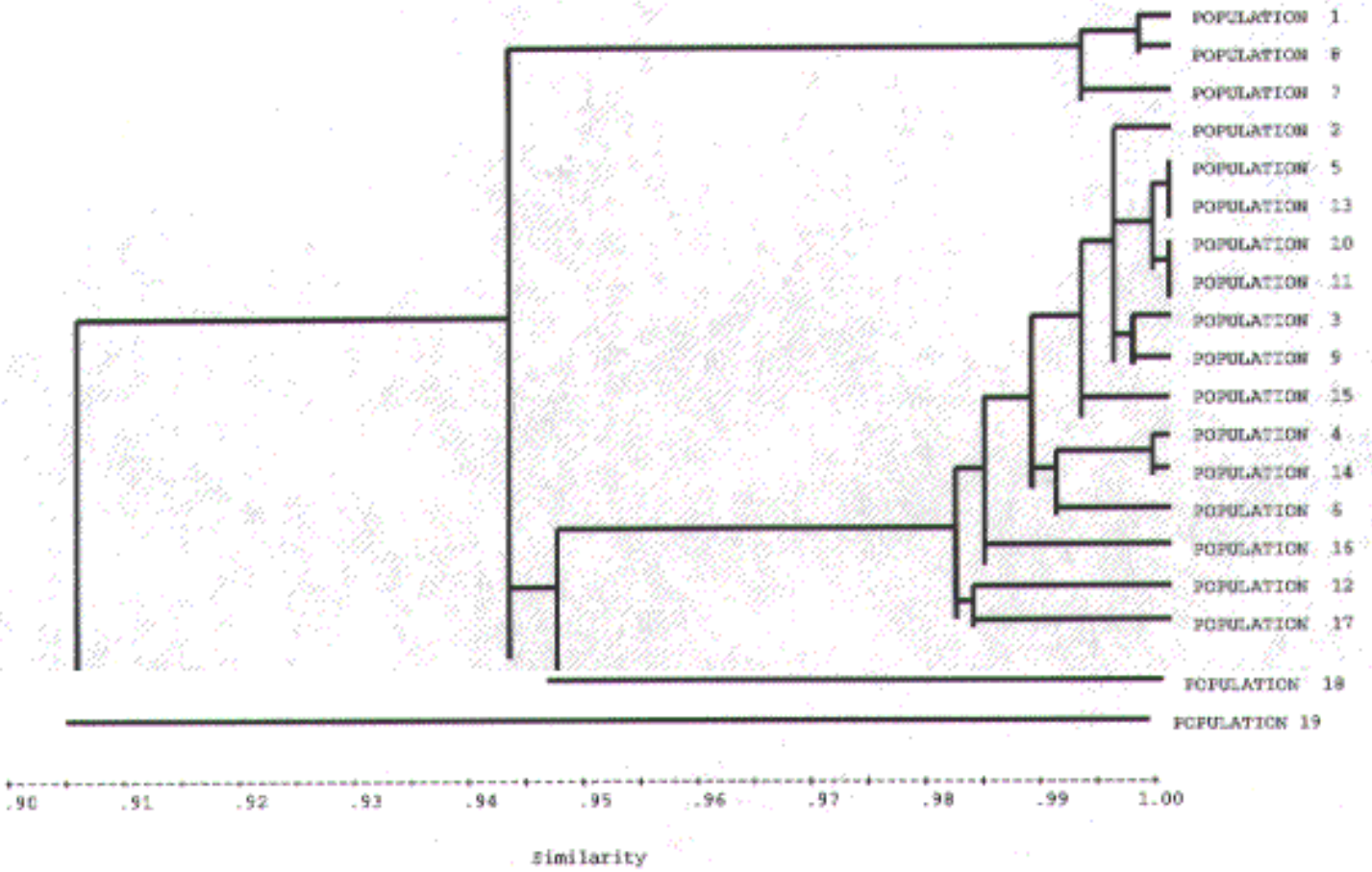


Figure 2. Cluster diagram based on Nei's (1978) genetic identities among *Lupinus latifolius* populations. Calculated using the 19-locus dataset (see results).

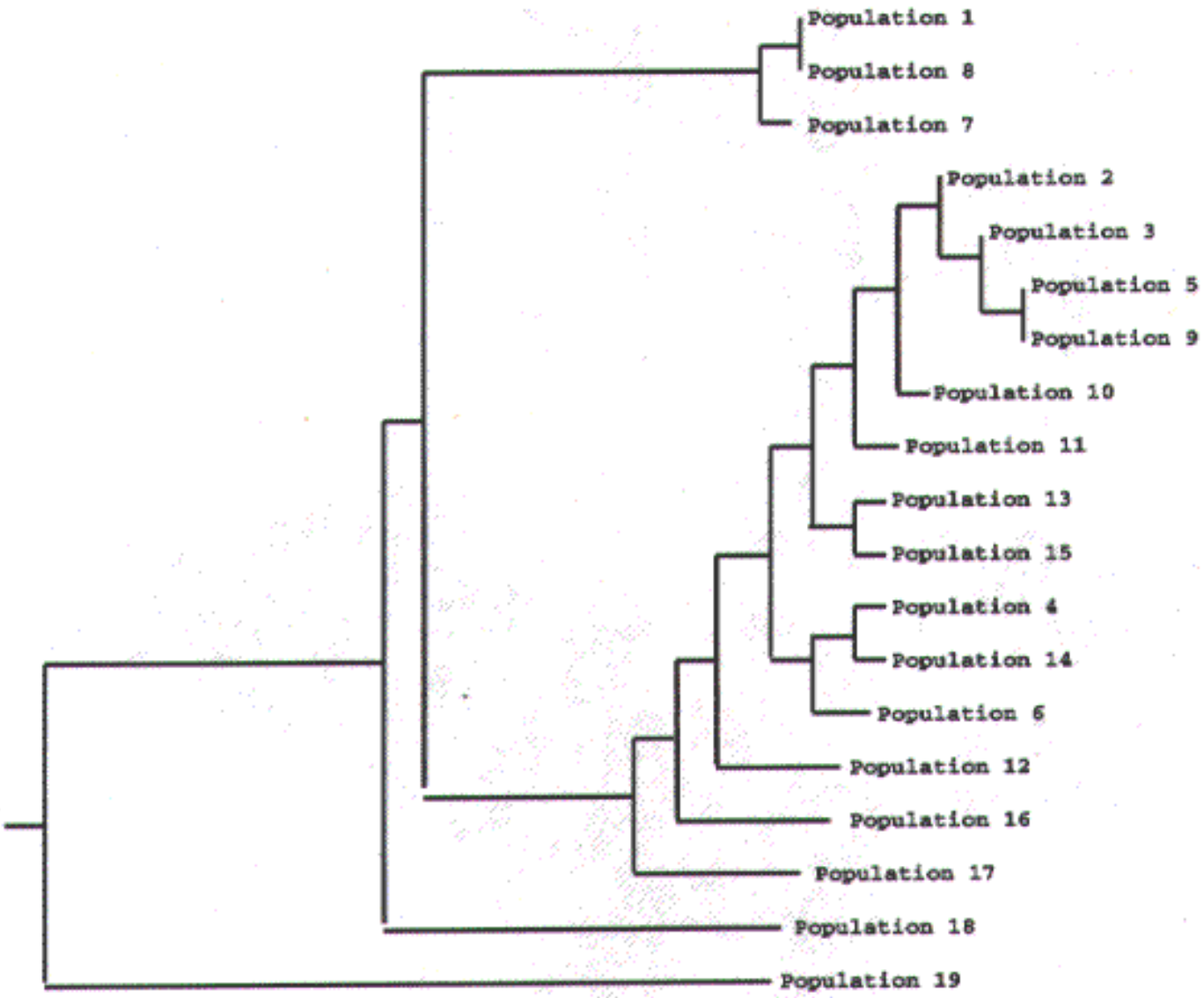
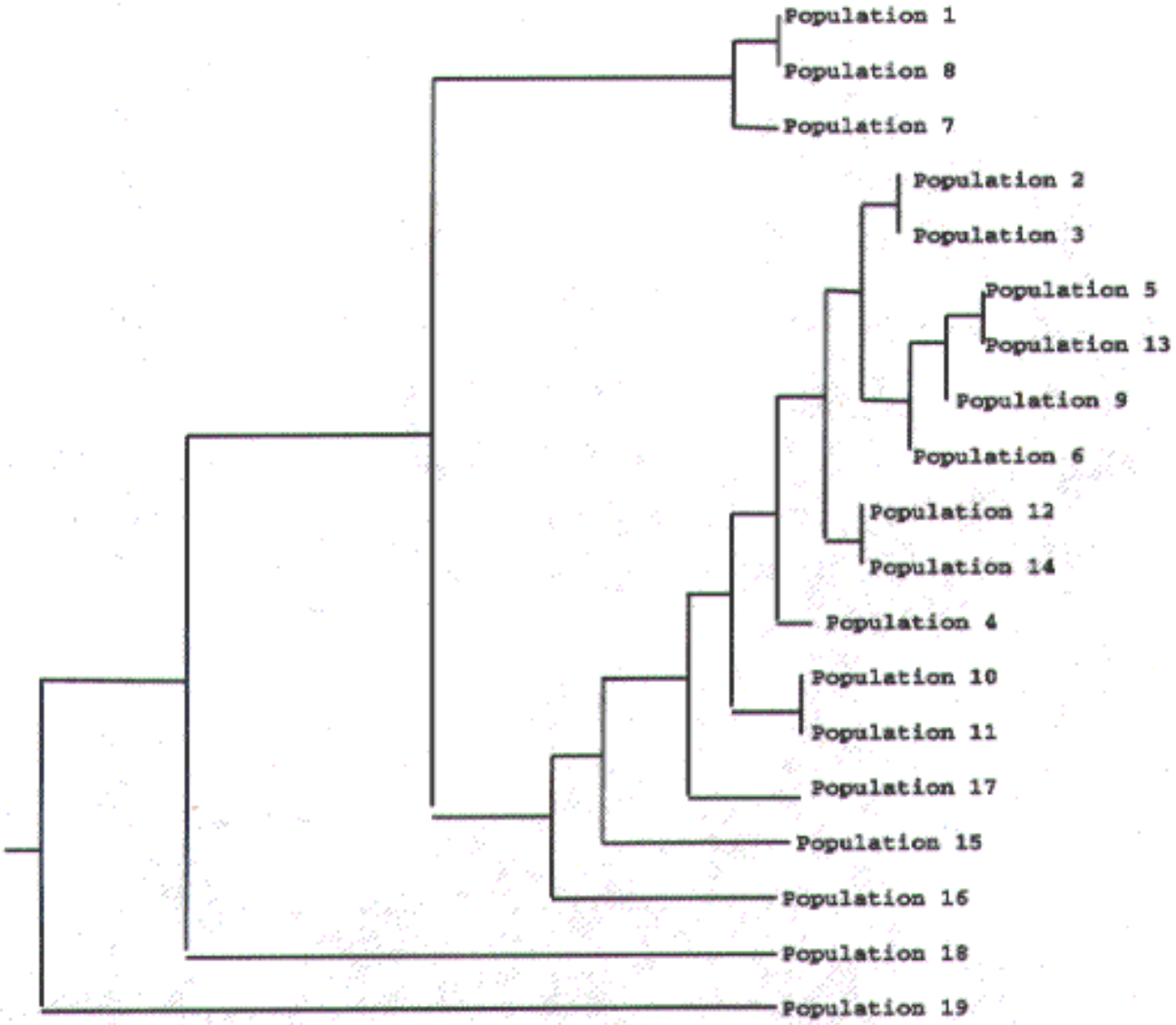


Figure 3. Cluster diagram based on Nei's (1978) genetic identities among *Lupinus latifolius* populations. Calculated using the 13-locus dataset (see results).



Staff Activities

Meetings, Shortcourses, and Workshops

Presentations

2000. V Hipkins. Issues of genetic purity and diversity for land managers. Native and Invasive Plant Conference, Ft. Collins, CO. November 29 - December 1.

2001. V. Hipkins. Gene conservation and genetic markers in the USDA Forest Service: From conifers to grasses. Western Forest Genetics Association. University of California, Davis, July 30 - August 2.

Posters

2001. Conservation Genetics - A History of NFGEL Projects. Western Forest Genetics Association. University of California, Davis, July 30 - August 2.

Attended

2001. Western Forest Genetics Association. University of California, Davis, July 30 - August 2. (R.Saich, S.Carroll, P.Guge).

2001. Southern Forest Tree Improvement Conference, Athens, GA, June 27 - 29. (V. Hipkins)

2001. Budget and Allocation Meeting. USDA Forest Service, Washington Office, Washington D.C., June 24 - 25. (V. Hipkins)

Professional Activities

Chen, Z., T.E. Kolb, K.M. Clancy, V.D. Hipkins, and L.E. DeWald. 2001. Allozyme variation in interior Douglas-fir: association with growth and resistance to western spruce budworm herbivory. Canadian Journal of Forest Research 31(10):1691-1700.

Wilson, B.L., J. Kitzmiller, W. Rolle, and V.D. Hipkins. 2001. Isozyme variation and its environmental correlates in Elymus glaucus from the California Floristic Province. Canadian Journal of Botany 79:139-153.

Samman, S., B.L. Wilson, and V.D. Hipkins. 2001. Genetic variation in Pinus ponderosa, Purshia tridentata, and Festuca idahoensis, community-dominant plants of California's yellow pine forest. Madrono 47(3):164-173.

Hipkins, V. 2001. NFGEL reaches milestone - its 100th project. Eldorado National Forests 'News Nuggets', June, and the Eldorado National Forest Interpretive Associations 'The Interpreter', September.

Peer reviewer for Canadian Journal of Forest Research, TAG, 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 (third year) (V.Hipkins).

Organization Committee for the 2001 Western Forest Genetics Association Meeting (V. Hipkins)

Participated in hosting the Institute of Forest Genetics 75th Anniversary Celebration, August 1, 2001 (R. Meyer, B. Carroll, P. Guge, S. Carroll, R. Saich)

2001 Western Forest Genetics Association conference web page;development and maintainance(R. Saich and V. Hipkins)

Developed NFGEL website (http://dendrome.ucdavis.edu/NFGEL/) (V. Hipkins and R. Saich)

Internal Activities

Member of Region 2,4,5, & 6 FFIS Fire Payment Team (S.Carroll). Member of the Eldorado National Forest Safety Committee (R.Meyer). Union Representative - Eldorado National Forest (R.Meyer). Participated in PSW Station Review (V. Hipkins)

Hosted

NFGEL continues to host a variety of visitors. Tours of the facility and operation were provided to (1) Forest Service employees representing the Research branch, the Washington Office, and three Regions of the National Forest System, (2) members of the public, both from within and outside of California, (3) private industry, (4) university faculty, (5) foreign scientists from Korea, Poland, Australia, Mexico and Canada, and (6) employees from other state and federal government agencies. NFGEL hosted groups from Modesto Jr. College and the Western Forest Genetics Conference, and a graduate student from Northern Arizona University.

Collaborations and Cooperations

NFGEL formed collaborations with FS Research Stations, Northern Arizona University (Flagstaff), Bureau of Land Management, California Department of Transportation, US Fish and Wildlife Service, University of California at Davis, and private companies. We also collaborate internally within the Agency to lend expertise in the area of genetics. This took the form of reviewing contracted work reports on the genetics of mammals and plants.

Current Staffing

Name	Position	Term	E-mail Adress
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
Robert Saich	Lab Biotechnician	Temp (10/00 - 10/01)	rcsaich@fs.fed.us
Barbara Wilson	Associate Director	Temp (8/01 - 10/01)	blwilson@fs.fed.us
Brady Carroll	Lab Biotechnician	Temp (6/01 - 8/01)	

Budget

Activity		FY98	FY99	FY00	FY01
Receipts (in thousands)					
	Allocation	290.0	307.0	290.0	290.0
	Carryover	0.0	9.9	0.2	6.9
	Soft Money	22.7	25.8	26.5	169.8
	Total	**312.7**	**342.7**	**316.7**	**466.7**
Expenditures (in thousands)					
	Salary (permanant)	142.9	165.3	171.5*	185.7**
	(temperary)	29.5	58.5	25.6	19.2
	Overhead to ENF	67.3	69.0	59.0	60.0
	Overhead to P'ville Nursery	0.0	0.0	0.0	10.9
	Chemicals/Supplies	26.4	16.5	23.6	15.7
	Equipment	22.9	10.1	3.2	164.2
	Travel/Training	5.1	8.6	11.6	5.0
	Awards	4.4	4.4	0.0	2.5
	Fees	1.5	0.5	0.5	0.3
	Books/subscriptions	0.9	0.8	1.0	0.0
	Computers (not including FOR)	0.6	4.9	3.7	0.8
	Repair	0.5	0.3	1.2	0.8
	Photos/Slides/Publications	0.4	0.5	1.2	1.2
	Postage	0.4	0.4	0.9	0.1
	Office Supplies	0.0	0.8	0.2	0.1
	Furniture	0.0	1.5	0.4	0.0
	Lab Relocation	0.0	0.0	0.0	9.7
	Total	**302.8**	**342.1**	**303.6**	**476.2**

*$5.2 salary savings due to alternate salary sources
**$11.7 salary savings due to alternate salary sources

FY 01 Soft Money

Source		Amount ($)	Percentage
FS-NFP (RMRS)	(Native Plants)	72,254	42.6%
FS-NFP (WO)	(Equipment)	85,000	50.1%
FS-SRS	(Southern pines)	5,000	2.9%
FS-R6	(Aspen)	2,500	1.5%
FS-R4	(Lewisia)	5,000	2.9%
Total		**169,754**	**100.0%**

NFGEL Projects (2001)

Project#	Collaborator	*Species*	Objective	Sample Type	Sample Size	Submission Dates	Preparation Dates	Electrophoresis Dates	Marker System	#Loci
74	Teresa Prendusi, FS-R4	*Lewisia kelloggii*	(1) Assess levels and structure of genetic variation in and among populations of Lewisia kelloggii growing in Idaho, and (2) determine genetic similarity between Idaho and California populations of L. kelloggii	leaves	385 indiv.	6/99 - 6/00	7/99 - 6/00	10/00 - 12/00	isozymes	19
94	Nick Wheeler, Weyerhaeuser Co.	*Pseudotsuga menziesii*	To determine the efficancy of supplemental mass pollination methods in a Douglas-fir seed orchard	seed (meg/embryo pairs)	5 indiv., 6 seedlots (328 seed)	3/00, 12/00	3/00, 12/00 - 2/01	3/00, 12/00 -2/01	isozymes	15
99	Kristin Kolanowski, NAU	*Pinus ponderosa*	(1) Determine the genetic variation partitioning of clumps of trees that established prior to Euro-American settlement (1876),(2) determine the genetic composition and structure of pre- and post -settlement tree,(3) compare allozyme variation of the two age groups, and(4) compare the genetic composition and structure of 5 stands prior to and after simulated random thinnings having 50,25,and 10% post-settlement retaining percentages	vegetative buds	465 indiv.	2/01	2/01	2/01	isozymes	22

98	Lisa Schicker, CalTrans	*Pinus radiata*	(1) Did the planted Monterey pine trees along Highway 1 in Monterey county near Carmel originate from one of the three natural mainland P. radiata populations (Monterey, Cambria, or Ano Nuevo)? (2) How genetically similar is the Hatton Canyon stand relative to other stands in the Monterey population? (3) Is overall genetic variation reduced in selected pitch canker resistant material compared to the species as a whole, or compared to susceptible material? (Contingent on common garden material being available at a latter date for genetic testing).	seed (megs)	254 indiv.	1/01	2/01 - 3/01	3/01 - 8/01	isozymes	26
104-119	Durant MacArthur, FS-RMRS	*Bromus carinatus, Viguiera multiflora, Astragalus utahensis, Crepis acuminata, Eriogonum umbellatum, Lupinus argenteus, Erigeron pumilus, Vicia americana*	Methods development	leaves	--	5/01 - 7/01	5/01 - 7/01	5/01 - 7/01	isozymes	--
112	Bryan Schulz, Olympic Resource Management	*Pseudotsuga menziesii*	Clonal identification. Progeny to parent identification.	vegetative buds	44 indiv.	6/01	6/01	8/01	isozymes	18

102	Richard Sniezko, FS-R6	*Chamaecyparis lawsoniana, Chamaecyparis obtusa, Chamaecyparis nootkatensis, Chamaecyparis thyoides*	Verify hybrid seedlings between CHLA, CHNO, CHOB, and CHTH	needles	52 indiv.	5/01	3/01	8/01 - 9/01	isozymes	19
97	Richard Sniezko, FS-R6	*Chamaecyparis lawsoniana, Ch. obtusa, Ch. leylandii, Ch. nootkantensis, Ch. thyoides, Cupressus macrocarpa, C. tortulosa*	Ramet/hybrid identification	needles	34 indiv., 45 primers	3/00 - 5/00	--	10/00 - 12/00	RAPDs	--
102	Richard Sniezko, FS-R6	*Chamaecyparis lawsoniana, Chamaecyparis obtusa, Chamaecyparis nootkatensis, Chamaecyparis thyoides*	Verify hybrid seedlings between CHLA, CHNO, CHOB, and CHTH	needles	54 indiv.	5/01	6/01	--	DNA Extraction	--
105	Durant MacArthur, FS-RMRS	*Viguiera multiflora*	To examine (1) gene flow and genetic population structure of source populations, seeded populations, and indigenous populations adjacent to the seeded populations, and (2) the intrapopulation and interpopulation genetic structure and variation of material being introduced to revegation and restoration treatments of disturbed wildlands.	leaves	53 indiv.	5/01	5/01 - 8/01	--	DNA Extraction	--

107	Durant MacArthur, FS- RMRS	*Erigeron pumilus*	To examine (1) gene flow and genetic population structure of source populations, seeded populations, and indigenous populations adjacent to the seeded populations, and (2) the intrapopulation and interpopulation genetic structure and variation of material being introduced to revegation and restoration treatments of disturbed wildlands.	leaves	65 indiv.	5/01	6/01 - 8/01	--	DNA Extraction	--
109	Durant MacArthur, FS- RMRS	*Crepis acuminata*	To examine (1) gene flow and genetic population structure of source populations, seeded populations, and indigenous populations adjacent to the seeded populations, and (2) the intrapopulation and interpopulation genetic structure and variation of material being introduced to revegation and restoration treatments of disturbed wildlands.	leaves	49 indiv.	5/01	6/01 -8/01	--	DNA Extraction	--

111	Durant MacArthur, FS- RMRS	*Astragalus utahensis*	To examine (1) gene flow and genetic population structure of source populations, seeded populations, and indigenous populations adjacent to the seeded populations, and (2) the intrapopulation and interpopulation genetic structure and variation of material being introduced to revegation and restoration treatments of disturbed wildlands.	leaves	32 indiv.	5/01	6/01 - 8/01	--	DNA Extraction	--
114	Durant MacArthur, FS- RMRS	*Eriogonum umbellatum*	To examine (1) gene flow and genetic population structure of source populations, seeded populations, and indigenous populations adjacent to the seeded populations, and (2) the intrapopulation and interpopulation genetic structure and variation of material being introduced to revegation and restoration treatments of disturbed wildlands.	leaves	63 indiv.	6/01	6/01 - 8/01`	--	DNA Extraction	--

116	Durant MacArthur, FS- RMRS	*Lupinus argenteus, L. sericeus*	To examine (1) gene flow and genetic population structure of source populations, seeded populations, and indigenous populations adjacent to the seeded populations, and (2) the intrapopulation and interpopulation genetic structure and variation of material being introduced to revegation and restoration treatments of disturbed wildlands.	leaves	67 indiv.	7/01	7/01- 8/01	--	DNA Extraction	--
118	Durant MacArthur, FS- RMRS	*Bromus carinatus*	To examine (1) gene flow and genetic population structure of source populations, seeded populations, and indigenous populations adjacent to the seeded populations, and (2) the intrapopulation and interpopulation genetic structure and variation of material being introduced to revegation and restoration treatments of disturbed wildlands.	leaves	22 indiv.	7/01 - 8/01	7/01 - 8/01	--	DNA Extraction	--

120	Durant MacArthur, FS- RMRS	*Vica americana*	To examine (1) gene flow and genetic population structure of source populations, seeded populations, and indigenous populations adjacent to the seeded populations, and (2) the intrapopulation and interpopulation genetic structure and variation of material being introduced to revegation and restoration treatments of disturbed wildlands.	leaves	41 indiv	7/01 - 8/01	8/01	--	DNA Extraction	--
103	Mary Frances Mahalovich, FS-R1	*Pinus ponderosa*	Source identification	needles	158 indiv.	5/01 - 9/01	6/01- 8/01	--	DNA extraction	--

Workload by Region or Agency, FY01

(1) Isozymes (starch gel electrophoresis)

By Project

Region or Agency	Project #	Species	# gels	# days	# weeks
Forest Service					
R4	74	*Lewisia kelloggii*	75	20	10.0
R3/NAU	99	*Ponderosa pine*	63	9	3.0
R6/BLM	102	*Chamaecyparis*	12	2	1.0
RMRS	104-119	development	12	1	0.5
NFGEL	--	development	6	1	0.5
CalTrans	98	Monterey pine	153	24	15.0
Weyerhaeuser	94	Douglas fir	54	10	6.0
Olympic Resource Mgt.	112	Douglas fir	14	3	1.5

By Forest Service Region or Agency

Region or Agency		# gels	# days	# weeks
Forest Service				
NFS				
	R4	75	20	10.0
	R3/NAU	63	9	3
	R6/BLM	12	2	1
	NFGEL	6	1	0.5
FSR				
	RMRS	12	1	0.5
CalTrans		153	24	15.0
Weyerhaeuser Co		54	10	6.0
Olympic Resource Mgt		14	3	1.5
TOTAL		389	70	37.5

(2) DNA

Region or Agency	Project #	species	# DNA extractions	Extraction Method	# PCR reactions	# days	# weeks
FS-NFS-R6 / BLM	97	*Chamaecyparis lawsoniana, Ch. obtusa, Ch. leylandii, Ch. nootkantensis, Ch. thyoides, Cupressus macrocarpa, C. tortulosa*	34	FastPrep	21	27	8
FS-NFS-R1	103	*Pinus ponderosa*	158	FastPrep	--	20	7
FS-FSR-RMRS	105	*Viguiera multiflora*	53	FastPrep	--	30*	13*
FS-FSR-RMRS	107	*Erigeron pumilus*	65	FastPrep	--	30*	13*
FS-FSR-RMRS	109	*Crepis acuminata*	49	FastPrep	--	30*	13*
FS-FSR-RMRS	111	*Astragalus utahensis*	32	FastPrep	--	30*	13*
FS-FSR-RMRS	114	*Eriogonum umbellatum*	63	FastPrep	--	30*	13*

FS-FSR-RMRS	116	*Lupinus argenteus, L. sericeus*	67	FastPrep	--	30*	13*
FS-FSR-RMRS	118	*Bromus carinatus*	22	FastPrep	--	30*	13*
FS-FSR-RMRS	120	*Vica americana*	41	FastPrep	--	30*	13*

BLM=Bureau of Land Management
NAU=Northern Arizona University
CalTrans=California Department of Transportation
FS=Forest Service
  FSR=Forest Service Research
    RMRS=Rocky Mountain Research Station
  NFS=National Forest System
    R#=Region