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NFGEL Annual Report 2002 – 2003 (FY03)

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Report prepared March 2004

Overview

NFGEL projects were processed to meet a variety of management objectives. Project results were used to guide restoration and conservation projects, and assist in silviculture and tree improvement activities. During FY 2003, NFGEL continued to follow its mission to "provide state-of-the-art molecular genetic information to the National Forests and other cooperating agencies for the evaluation and protection of our nation's genetic resource".

Thirteen reports, including results from 24 projects, follow.

Silviculture and Tree Improvement

(1) Clonal identification in a Douglas-fir orchard using isozymes
(2) Clonal identification in Port-Orford cedar
(3) Determining supplemental mass pollination success in Douglas-fir using SSR markers
(4) Clonal identification in a Douglas-fir orchard using isozyme and SSR markers
(5) Validation of parental material and crosses used to test for a major gene type of inheritance for resistance to the root pathogen *Phytophthora lateralis* in Port-Orford cedar
(6) Assessing SSR markers for paternity analysis in *Populus* species
(7) Identification of twelve unknown samples from Mt Ashland, Rogue River National Forest, as *Pinus albicaulis* or *P. monticola*

Conservation and Restoration

(1) Isozyme analysis of intermountain plants: progress report
(2) Genetic distribution of trembling aspen (*Populus tremuloides*) clones in the central Sierra Nevada, California
(3) Expanded study of the genetic diversity in *Perideridia erythrorhiza*: a rare plant in southern Oregon
(4) Implications of isozyme variation for the taxonomy of the rare california plant *Silene campanulata* ssp. *campanulata*
(5) Expanded evaluation of genetic diversity in tahoe yellow cress (*Rorippa subumbellata*)
(6) Characterizing ploidy level variation using flow cytometry

CLONAL IDENTIFICATION IN A DOUGLAS-FIR ORCHARD USING ISOZYMES

NFGEL Project #145

January 30, 2003

Five individuals of Douglas-fir were genotyped at 19 isozyme loci to assess their clonal identity. Data indicated that all ramets of a given clone had matching genotypes. Therefore, it appears that trees R14-C38 and R7-C45 are ramets of clone 108, and trees R13-C5, R13-C6, and R14-C36 are ramets of clone 105.

Final Data Set

Each sample was run twice. Samples were genotyped at 19 isozyme loci.

Sample	LAP	PGI	DIA1	DIA2	FEST	UGPP1	UGPP2	GLYDH	PGM1	PGM2
R14-C38-108	27	11	11	11	33	35	22	11	11	12
R7-C45-108	27	11	11	11	33	35	22	11	11	12
R13-C6-105	24	11	11	11	33	55	22	11	11	12
R14-C36-105	24	11	11	11	33	55	22	11	11	12
R13-C5-105	24	11	11	11	33	55	22	11	11	12

Sample	GOT1	GOT2	GOT3	GDH	MDH1	MDH3	6PGD	IDH	SKD
R14-C38-108	11	11	11	11	11	11	11	14	22
R7-C45-108	11	11	11	11	11	11	11	14	22
R13-C6-105	11	11	11	11	11	11	11	44	22
R14-C36-105	11	11	11	11	11	11	11	44	22
R13-C5-105	11	11	11	11	11	11	11	44	22

Date Submitted: 9/25/02 Date Prepared: 9/27/02 Date Analyzed: 01-14-03

Client: Private Company

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CLONAL IDENTIFICATION IN PORT-ORFORD CEDAR

NFGEL Project #146

March 11, 2003

Of 19 Port-Orford cedar trees received on 12/11/02 for genetic analysis, five of them do not match their respective clonal ramets based on a combination of isozyme and RAPD data. We genotyped the trees at 18 isozyme loci using starch gel electrophoresis. We also extracted genomic DNA from 17 of the trees and obtained RAPD data using 6 primers.

MATERIAL ANALYZED:

Accession #	Number of Ramets
PO-117503	3
PO-117509	2
PO-118568	3
PO-118838	3

Accession #	Number of Ramets
PO-510005	2
PO-510008	3
PO-DOR-70006	3

RESULTS:

Isozyme analysis clearly shows that:

1. 117503-1 does not match the genotype shared by ramets 117503-2 and 117503-3 (at 4 loci, see Table below), and
2. 118568-3 does not match the genotype shared by ramets 118568-1 and 118568-2 (at 3 loci).

Because these mismatches were clear, we did not assess these samples with RAPDs. We did extract DNA from the remaining 17 trees and generate RAPD markers using six primers.

RAPD data show that:

1. 118838-1 does not match the pattern shared by ramets 118838-2 and 118838-3 (at two primers, see Table below),
2. 70006-2 does not match the pattern shared by ramets 70006-1 and 70006-3 (at two primers), and
3. 510008-3 does not match the pattern shared by ramets 510008-1 and 510008-2 (at two primers).

All RAPD bands were confirmed by running samples twice. Individuals 118838-1 and 70006-2 also differed from their clonal ramets at one and two isozyme loci, respectively. Isozyme data did not distinguish any differences among the three 510008 ramets.

Mismatch	Confirmed with Isozymes:	Confirmed with RAPD primers:
117503-1	PGI-2, UGPP-1, IDH, MDH-1	not assessed
118568-3	PGI-2, TPI-1, MDH-1	not assessed
118838-1	UGPP-1	OPK-8, OPA-19
70006-2	UGPP-1, TPI-1	OPB-3, OPA-19
510008-3	-----	OPH-11, OPA-19

Date Submitted: 12/11/02 Date Report Prepared: 3/11/03

Client: USDA Forest Service, Region 6, Richard Sniezko

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DETERMINING SUPPLEMENTAL MASS POLLINATION SUCCESS IN DOUGLAS-FIR USING SSR MARKERS

NFGEL Project #148
June 18, 2003

The project objective is to determine the efficacy of supplemental mass pollination methods in a Douglas-fir (*Pseudotsuga menziesii*) seed orchard by applying SSR markers to seedlots resulting from six crosses. A secondary objective is to check the clonal identification of the various ramets. This work was performed with SSR markers developed by the Pacific Northwest Tree Improvement Research Cooperative (PNWTIRC), Oregon State University, Corvallis, OR, and the data analyzed with the assistance of Gancho Slavov and Glenn Howe (PNWTIRC).

METHODS

Stratification and Germination. Seed were soaked in cotton bags for 24 hours in a 4L beaker with airation at 21° C. Seed bags were removed from soaking, squeezed lightly to remove excess water, and then placed in each of two 4" X 8" plastic bags, the open end wrapped slightly loose with rubber band to allow for oxygen exchange. Bags were set flat on walk in refrigerator shelf for a period of 30 days at 1 – 3° C. After stratification, seed were plated in 4"WX5"L plastic germ boxes containing kim-pak that was dampened with ddH2O. Germ boxes were placed in a germinator that was set to expose 12 hours of light at 30° C, and 12 hrs of dark at 20° C.

DNA Extraction. Embryos were removed from germinated seed when they reached approx. 2 cm in length (30-40 mg) and extracted using the Qiagen DNeasy 96 fresh tissue protocol for a minimum of 81 progeny per cross. DNA from nine samples of needle &/or bud tissue for clonal identification purposes was extracted using the Qiagen DNEasy Maxi-kit.

Isozyme Analysis. Nine samples of needle tissue were prepped for isozyme analysis using NFGEL Standard Operating Procedures.

SSR Analysis. Methodology is outlined in "Highly variable SSR markers in Douglas-fir: Mendelian inheritance and map locations", GT Slavov, GT Howe, I Yakovlev, KJ Edwards, KV Krutovskii, GA Tuskan, JE Carlson, SH Strauss, and WT Adams. Pacific Northwest Tree Improvement Research Cooperative, Oregon State University, Corvallis, OR. March 2003.

RESULTS

Precise estimates of SMP success were obtained for each cross using three SSR markers.

CROSS	FEMALE	MALE	# EMBRYOS GENOTYPED	% SMP SUCCESS (SE)
1	1	4	72	44.2 (6.0)
2	2	4	76	57.9 (5.8)
3	3	4	54	71.4 (6.3)
4	1	4	71	76.8 (5.1)
5	2	4	91	69.4 (4.9)
6	3	4	91	86.4 (3.7)

Date Submitted: 3/10/03; Date Report Prepared: 6/18/03
Client: Private Company

CLONAL IDENTIFICATION IN A DOUGLAS-FIR ORCHARD USING ISOZYME AND SSR MARKERS

NFGEL Project #154
October 3, 2003

Three individuals of Douglas-fir were genotyped at isozyme and SSR loci to assess their clonal identity. Data show that the three individuals have matching genotypes at all loci measured. Trees were genotyped at 18 isozyme loci using starch gel electrophoresis, and three SSR markers analyzed on an ABI-3100. Both techniques were unable to detect any genetic differences among the three Douglas-fir individuals.

Final SSR Data

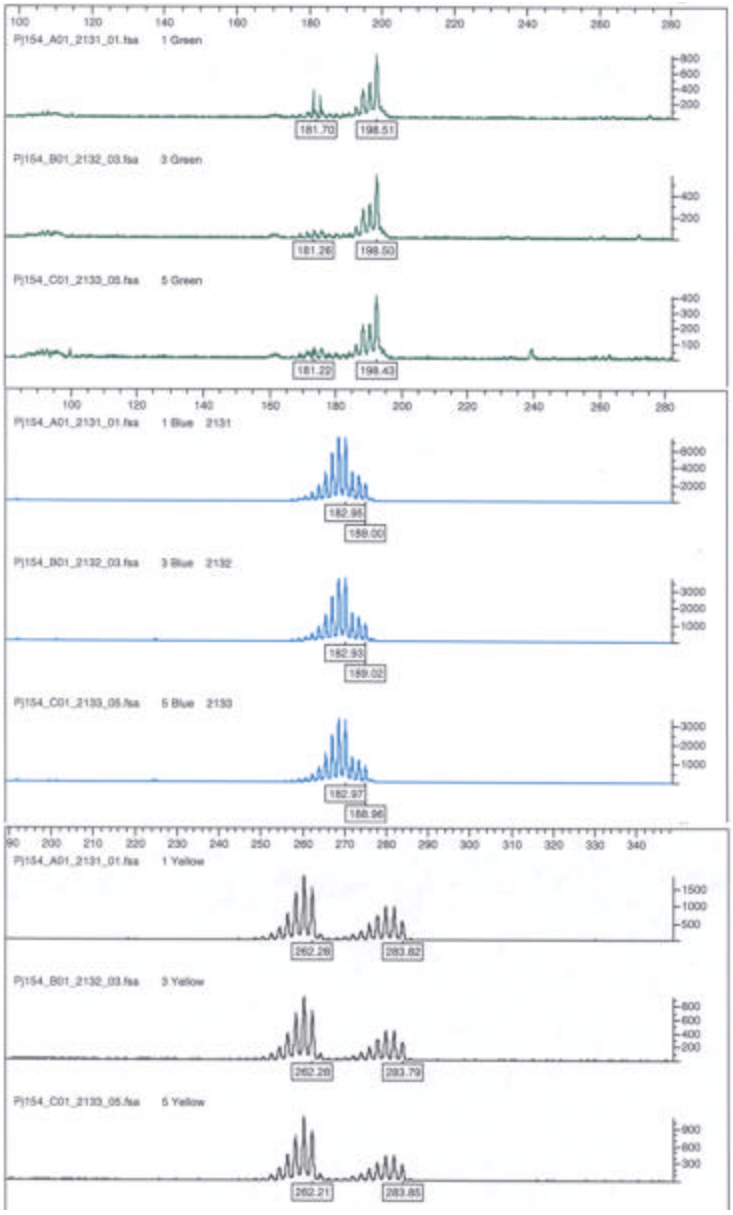
The tree identities are listed along the left side of the figure. Isozyme data is not shown.

TREE #

152-11-17

152-11-41

152-17-42



152-11-17

152-11-41

152-17-42

152-11-17

152-11-41

152-17-42

Date Submitted: 5/30/03 Date Prepared: 6/03 Date Analyzed: 9/03
Client: Private Company

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VALIDATION OF PARENTAL MATERIAL AND CROSSES USED TO TEST FOR A MAJOR GENE TYPE OF INHERITANCE FOR RESISTANCE TO THE ROOT PATHOGEN *PHYTOPHTHORA LATERALIS* IN PORT-ORFORD CEDAR

NFGEL Project #161

January 19, 2004

In order to validate parents and crosses in Port-Orford cedar, three types of genetic data were generated on twenty-six trees: isozymes at 19 loci, RAPD's using three primers, and SSR's using three primer sets. The isozymes provided the cleanest results. RAPD data was used for confirmation of the allozyme results. The SSR's would need further development before resulting peaks could be interpreted with confidence (SSR markers were obtained from the literature as being useful on other *Chamaecyparis* species – they may not be as useful for these Port-Orford-cedar).

Nine project hypotheses were addressed.

1) Ho: Is PO-117490 the same genotype as 117490 field collection?
   YES. The samples match at 18 isozyme loci and three RAPD markers.
2) Ho: Is PO-510015 box 22 the same ramet as PO-510015, box 23?
   YES. The samples match at 19 isozyme loci and three RAPD markers.
3) Ho: Are progeny from the self cross PO-510015 x PO-510015 similar, and are they similar to the parent?
   The progeny are similar, however, two of them cannot be the product of a 510015 self. 'PO-DOR-70785 box 196 SH8' and 'PO-DOR-70787 box 196 SH8' can't be progeny of a 510015 self (shown at the 6PGD-1 isozyme locus). It is possible that 'PO-DOR-70786 box 196 SH8' can be produced by a 510015 self cross.
4) Ho: Are progeny from the self cross PO-OSU-CF1 x PO-OSU-CF1 similar, and are they similar to the parent?
   YES. The progeny all share the same genotype (common allele for all isozymes loci), and match the genotype of the putative parent.
5) Ho: Is PO-117502 box 51 the same ramet as PO-117502 box 52?
   NO. These two individuals have different genotypes (at one isozymes locus, and at two RAPD markers).
6) Ho: Are progeny from the self cross PO-117502 x PO-117502 similar, and are they similar to the parent?
   Two of the progeny have identical isozymes genotypes (trees 'PO-DOR-70512 box 330 GH14' and 'PO-DOR-70513 box 328 GH14'). The third progeny ('PO-DOR-70511 box 330 GH14') differs from the other two at two isozymes loci. This is confirmed by RAPDs. None of these progeny can be from a selfed 'PO-117502 box 52 GH2' parent (see UGPP isozyme locus). It is possible that 'PO-117502 box 51 GH2' can serve as the selfed parent of these progeny.
7) Ho: Is PO-117499 box 1-3, PO-117499 box 24, and PO-117499 box 51 the same ramet?
   YES. Isozyme genotypes match at 19 loci.
8) Ho: Are progeny from self cross PO-117499 x PO-117499 similar, and are they similar to the parent?
   YES. The progeny are similar and can be the offspring of a selfed 'PO-117499' parent.
9) Ho: Is PO-DOR-70020 box 44, PO-DOR-70020 box 50, and PO-DOR-70020 box 476 the same ramet?
   YES. The samples match at 16 isozyme loci.

Date Submitted: August and September, 2003 Date Report Prepared: 1/19/04
Client: USDA Forest Service, Region 6, Richard Sniezko and Scott Kolpak

Isozyme data at 19 loci. 1/19/04

Sample	fest1	lap	pgm1	me7	pgi1	pgi2	ugpp1	tpi1	aat1	aat2	g6pd	gdh	mdh1	mdh2	6pgd1	6pgd2	idh	skd2	fdp1
PO-510015 box 22 GH1	11	11	11	11	11	11	12	11	11	11	22	11	11	11	11	11	11	11	11
PO-DOR-70516 box T-4 GH1	11	11	11	11	11	11	22	11	11	11	22	11	13	11	11	11	11	11	11
PO-117490 box 12 GH11	11	11	11	11	11	12	11	11	11	11	22	13	11	11	11	11	11	11	11
PO-117499 box 13 GH11	12	11	11	11	11	11	22	11	11	11	22	11	13	11	11	11	11	11	11
PO-DOR-70020 box 476 GH13	00	00	11	00	11	11	11	11	11	11	22	11	44	11	11	11	11	11	11
PO-DOR-70514 box 365 GH14	12	11	11	11	11	11	22	11	11	11	22	11	13	11	11	11	11	11	11
PO-DOR-70511 box 330 GH14	11	11	11	11	11	11	12	11	11	11	22	11	11	11	11	11	11	11	11
PO-DOR-70512 box 330 GH14	11	11	11	11	11	22	12	11	11	11	22	11	11	11	22	11	11	11	11
PO-DOR-70513 box 328 GH14	11	11	11	11	11	22	12	11	11	11	22	11	11	11	22	11	11	11	11
PO-DOR-70515 box 328 GH14	11	11	11	11	11	11	22	11	11	11	22	11	11	11	11	11	11	11	11
PO-OSU-CF1 box 13 GH2	00	11	00	11	11	11	11	11	11	11	22	11	11	11	00	11	11	11	00
PO-117502 box 51 GH2	11	11	11	11	11	12	12	11	11	11	22	11	11	11	12	11	11	11	11
PO-117502 box 52 GH2	11	11	11	11	11	12	11	11	11	11	22	11	11	11	12	11	11	11	11
PO-DOR-70020 box 44 GH2	11	11	11	11	11	11	11	11	11	11	22	11	44	11	11	11	11	11	11
PO-DOR-70020 box 50 GH2	11	11	11	11	11	11	11	11	11	11	22	11	44	11	11	11	11	11	11
PO-117499 box 24 GH2	12	11	11	11	11	11	22	11	11	11	22	11	13	11	11	11	11	11	11
PO-117499 box 51 GH2	12	11	11	11	11	11	22	11	11	11	22	11	13	11	11	11	11	11	11
PO-510015 box 23 GH2	11	11	11	11	11	11	12	11	11	11	22	11	11	11	11	11	11	11	11
PO-DOR-70773 box 195 SH8	11	11	11	11	11	11	11	11	11	11	22	11	11	11	11	11	11	11	11
PO-DOR-70774 box 195 SH8	11	11	11	11	11	11	11	11	11	11	22	11	11	11	11	11	11	11	11
PO-DOR-70775 box 195 SH8	11	11	11	11	11	11	11	11	11	11	22	11	11	11	11	11	11	11	11
PO-DOR-70785 box 196 SH8	11	11	11	11	11	11	12	11	11	11	22	11	11	11	12	11	11	11	11
PO-DOR-70786 box 196 SH8	11	11	11	11	11	11	22	11	11	11	22	11	11	11	11	11	11	11	11
PO-DOR-70787 box 196 SH8	11	11	11	11	11	11	12	11	11	11	22	11	11	11	12	11	11	11	11
PO-117490 parent	11	11	11	11	11	12	11	11	11	11	22	13	11	11	11	11	11	11	11
“Big POC” from Ron Powers	11	11	11	11	11	11	11	00	11	11	22	00	11	11	12	11	11	11	11

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ASSESSING SSR MARKERS FOR PATERNITY ANALYSIS IN *POPULUS SPP.*

NFGEL Final Report, Project #162
February 18, 2004

Report Prepared by: Robert C. Saich and Valerie D. Hipkins
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PROJECT GOALS

The project cooperator is testing the hypothesis of whether progeny generated by polymix breeding are represented equally by each pollen parent. Markers are needed that can adequately identify paternity of each individual tree in the progeny. Once developed, these markers can be used for a variety of purposes including tree breeding, conservation of the species, and assessment of genetic diversity. The objective of this project was to evaluate previously developed *Populus* SSR loci for their potential use as markers for paternity analysis in the species *Populus trichocarpa*, *P. deltoides*, and *P. nigra*.

MATERIALS

Samples of fresh mature leaf tissue (3-5 leaves/individual) from three *Populus* species were received on September 4, 2003 at NFGEL, Placerville, CA. Leaf material was received from:

(1) 16 individuals from each of three populus species, trees within a given species are related by no more than 25% (48 individuals), and
(2) eight progeny per each of six families; two families from each of the three species (48 individuals).

Leaf tissue from the parents of the six families, excluding the male parent of *P. trichocarpa* family 1, was received October 17, 2003 (11 individuals). In addition, in order to positively identify a maternal parent of *P. deltoides* family 9, bud tissue was supplied from 14 individual on November 4, 2003.

METHODS

DNA Extraction. DNA extraction was carried out on leaf tissue using the DNeasy-96 Frozen Leaf Tissue Protocol following manufacturers instructions with tissue homogenization achieved via the Mixer Mill 300 (QIAGEN Feb. 2002). DNA was extracted from bud tissue using the DNeasy Plant Mini Kit (QIAGEN 2000) following manufacturers instructions. DNA quality and approximate quantity was assessed by visualizing all samples against 50ng of Lambda DNA standard on 0.8% agarose gels stained with EtBr under UV light.

SSR Amplification and Electrophoresis. A total of 28 candidate SSR loci were evaluated. Aliquots of primers needed to amplify 18 of the loci were provided by Gerald Tuskan and Steve DiFazio, Environmental Sciences Division, Oak Ridge National Laboratory. Steve Strauss, Department of Forest Science, Oregon State University provided primer aliquots for the other 10 SSR loci screened.

SSR screening was achieved by amplifying approximately 1.5 - 2.5 ng of template DNA in a 10 ul final volume including 1X PCR buffer, 2.0 mM $MgCl_2$, 0.4 uM of each dNTP, 0.4 uM

of the forward and reverse primers, and 1 U of HotStarTaq DNA Polymerase (QIAGEN Nov. 2000). Amplifications were performed using a MJ Research PT-100 thermal controller with the following touchdown conditions: 15 min at 95°C, 94°C for 30 s, 55°C for 30 s, 72°C for 1 min (3X), 94°C for 30 s, 52°C for 30 s, 72°C for 1 min (3X), 94°C for 30 s, 50°C for 30 s, 72°C for 1 min (29X), followed by a final extension of 72°C for 7 min. The amplification product was then diluted to a ratio of 1:50 (amplification:ddH_2O) and 1ul of dilute amplification product was added to 10ul of Hi-Di™ Formamide containing 1.2% GeneScan®-500 [ROX]™ size standard. Samples were then denatured at 95°C for 2 min, and placed immediately on ice for 3 min before the sample plate was loaded on an ABI Prism 3100 Genetic Analyzer for detection of SSR product. ABI software packages, GeneScan® Analysis Software and Genotyper® Software v 3.7, were used to visualize and evaluate alleles at each locus.

Analysis. The parental genotypes were confirmed by simple exclusion analysis of the paternal contribution to each progeny in each species family, thus providing a basis for the evaluation of each locus in the areas of interpretability and presence of null alleles. Datum from each species were scored using Genotyper® Software v 3.7. CERVUS 2.0 (Marshall 1998) was used to calculate heterozygosity, parental exclusion probabilities, and estimated null allele frequencies.

RESULTS AND DISCUSSION

SSR Marker Selection. The 28 candidate SSR loci were initially screened on pedigree family progeny of each species: five progeny of *P. deltoides,* five *P. nigra* progeny, and six *P. trichocarpa* progeny. Based on their inability to amplify and/or their lack of producing interpretable peaks (potential alleles), 13 of the 28 candidate SSR loci were discarded from further study. The remaining 15 candidate loci (data available upon request) were determined to be potentially useful for paternity analysis, and were amplified on the total set of 107 *Populus* individuals.

Populus trichocarpa contains the greatest level of diversity compared to the other two *Populus* species (Table 1). Slightly less variation was found in *P. deltoides* compared to *P. trichocarpa*, and *P. nigra* was found to be the least variable and have the lowest exclusionary power of the three species.

Each SSR locus was assessed for its utility to assess paternity in each of the three species. Criteria used included: heterozygosity level, exclusion probability, expected null allele frequency, and genetic interpretability. Taking into account all criteria, SSR loci were placed in three catogories for each species: loci 'likely useful' for paternity analysis, loci that are 'potentially useful', and those loci that will not be useful. Six of the 15 loci can be found within the "likely useful" category for all three species of poplar tested.

Populus trichocarpa

Nine of the fifteen SSR's were found to be likely useful for paternity analysis in future projects. The three most informative primers yield 41 total alleles, mean H(E) = 0.941, and Excl(1) = 0.975.

Populus deltoides

Ten of the fifteen SSR's were found to be likely useful for paternity analysis. The three preferred primers in this species yield 34 total alleles, mean H(E) = 0.871, and Excl(1) = 0.908.

Populus nigra

Ten of the fifteen SSR's were found to be likely useful for paternity analysis. The three preferred primers yield 26 total alleles, mean H(E) = 0.826, and mean Excl(1) = 0.842.

Misidentified Plant Material. Analysis of the pedigree parents and progeny of each species revealed individual identification error.

P. deltoides, family 9

While screening the SSR primers against progeny arrays, it became apparent that the female received on 10/17/03, was not the mother of the #9 family. Additional plant tissue from ramets, rootstocks, and other candidate mother trees was sent for analysis. It was determined that the original shipment was actually a rootstock collection.

P. deltoides, family 1

Family paternal parent can not have served as the father to progeny #13 (possible pollen contamination in the family).

P. nigra, family 3

The male received on 10/17/03, is not the father of the #3 family. (Perhaps this is another example of an inadvertant rootstock collection).

P. trichocarpa, family 2

The identity of the male parent is suspect due to eight of the fifteen loci not segregating as expected in this family. In addition, pollen contamination is occuring among the progeny. Progeny #46 and #47 do not have the same father as the other six progeny analyzed.

REFERENCES

Marshall, TC, Slate, J, Kruuk, L & Pemberton, JM. 1998. Statistical confidence for likelihood-based paternity inference in natural populations. *Molecular Ecology* **7**(5): 639-655.

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QIAGEN. Feb 2002. DNeasy 96 Plant Kit Handbook For high-throughput isolation from plant tissue. Qiagen, Inc., Valencia, California, available at http://www1.qiagen.com/HB/DNeasy96Plant

QIAGEN. 2000. DNeasy Plant Mini Kit and DNeasy Plant Maxi Kit Handbook for DNA Isolation from Plant Tissue. Qiagen, Inc., Valencia, California, available at http://www1.qiagen.com/HB/DNeasyPlantMiniMaxi

Table 1. Diversity levels in three species of *Populus* at 15 SSR loci.

Species	Mean number of alleles per locus	Mean proportion of individuals typed	Mean H(E)	Mean Excl(1)	Mean Excl(2)
P. trichocarpa	11.1	0.93	0.823	1.000	1.000
P. deltoides	10.6	0.96	0.772	1.000	1.000
P. nigra	8.5	0.97	0.686	0.999	1.000

United States Department of Agriculture

Forest Service

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Final Report

Identification of Twelve Unknown Samples from Mount Ashland, Rogue River National Forest, as *Pinus albicaulis* or *P. monticola*

Report prepared by Jennifer DeWoody and Valerie D. Hipkins

Project submitted by: Wayne Rolle, USDA Forest Service,
Rogue River National Forest

January 5, 2004

Summary

Genetic testing confirmed the presence of three Whitebark pine trees on Mt. Ashland. The Mt. Ashland Whitebark pine samples are:

Tree 35 (one of 12 submitted Unknown Samples),
Tree 33 (one of six submitted Blind Samples), and
Tree 29 (a tree submitted as *Pinus albicaulis* based on morphological characteristics).

Two other Blind Samples (Tree 8 from East of Boulder Peak, and Tree 18 from Mt. McLoughlin) were also determined to be Whitebark pine. The remaining Unknown Samples (Trees 26, 27, 30, 31, 32, 34, 36, 37, 38, 39, and 40) and Blind Samples (Trees 11, 20, and 25) were identified as *P. monticola*.

Introduction

Twelve unknown samples of *Pinus spp.* were submitted for genetic analysis in order to determine whether each sample is *Pinus albicaulis* (Whitebark pine) or *P. monticola* (Western White Pine). Eleven samples of *P. albicaulis* and fifteen samples of *P. monticola*, all positively identified based on morphological characteristics, were provided for comparison and to determine appropriate species-specific markers. In addition, six "blind" samples, which were positively identified by W. Rolle but the identities not disclosed to NFGEL, were analyzed as a measure of quality assurance (Table 1).

Methods

DNA Analysis: DNA was extracted from 100 mg of needle tissue for each sample using Qiagen™ DNEasy Mini kits (Appendix: Part A). DNA concentration was quantified by fluorometry, and quality was assessed by agarose gel electrophoresis. Sufficient quantities of DNA were obtained so that no additional extractions were required.

Richardson *et al.* (2002) identified variation in the nad5a intron of the mitochondrial genome in *P. albicaulis*. This variation was found to occur in a sequence recognized by the restriction endonuclease MseI, resulting in two haplotypes at the locus. After amplifying the nad5a intron, one haplotype is cut by MseI and can be identified by the presence of two bands, while the other haplotype does not contain a restriction site and produces a single band.

Amplification of the nad5a intron was completed using primers designed by Wu *et al.* (1998). For each sample, 2.0 ng of DNA was amplified following the reaction conditions described by Richardson *et al.* (2002). Amplification was carried out on a MJ Research® PTC-100 thermalcycler (Appendix: Part B).

Following amplification of the nad5a intron, the product was purified using the Qiagen™ Qiaquick PCR Purification Kit following the recommended protocols. Samples were then restricted with MseI (Appendix: Part C). Restriction products were separated via electrophoresis on a 1% agarose gel using 1X TBE buffer (0.045 M Tris-borate, 0.001 M EDTA pH 8) and visualized using ethidium bromide under UV light.

Isozyme Analysis: Both needle and bud tissues were prepared for isozyme analysis. Needle tissue was prepared using a liquid nitrogen protocol, and bud tissue was prepared following an

uncrushed frozen protocol. Both tissues were prepared using Melody-Neale extraction buffer following the standard procedures described in the NFGEL Standard Operating Procedure (SOP) (USDA Forest Service 2003). Tissue was frozen at –80°C until electrophoresis. As part of the NFGEL quality assurance (QA) program, 16 samples (36%) were prepared and analyzed twice (Table 1).

Starch gel electrophoresis took place following the NFGEL SOP (USDA Forest Service 2003) using three buffer systems, lithium borate (LB), sodium borate (SB), and morpholine-citrate pH 6.1 (MC6). In order to identify the most informative loci, nineteen stain-buffer combinations were screened on both bud and needle tissue (data available upon request). After initial testing, eleven loci were visualized in three buffer systems (Table 2) for analysis.

Data analysis: Analysis of DNA data was completed by assigning a haplotype to each individual based on the presence of a single band (haplotype 1) or two bands (haplotype 2) following restriction analysis.

Analysis of isozyme data was completed independently by three staff members of NFGEL. Unknown and blind samples were identified as either *Pinus albicaulis* or *P. monticola* based on each multilocus genotype and the allele frequencies observed in the known samples for each species. These analyses were completed using direct assignment tests using the Bayesian likelihood algorithm employed by the program GeneClass (v. 1.0.02; Cornuet 1999).

Results

DNA analysis: The nad5a intron revealed fixed differences between *Pinus albicaulis* and *P. monticola* based the 26 known samples identified (Figure 1). All *P. albicaulis* individuals contained haplotype 2, while all *P. monticola* individuals contained haplotype 1. Based on this diagnostic marker, one of the unknown samples is *Pinus albicaulis* (tree 35). The remaining unknown samples are *P. monticola* (trees 26, 27, 30, 31, 32, 34, 36, 37, 38, 39, 40). Three of the blind samples are *P. albicaulis* (trees 8, 18, 33) and three are *P. monticola* (trees 11, 20, 25).

Isozyme analysis: All isozyme loci produced banding patterns consistent with published protein structures and diploid, Mendelian inheritance. Four loci in particular displayed species-specific alleles: AAT2, PGM1, SKD2, and TPI1 (detailed results available upon request).

Four trees were identified as *P. albicaulis* using the Bayesian likelihood tests based on the eleven isozyme loci: Unknown tree 35, and Blind trees 8, 18, and 33 (Table 3).

Discussion

The nad5a intron acts as a diagnostic marker to distinguish between *Pinus albicaulis* and *P. monticola* in the Mount Ashland region. Haplotypes at this locus were fixed for different alleles in each species: haplotype 2 in *P. albicaulis* and haplotype 1 in *P. monticola*. Applying this diagnostic marker to the unknown and blind samples allowed the unambiguous identification of each sample (Figure 1): one of the unknown samples from Mt. Ashland

contains haplotype 2, and is therefore identified as *P. albicaulis* (tree 35). The remaining unknown samples (trees 26, 27, 30, 31, 32, 34, 36, 37, 38, 39, and 40) displayed haplotype 1 and are therefore identified as *P. monticola*. Three of the blind samples were identified as *P. albicaulis* (trees 8, 18, and 33), and three were identified as *P. monticola* (trees 11, 20, and 25).

The results of the individual assignment tests based on multilocus isozyme data further supports these conclusions. While four loci resolved species-specific alleles in the known samples (AAT2, PGM1, SKD2, and TPI1), the overall differences in allele frequency allowed the direct assignment of Unknown and Blind trees to either the *P. monticola* or the *P. albicaulis* group. These analyses confirmed the DNA results, and identified four trees as *P. albicaulis*: one of the Unknown samples (tree 35) and three of the Blind samples (trees 8, 18, and 33). The remaining samples were identified as *P. monticola*.

In conclusion, genetic analyses identified one unknown sample as *P. albicaulis*, and the remaining eleven unknown samples as *P. monticola*. These findings are consistent with the recent identification of *P. albicaulis* on Mt. Ashland based on morphological attributes (tree 29).

Literature Cited

Cornuet J.M., S. Piry, G. Luikart, A. Estoup, and M. Solignac. *1999*. New methods employing multilocus genotypes to select or exclude populations as origins of individuals. Genetics 153(4): 1989-2000.

Richardson, B. A., N. B Klopfenstein, and S. J. Brunsfeld. *2002*. Assessing Clark's nutcracker seed-caching flights using maternally inherited mitochondrial DNA of whitebark pine. Can. J. For. Res. 32: 1103-1107.

USDA Forest Service. *2003*. National Forest Genetic Electrophoresis Laboratory Standard Operating Procedures. NFGEL, Placerville, California.

Wu, J., K. V. Krutovskii, and S. H. Strauss. *1998*. Abundant mitochondrial genome diversity, population differentiation and convergent evolution in Pines. Genetics 150: 1605-1614.

Table 1. *Pinus* samples submitted for genetic analysis. Samples indicated in the QA column were analyzed twice as part of the Quality Assurance program. ID numbers and Pine Species Name were submitted by W. Rolle.

ID Number (bag #)	Pine Species Name	Location	Date Collected	QA
1	*Pinus monticola*	East of Boulder Peak	10/20/2003	
2	*Pinus monticola*	East of Boulder Peak	10/20/2003	Y
3	*Pinus monticola*	East of Boulder Peak	10/20/2003	
4	*Pinus albicaulis*	East of Boulder Peak	10/20/2003	Y
5	*Pinus albicaulis*	East of Boulder Peak	10/20/2003	Y
6	*Pinus albicaulis*	East of Boulder Peak	10/20/2003	Y
7	*Pinus monticola*	East of Boulder Peak	10/20/2003	Y
8	blind sample	East of Boulder Peak	10/20/2003	
9	*Pinus albicaulis*	East of Boulder Peak	10/20/2003	Y
10	*Pinus albicaulis*	East of Boulder Peak	10/ 20/2003	
11	blind sample	East of Boulder Peak	10/20/2003	
12	*Pinus monticola*	East of Boulder Peak	10/20/2003	
13	*Pinus albicaulis*	Mt. McLoughlin	10/21/2003	Y
14	*Pinus albicaulis*	Mt. McLoughlin	10/21/2003	Y
15	*Pinus monticola*	Mt. McLoughlin	10/21/2003	Y
16	*Pinus albicaulis*	Mt. McLoughlin	10/21/2003	Y
17	*Pinus monticola*	Mt. McLoughlin	10/21/2003	
18	blind sample	Mt. McLoughlin	10/21/2003	
19	*Pinus monticola*	Mt. McLoughlin	10/21/2003	
20	blind sample	Mt. McLoughlin	10/21/2003	
21	*Pinus albicaulis*	Mt. McLoughlin	10/21/2003	Y
22	*Pinus albicaulis*	Mt. McLoughlin	10/21/2003	Y
23	*Pinus monticola*	Mt. McLoughlin	10/21/2003	Y
24	*Pinus monticola*	Mt. McLoughlin	10/21/2003	Y
25	blind sample	Mt. Ashland	10/21/2003	
26	Unknown	Mt. Ashland	10/21/2003	
27	Unknown	Mt. Ashland	10/21/2003	
28	*Pinus monticola*	Mt. Ashland	10/21/2003	
29	*Pinus albicaulis*	Mt. Ashland	10/21/2003	
30	Unknown	Mt. Ashland	10/21/2003	Y
31	Unknown	Mt. Ashland	10/21/2003	Y
32	Unknown	Mt. Ashland	10/21/2003	
33	blind sample	Mt. Ashland	10/21/2003	
34	Unknown	Mt. Ashland	10/21/2003	
35	Unknown	Mt. Ashland	10/21/2003	
36	Unknown	Mt. Ashland	10/21/2003	
37	Unknown	Mt. Ashland	10/21/2003	
38	Unknown	Mt. Ashland	10/21/2003	
39	Unknown	Mt. Ashland	10/21/2003	
40	Unknown	Mt. Ashland	10/21/2003	
41	*Pinus monticola*	Mt. Ashland	10/21/2003	
42	*Pinus monticola*	Mt. Ashland	10/21/2003	
43	*Pinus monticola*	Mt. Ashland	10/21/2003	
44	*Pinus monticola*	Mt. Ashland	10/21/2003	

Table 2. Stain and buffer systems for 11 loci resolved in 44 samples of *Pinus albicaulis* and *P. monticola*.

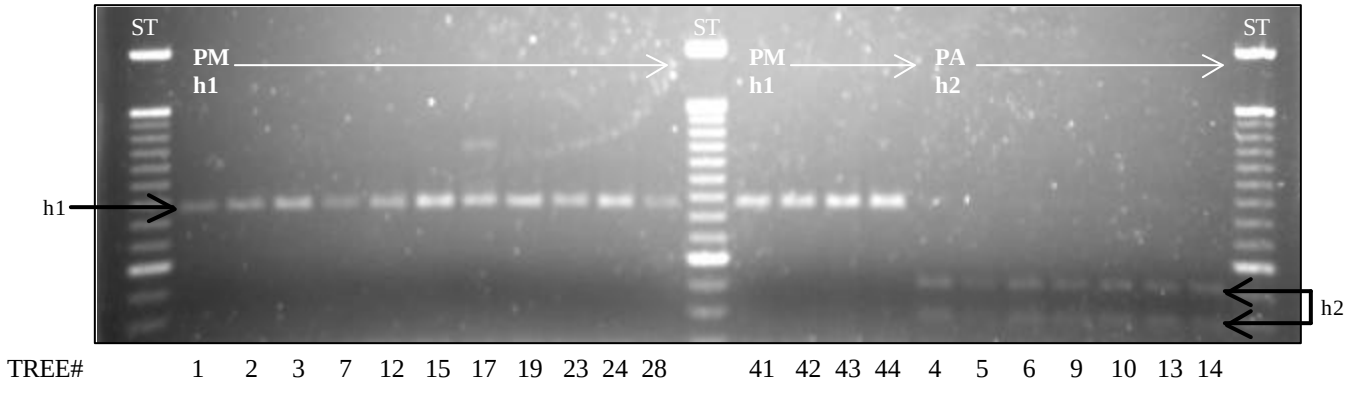
Buffer System	Stain Name (Abbreviation)	Loci
LB	Phosphoglucomutase (PGM)	PGM-1
LB	Phosphoglucoisomerase (PGI)	PGI-1, PGI-2
SB	Aspartate aminotransferase (AAT)	AAT-1, AAT-2, AAT-3
SB	Triose-phosphate isomerase (TPI)	TPI-1
SB	UTP-glucose-1-phosphate uridylyltransferase (UGPP)	UGPP-1
MC6	6-phosphogluconate dehydrogenase (6PGD)	6PGD-2
MC6	Shikimic dehydrogenase (SKD)	SKD-1, SKD-2

Table 3. Results of Bayesian likelihood assignment test based on eleven isozyme loci. PM Distance and PA Distance is minus the decimal logarithm of the likelihood of the unknown tree belonging to *P. monticola* and *P. albicaulis*, respectively. This statistic can be compared to the genetic distance between the unknown tree and each species.

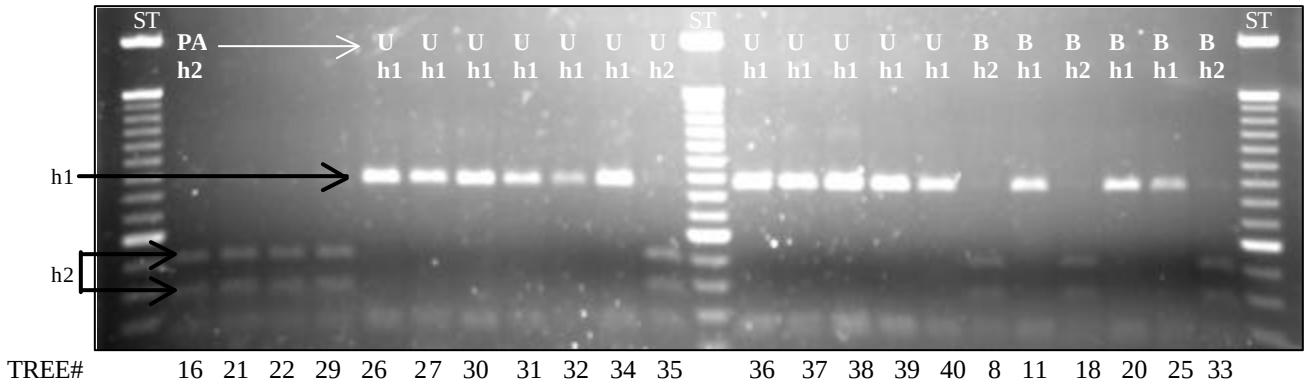
Tree	PM Distance	PA Distance	Identified As
Unknown-26	2.43	9.60	*P. monticola*
Unknown-27	3.82	11.27	*P. monticola*
Unknown-30	4.18	11.32	*P. monticola*
Unknown-31	3.08	7.67	*P. monticola*
Unknown-32	3.08	9.05	*P. monticola*
Unknown-34	4.72	12.21	*P. monticola*
Unknown-35	**15.28**	**8.62**	***P. albicaulis***
Unknown-36	1.65	8.56	*P. monticola*
Unknown-37	2.74	9.82	*P. monticola*
Unknown-38	4.13	10.57	*P. monticola*
Unknown-39	3.99	10.84	*P. monticola*
Unknown-40	2.01	7.59	*P. monticola*
Blind-8	**13.53**	**4.54**	***P. albicaulis***
Blind-11	2.87	9.37	*P. monticola*
Blind-18	**8.43**	**1.50**	***P. albicaulis***
Blind-20	2.25	7.72	*P. monticola*
Blind-25	2.30	6.63	*P. monticola*
Blind-33	**9.71**	**3.37**	***P. albicaulis***

Figure 1. Identification of unknown and blind samples based on nad5a haplotypes. Haplotype 1 (h1) does not contain a restriction site for the endonuclease MseI and appears as one band. Haplotype 2 (h2) does contain a restriction site for MseI, producing two bands from the PCR product. **a)** Samples morphologically identified as either *P. monticola* (PM) or *P. albicaulis* (PA). **b)** Samples either morphologically identified as *P. albicaulis* (PA), or as unknown (U) or blind samples (B). The tree number of each sample appears below each lane. ST = 100 bp ladder size standard.

a)



b)



Appendix. Procedures used in DNA analysis.

Part A. Qiagen® DNEasy Mini Kit extraction protocol adapted from manufacturer's instructions.

Before beginning extraction:

a. Read the "Technical Information" in the Kit's handbook.
b. Ensure that ethanol has been added to buffers AW and AP3/E.
c. Preheat water bath to 65°C.
d. Preheat AP1 and AE to 65°C. (AP1 when precipitates form)
e. Label QIAshredder spin column (lilac), DNeasy spin column (clear) and the supplied 2ml collection tube.
f. Label three additional 1.5ml eppendorf tubes per sample. Final tube with tough spot and tag.
g. Collect needed equipment for LqN grind. (Mortar and Pestles, adequate supply of Liquid Nitrogen)

Extraction protocol:

Note: Use approximately 100mg plant tissue.

1. Add 400µl of buffer AP1 to a pre-labeled 1.5ml eppendorf tube and place on crushed ice. Repeat for all tubes in run.
2. Grind tissue under LqN to a fine powder (100mg fresh, 20mg dried tissue). DO NOT ALLOW TO THAW. Transfer powder to tube from step 1 and mix thoroughly with spatula. Keep samples on ice until all individuals in run are ground.
3. Add 4ul of RNase A and mix with pipette tip.
4. Incubate @ 65°C for 10 minutes. Vortex 2-3X during incubation.
5. Add 130µl of buffer AP2 to lysate and mix with pipette tip. Invert 2-3X and incubate on ice 5 minutes.
6. Spin @ high speed for 5 minutes.
7. Transfer lysate to lilac spin column. Spin @ high speed for 2 minutes.
8. Transfer supernatant to new, labeled, eppendorf tube, taking care not to disturb the pellet.
9. Add 1.5 volume of buffer AP3/E to supernatant. Immediately mix by pipetting.
10. Transfer 650µl of supernatant to the clear DNeasy spin column. Spin @ 8000rpm for 1 minute, discarding flow-through at end of spin.
11. Repeat step 10 with remaining supernatant.
12. Add 500µl of buffer AW. Spin @ 8000rpm for 1 minute, discarding collection tube and flow-through. Place spin column into new collection tube.
13. Add 500µl of buffer AW. Spin @ 8000rpm for 1 minute, discarding flow-through at end of spin.
14. Repeat step 13 with 500µl ethanol if insufficient washing of spin column is noted. Spin @ high speed for 2 minutes to dry the column membrane.
15. Place spin column in labeled (tough spot and/or tag) 1.5ml eppendorf tube. Place in fume hood (set @ 150 feet/min velocity) for 30 minutes to air dry the spin column membrane.
16. Add 50µl of (65° C) buffer AE. Incubate @ room temperature for 5 minutes. Spin @ 8000rpm to elute.
17. Repeat step 16. Discard spin column and store DNA @ 4°C short term.

Appendix continued.

Part B. Amplification conditions for nad5a intron.

Amplification reaction conditions.

Reagent	Working concentration	Final concentration	Amount (uL)
Sample DNA	0.5 ng/uL	2.0 ng	4.0
Reaction Buffer*	10 X	1 X	2.0
Magnesium chloride*	25 mM	2.5 mM	2.0
dNTPs (each)	1.25 mM	0.2 mM	3.2
Forward primer	10.0 uM	0.5 uM	1.0
Reverse primer	10.0 uM	0.5 uM	1.0
dH_2O	--	--	6.6
Taq	5 Units/uL	1 Unit	0.2
Total Volume			20.0

*Provided by manufacturer. Reaction Buffer contains 1.5 mM final concentration magnesium chloride. Additional magnesium chloride was added for final concentration of 2.5 mM.

Primer Sequences (5' – 3')

```
Forward Primer:  GAA ATG TTT GAT GCT TCT TGG G
Reverse Primer:  ACC AAC ATT GGC ATA AAA AAA GT
```

Amplification program.

Step	Temperature (°C)	Time (mm:ss)
1	95	15:00
2	95	0:30
3	55	0:30
4	72	2:00
5	GOTO Step 2	34 more times
6	72	5:00
7	4	0:00 (indefinite)
8	END	

Appendix continued.

Part C. Purification and restriction of nad5a intron amplification product.

Purification of amplification product

The total volume of the nad5a amplification reaction was purified using Qiagen® Qiaquick 96 PCR Purification Kit, following manufacturer's instructions. Specifically, purification was completed using the vacuum manifold procedure, and samples were eluted in the provided RNAse-free water. Approximately 60 uL of product was recovered following purification, and samples were stored at –20°C until restriction.

Restriction of purified product

The total volume recovered from the purification procedure was used in the restriction reactions. After adding all ingredients, samples were incubated at 37°C for 2 hours.

Restriction reaction conditions.

Reagent	Working concentration	Final concentration	Amount (uL)
Amplification product	--	--	60.0
Buffer 2*	10 X	1 X	10.0
BSA	1 mg/1000 uL	0.1 mg/1000 uL	10.0
dH_2O	--	--	19.9
MseI	10 Units/uL	1 Unit	0.1
Total Volume			100.0

* Provided by manufacturer.

Conservation and Restoration

(1) Isozyme analysis of intermountain plants: progress report
(2) Genetic distribution of trembling aspen (*Populus tremuloides*) clones in the central Sierra Nevada, California
(3) Expanded study of the genetic diversity in *Perideridia erythrorhiza*: a rare plant in southern Oregon
(4) Implications of isozyme variation for the taxonomy of the rare california plant *Silene campanulata* ssp. *campanulata*
(5) Expanded evaluation of genetic diversity in tahoe yellow cress (*Rorippa subumbellata*)
(6) Characterizing ploidy level variation using flow cytometry

ISOZYME ANALYSIS OF INTERMOUNTAIN PLANTS: PROGRESS REPORT

NFGEL Projects 104, 106, 108, 110, 113, 115, 117, 119, 132, and 134
March 1, 2004

Collaborators: Durant McArthur, USDA Forest Service, Rocky Mountain Research Station
Richard Cronn, USDA Forest Service, Pacific Northwest Research Station

This isozyme study uses two approaches to explore the genetic effects of using non-local native plants in habitat restoration projects. First, gene flow among indigenous populations is assessed. Second, the genetic diversity of certain restored plant populations is compared with variation in local indigenous populations and with the native populations that were sources of the seed for restoration.

Twelve species were chosen for this study (Table 1), and in 2003 a species of *Balsamorhiza* was added (not shown). These species are all common perennial species of the Intermountain West. Therefore, they are frequently used in habitat restoration projects. These species differ in important life history components, including habit, polyploidy, and breeding system (Table 2).

METHODS

A total of 1,612 individuals were sampled between 2001 and 2003. DNA extraction was carried out on leaf tissue using either the (1) DNeasy-96 Frozen Leaf Tissue Protocol, or DNeasy Plant Mini Kit following manufacturer's instructions with tissue homogenization achieved via the Mixer Mill 300 (Qiagen), or (2) FastPrep DNA Extraction (Bio-101). DNA quantity was assessed by fluorometry, and quality determined by visualizing all samples against 50ng of Lambda DNA standard on 0.8% agarose gels stained with EtBr under UV light. DNA samples were shipped overnight on dry-ice to Richard Cronn, PNW, USDA Forest Service. Isozyme preparation followed the NFGEL Standard Operating Procedures. Extracts were electrophoresed on 11% starch gels, and stained for a suite of enzyme systems.

RESULTS AND DISCUSSION

To date, a total of 1034 individuals in 52 populations of ten species were analyzed for isozymes (Table 3). Material collected during the 2003 field season has not yet been analyzed for isozyme variation. All but two of the analyzed populations were indigenous (Table 4). Quality of the samples submitted for analysis varied greatly.

Genetic Diversity.

Descriptive statistics for populations varied widely (Table 5). Percent polymorphic loci vary from 4.6% in *Stipa comata*, a cleistogamous selfer, and 7% in *Bromus carinatus* and one population of *Atriplex canescens*, to 91% in the Oak Spring population of *Lupinus*, which may be a mixed collection, 73.7% in *Erigeron pumilus*, and 72.2% in *Eriogonum umbellatum*. Alleles per locus follow a similar pattern. Observed heterozygosity was somewhat less than expected heterozygosity, suggesting some degree of selfing, except in one *Crepis acuminata* population, two *Erigeron pumilus* populations, three *Lupinus* populations, and *Vicia* sp. The *Crepis* is apomictic.

Descriptive statistics for species were also variable (Table 6). Percent polymorphic loci varied from 19% in largely selfing *Bromus carinatus* to 84% in

Erigeron pumilus and 100% in *Lupinus,* which may include two species. Allele per locus varied from 1.2 in *Stipa comata* to 2.8 in *Lupinus.* Observed heterozygosity was less than expected except in *Vicia americana*.

Genetic Structure.

Statistics rega rding differentiation and inferred gene flow among populations are provided for diploids and plants that were treated as diploids for analysis (Table 7). Fst varied greatly, from 0.05 between the two populations of *Eriogonum umbellatum* we were confident were that species to 0.78 in *Stipa comata.* (When the three population of *Eriogonum* are analyzed together, Fst -= 0.44.) Inferred gene flow varied inversely with Fst (Table 7).

In all species for which it could be calculated, genetic identity of conspecific populations averaged above 0.9, as expected (Crawford 1989), except in *Lupinus argenteus,* where genetic identities averaged 0.88 (Table 7). Morphological diversity among *Lupinus* samples suggests that some were misidentified. Data for *Stipa comata* may seem contradictory because genetic identities were as expected for conspecific populations (averaging 0.907) but Fst was very high (0.78). This resulted from the fact that each population in this selfing species was extremely uniform. The species was monomorphic at most loci, but there were fixed differences among populations at certain loci.

Taxonomy.

One population each among the samples submitted as *Eriogonum umbellatum, Lupinus argenteus,* and *Vicia americana* populations are so divergent that we must assume they are different species unless examination of voucher specimens proves otherwise (genetic identities in Table 8, allele frequencies in Appendix A). One individual submitted as *Stipa comata* was a different species, with 9 unique alleles (Appendix A).

The *Lupinus* samples are highly variable. Morphological differences noted among the samples indicated that there might well be more than one species included in the study. If the study includes two species but each population has only a single species, we would expect to see fixed differences among populations. We do not. However, closely related species may lack fixed isozyme differences, especially if they diverged recently or hybridization is ongoing. Alternatively, perhaps one or more of the populations is a mixed collection, containing two or more species. That might account for the unusually high diversity observed in *Lupinus,* including the 100% percent polymorphic loci. The odd 'Eureka' *Lupinus argenteus* population is genetically similar to other populations submitted as the same species. It must be a close relative or a divergent conspecific, because it shares the duplicated GLYDH locus observed in all populations. The 'Eureka' population has evidence of fixed heterozygosity, which is lacking in the other *Lupinus* samples. If the SKD locus that resolves in 'Eureka' and three other individuals (but not most samples) is a marker for the different taxon, then the Oak Springs sample may be a mixed collection.

Isozyme analysis confirms that certain species or populations are polyploid, and suggests that others considered diploid are in fact polyploid (Appendix B)

Table 1. Taxonomy of species used in this study. Intraspecific taxa have not yet been determined for most populations. * This information submitted for 10 of the 11 *Atriplex* populations.

Original name	Current name	author	v/s	subtaxon	author	subtaxa?	Family
Artemisia tridentata	Artemisia tridentata	Nutt.				yes	Asteraceae
Astragalus utahensis	Astragalus utahensis	(Torr.) Torr. & Gray		----------	----------	no	Fabaceae
Atriplex canescens	Atriplex canescens	(Pursh) Nutt.		*		yes	Chenopodiaceae
Bromus carinatus	Bromus carinatus	Hook. & Arn.				yes	Poaceae
Chrysothamnus nauseosus	Ericameria nauseosa	(Pall. ex Pursh) G. L. Nelson & G. I. Baird				yes	Asteraceae
Crepis acuminata	Crepis acuminata	Nutt.		----------	----------	no	Asteraceae
Erigeron pumilus	Erigeron pumilus	Nutt.				yes	Asteraceae
Eriogonum umbellatum	Eriogonum umbellatum	Torrey				yes	Polygonaceae
Lupinus argenteus	Lupinus argenteus	Pursh				yes	Fabaceae
Stipa comata	Hesperostipa comata	(Trin. & Rupr.) Barkworth				yes	Poaceae
Vicia Americana	Vicia americana	Willd.				yes	Fabaceae
Viguiera multiflora	Heliomeris multiflora	Nutt.				yes	Asteraceae

Table 2. Chromosome numbers, inheritance, and breeding systems of species used in this study. Chromosome numbers provided by E. Durant McArthur except as noted.

Original name	Ploidy	Chromosomes	inheritance	scored as	Breeding system	Habit
Artemisia tridentata	2X and 4X	2n = 18, 36*; x = 9, 2 x and 4 x	auto			shrub
Astragalus utahensis	2X	2n = 22[2]: n = 11	-- auto[3]			herb
Atriplex canescens	2X – 6X (– 20X)	2n = 18, 36*; x = 9 (2x to 20 x)	auto			shrub
Bromus carinatus	4X – 10X	2n = 56*; x = 7, n = 14, 21, 24, 35	auto		outcrossing	herb
Chrysothamnus nauseosus	2X	2n = 18*; x = n = 9	--			shrub
Crepis acuminata	high polyploid	2n = 22, 33, 44, 55, 88*; 2n = 22, 33, 44 . . .	(?)		apomictic	herb
Erigeron pumilus	2X	2n = 18, 36*; n = 9	--			herb
Eriogonum umbellatum	2X (?)	n = 20	--			shrub?
Lupinus argenteus	4X (?)	n = 24	allo (?)	diploid		herb
Stipa comata	4X (?)	2n = 38, 44, 46*; n = 22	auto (?)	allotetraploid	often selfing[4]	herb
Vicia americana	2X	2n = 14*; x = n = 7	--			herb
Viguiera multiflora	2X (?)	2n = 16*; n = 8	--			herb

* Chromosome numbers from Jepson Manual. All references *in* James C. Hickman, ed., The Jepson Manual: Higher Plants of California. University of California Press, Berkeley, California.

Artemisia tridentata: Schultz, Leila M. 1993. *Artemisia;* Sagebrush. pp. 202 – 205

Atriplex canescens: Taylor, Dean, and Deiter H. Wilkin. 1993. *Atriplex;* Saltbush. pp. 501 – 505

Bromus carinatus: Wilken, Dieter H. and Elizabeth L. Painter. 1993. *Bromus*; Brome. pp. 1239 – 1243

Crepis acuminata: Stebbins, G. Ledyard. 1993. *Crepis*; Hawkweed. p. 242 – 245

Ericameria nauseosa: Anderson, Loran C. 1993. *Chrysothamnus*, Rabbitbrush. pp. 229 – 232

Erigeron pumilus: Nesom, Guy L. 1993. *Erigeron*; Fleabane Daisy. pp. 253-261

Heliomeris multiflora: Keil, David J. 1993. Asteraceae [Compositae] Sunflower Family. p. 280

Hesperostipa comata: Barkworth, Mary E. 1993. *Hesperostipa*. p. 1263

Vicia americana: Isely, Duane. 1993. *Vicia*; Vetch. pp. 654 – 657

[2] Intermountain Flora

[3] hypothesis from isozyme gels

[4] Personal communication from Mary Barkworth, 5 May 2003, to Barbara Wilson: "judging by anther length, the species is frequently but not always cleistogamous"

Astragalus utahensis, from Aaron Liston: Spellenberg counted 2n=22 for one pop. The new world Astragalus spp. are considered aneuploids, derived from an ancestral tetraploid - auto or allo is not known.

Table 3. Populations used in this study. loci seen = percent of all loci used in that species that were resolved.

Species	Name (locality)	State	EDM#	Date Coll.	N	Category	loci seen	loci	Comments
Astragalus utahensis	Jake's Valley	NV	2572	29 May 2001	19	indig.	64%	22	small #'s in some loci
Astragalus utahensis	Orem	UT	2700	30 Apr 2002	20	indig.	100%	22	
Astragalus utahensis	Wolverine Canyon	ID	2715	15 May 2002	20	indig.	91%	22	
Astragalus utahensis	Mt. Moriah	NV	2735	31 May 2002	20	indig.	91%	22	small #'s in some loci
Atriplex canescens 2X	Little Sahara	UT	2702	30 Apr 2002	20	indig.	100%	20	
Atriplex canescens 2X	Wilson Creek Road	NV	2723	17 May 2002	20	indig.	55%	20	
Atriplex canescens 2X	San Antonio	NM	2736	4 June 2002	20	indig.	75%	20	
Atriplex canescens 2X	Cerro de la Olla	NM	2738	7 June 2002	20	indig.	65%	20	
Atriplex canescens 4x	Jericho Dunes	UT	2704	01 May 2002	20	indig.	100%	20	small #'s in 3 loci
Atriplex canescens 4X	Panaca	NV	2721	17 May 2002	20	indig.	65%	20	
Atriplex canescens 4X	Sand Pit, Twist H.	UT	2724	17 May 2002	20	seeded	65%	20	
Atriplex canescens 4X	Twist Hollow	UT	2725	17 May 2002	20	source	65%	20	
Atriplex canescens 4X	Wales	UT	2729	22 May 2002	20	indig.	75%	20	
Atriplex canescens 6X	Tonopah	NV	2730	30 May 2002	20	indig.	65%	20	
Atriplex canescens 6X	Bernardo	NM	2737	7 June 2002	19	indig.	65%	20	
Bromus carinatus	Broad Canyon	UT	2578	17 July 2001	21	indig.	95%	21	
Bromus carinatus	Little Valley	UT	2615	9 July 2002	20	indig.	33%	21	EDM said to remove
Crepis acuminata	Jake's Valley	NV	2571	29 May 2001	19	indig.	59%	17	
Crepis acuminata	Grouse Creek	UT	2610	5 June 2001	21	indig.	65%	17	small #'s in 3 loci
Crepis acuminata	Black Rock Canyon	UT	2706	30 Apr 2002	20	indig.	71%	17	small #'s in 3 loci
Crepis acuminata	Woodruff Road	ID	2712	14 May 2002	20	indig.	100%	17	
Crepis acuminata	Kingston Canyon	NV	2731	30 May 2002	20	indig.	82%	17	small #'s in 3 loci
Erigeron pumilus	Jake's Valley	NV	2570	29 May 2001	21	indig.	89%	18	
Erigeron pumilus	Sheep Creek	UT	2607	(2001)	20	indig.	17%	18	
Erigeron pumilus	Cedar Grove	UT	2611	11 July 2001	22	indig.	72%	18	small #'s in some loci
Erigeron pumilus	Topaz Mtn.	UT	2705	01 May 2002	20	indig.	89%	18	
Erigeron pumilus	Black Rock Canyon	UT	2707	30 Apr 2002	21	indig.	100%	18	
Erigeron pumilus	Curlew	ID	2713	14 May 2002	7	indig.	94%	18	sample size small
Erigeron pumilus	Crystal Ice	ID	2717	16 May 2002	20	indig.	100%	18	

Erigeron pumilus	Welcome	NV	2719	16 May 2002	20	indig.	100%	18	
Eriogonum umbellatum	Underdown	NV	2573	31 May 2001	20	indig.	20%	20	small #'s in some loci
Eriogonum umbellatum	Tintic - 1	UT	2579	17 July 2001	21	indig.	20%	20	small #'s in some loci
Eriogonum umbellatum	Indian Peaks	UT	2612	23 July 2001	20	indig.	15%	20	
Eriogonum umbellatum	Tintic - 2	UT	2701	30 Apr 2002	20	indig.	95%	20	
Eriogonum umbellatum	Crystal Ice	ID	2716	16 May 2002	21	indig.	95%	20	
Eriogonum umbellatum?	Inkom	ID	2714	15 May 2002	21	indig.	90%	20	different species?
Lupinus argenteus?	Jackson Ridge	UT	2581	17 July 2001	20	indig.	100%	28	
Lupinus argenteus?	Salt Cave Hollow	UT	2586	24 July 2001	20	indig.	96%	28	
Lupinus argenteus?	Lost Peak	UT	2608	30 July 2001	20	indig.	93%	28	
Lupinus argenteus?	Eureka	UT	2703	01 May 2002	20	indig.	100%	30	different taxon?
Lupinus argenteus?	Crystal Ice	ID	2718	16 May 2002	20	indig.	100%	28	
Lupinus argenteus?	Oak Springs	NV	2720	16 May 2002	20	indig.	100%	28	mixed population?
Lupinus argenteus?	Jake's Valley	NV	2733	30 May 2002	20	indig.	93%	28	
Stipa comata	Modena	UT	2613	10 Jun 2002	20	indig.	100%	46	# loci = 23 doubled
Stipa comata	Cisco	UT	2614	10 Jun 2002	20	indig.	100%	46	
Stipa comata	Jake's Valley	NV	2732	30 May 2002	19	indig.	100%	46	
Vicia americana	Jackson Ridge	UT	2580	17 July 2001	20	indig.	96%	24	
Vicia americana	Lost Peak	UT	2609	(2001)	21	indig.	96%	24	
Vicia americana?	Egan Mountains	NV	2734	31 May 2002	20	indig.	100%	24	different species?
Viguiera multiflora	Rachel	NV	2569	May 2001	20	indig.		6	poor resolution
Viguiera multiflora	Broad Canyon	UT	2575	17 July 2001	20	indig.		6	poor resolution
Viguiera multiflora	Castleton Turnoff	NV	2722	17 May 2002	21	indig.		6	poor resolution

Table 4. Populations submitted to this study in the categories of “indigenous” (native to the local area), “seed” (plants seeded into the local area), and “source (plants native elsewhere that provided the “seed” population).

Species	Indigenous	Source	Seed
Astragalus utahensis	3		
Atriplex canescens 2X	4		
Atriplex canescens 4X	3	1	1
Atriplex canescens 6X	2		
Bromus carinatus	2		
Crepis acuminata	5		
Erigeron pumilus	8		
Eriogonum umbellatum	6		
Lupinus argenteus	7		
Stipa comata	3		
Vicia americana	3		
Viguiera multiflora	3		
Total:	49	1	1

Table 5. Genetic diversity statistics for populations used in this study. EDM# is an identification number. N = number of individuals collected. N/locus = average number of individuals scored per locus. P = percent polymorphic loci. A = alleles/locus. Ae = effective alleles/locus. Obs. Het. = observed heterozygosity. Exp. Het. = expected heterozygosity. * = recalculated with a different set of loci; not included in calculations for the entire species.

Species	Population	EDM#	N	N/locus	Loci	P (%)	A	Ae	Obs Het	Exp Het
Astragalus utahensis	Jake's Valley, NV	2572	19	8.2	14	28.6	1.4286		0.1561	
Astragalus utahensis	Orem, UT	2700	20	19.2	21	52.4	1.6667		0.2247	
Astragalus utahensis	Wolverine Canyon, ID	2715	20	19.8	21	42.9	1.5714		0.2139	
Astragalus utahensis	Mt. Moriah, NV	2735	20	17.4	20	50.0	1.6500		0.2432	
Atriplex canescens	Little Sahara, UT, 2X	2702	20	20	13	38.5	1.4615	1.1643	0.0783	0.1103
Atriplex canescens	Wilson Cr., NV, 2X	2723	20	16.5	13	7.7	1.0833	1.0233	0.0000	0.0194
Atriplex canescens	Cerro de la Olla, NM, 2X	2738	20	19.5	13	61.5	1.7692	1.3049	0.1542	0.1950
Atriplex canescens	San Antonio, NM, 2X	2736	20	19.5	13	38.5	1.5385	1.1532	0.0971	0.1011
Atriplex canescens	Jericho Dunes, UT, 4X	2704	20	20	13	46.2	1.5385		0.1500	
Atriplex canescens	Sand Pit (Twist), UT, 4X	2724	20	19	13	46.2	1.4615		1.0891	
Atriplex canescens	Twist Hollow, UT, 4X	2725	20	17.7	13	46.2	1.4615		0.1520	
Atriplex canescens	Panaca, NV, 4X	2721	20	18.2	13	46.2	1.4615		0.1441	
Atriplex canescens	Wales, UT, 4X	2729	20	19.7	13	61.5	1.6923		0.1914	
Atriplex canescens	Jericho Dunes*, UT, 4X	2704	20	17.8	19	57.9	1.8421		0.2920	
Atriplex canescens	Wales*, UT, 4X	2729	20	19.4	17	64.7	1.7647		0.2097	
Atriplex canescens	Tonopah, NV, 6X	2730	20	91.2	13	33.3	1.3333		0.1696	
Atriplex canescens	Bernardo, NM, 6X	2737	20	17.3	12	46.2	1.6154		0.1967	
Bromus carinatus	Broad Canyon, UT	2578	21	20.9	20	15.0	1.1500		0.0861	
Bromus carinatus	Little Valley, UT	2615	20	18.8	14	7.1	1.0714		0.0228	
Crepis acuminata	Jake's Valley, NV	2571	19	17	11	18.2	1.2727	1.2793	0.0909	0.1071
Crepis acuminata	Grouse Creek, UT	2610	21	16	12	8.3	1.0833	1.0833	0.0833	0.0427

Crepis acuminata	Black Rock Canyon, UT	2706	20	15	12	41.7	1.4167	1.1727	0.0833	0.1104
Crepis acuminata	Woodruff, ID	2712	20	19	12	25.0	1.3333	1.1779	0.0426	0.1024
Crepis acuminata	Woodruff, ID*	2712	20	19	18	22.2	1.2778	1.2571	0.0340	0.0910
Crepis acuminata	Kingston Canyon, NV	2731	20	18	12	25.0	1.3333	1.1027	0.0417	0.0665
Erigeron pumilus	Jake's Valley, NV	2570	21	19.5	17	52.9	1.5882	1.2502	0.0996	0.1575
Erigeron pumilus	Cedar Grove, UT	2611	22	14	13	30.8	1.3007	1.0892	0.0306	0.0684
Erigeron pumilus	Black Rock Canyon, UT	2707	20	15.5	19	73.7	2.0566	1.2719	0.1240	0.1930
Erigeron pumilus	Topaz Mountain, UT	2705	20	19	18	61.1	1.8889	1.2604	0.1799	0.1661
Erigeron pumilus	Crystal Ice, ID	2717	20	17.5	19	57.9	1.7368	1.1920	0.1321	0.1219
Erigeron pumilus	Curlew Grasslands, ID	2713	7	6.5	18	55.6	1.6667	1.2343	0.1151	0.1676
Erigeron pumilus	Welcome, Elko Co., NV	2719	20	19	19	57.9	1.8421	1.2340	0.1216	0.1618
Eriogonum umbellatum	Tintic-2, UT	2701	21	16.5	18	61.1	1.8889	1.4044	0.1637	0.2199
Eriogonum umbellatum	Crystal Ice, ID	2716	21	20	18	50.0	1.7778	1.2891	0.1680	0.1661
Eriogonum sp.	Inkom, Elko Co., ID	2714	21	16.5	18	72.2	2.1765	1.3550	0.1670	0.2187
Lupinus argenteus	Jackson Ridge, UT	2581	20	19	23	52.2	1.6522	1.2407	0.1603	0.1543
Lupinus argenteus	Salt Cave Hollow, UT	2586	20	19	23	56.5	1.7391	1.3145	1.1543	1.1686
Lupinus argenteus	Lost Peak, UT	2608	20	18.5	23	52.2	1.6957	1.2683	0.1343	0.1435
Lupinus argenteus	Crystal Ice, ID	2718	20	18.5	23	65.2	2.0870	1.4873	0.2639	0.2548
Lupinus argenteus	Oak Springs, NV	2720	20	19	23	91.3	2.3043	1.4773	0.2225	0.2726
Lupinus argenteus	Jake's Valley, NV	2733	20	19	23	65.2	1.9524	1.3321	0.1629	0.2054
Lupinus sp.	Eureka, UT	2703	20	19.5	23	47.8	1.6957	1.3966	0.271	0.2002
Stipa comata	Modena, UT	2613	19	18.5	44	11.4	1.1136	1.0155	0.0024	0.0135
Stipa comata	Cisco, UT	2614	20	19.5	44	4.6	1.0455	1.0247	0.0000	0.0138
Stipa comata	Jake's Valley, NV	2732	19	17.5	44	9.1	1.0909	1.0389	0.0203	0.0244
Vicia americana	Jackson Ridges, UT	2580	20	20	24	37.5	1.3750	1.1311	0.0750	0.0817
Vicia americana	Lost Peak, South UT	2609	21	20.5	24	33.3	1.4167	1.1366	0.0754	0.0868
Vicia sp.	Egan Mountains, NV	2734	20	20	24	45.8	1.5833	1.3093	0.1646	0.1618

Table 6. Genetic diversity statistics for species used in this study. Project is an NFGEL identification number. N = number of individuals collected. N/locus = average number of individuals scored per locus. Pops = # of populations included. P = percent polymorphic loci. A = alleles/locus. Ae = effective alleles/locus. Obs. Het. = observed heterozygosity. Exp. Het. = expected heterozygosity.

Species	Proj.	Pops	Ploidy	N	N/locus	Loci	P	A	Ae	Obs Het	Exp Het
Astragalus utahensis	110	4	autotetraploid	79	64.8	19	63.2	2.0952		0.2176	
Atriplex canescens	132	4	diploid	40	74.0	13	61.5	1.9231	1.2302	0.0886	0.1395
Atriplex canescens	132	5	autotetraploid	100	96.1	13	84.6	2.5271		0.1457	
Atriplex canescens	132	2	autohexaploid	39	36.5	13	53.8	1.8452		0.1835	
Bromus carinatus	117	2	autotetraploid	41	32.4	21	19.0	1.2381		0.0617	
Crepis acuminata	108	5	“diploid”	100	83.0	12	58.3	1.7500	1.1917	0.0682	0.1167
Erigeron pumilus	106	7	diploid?	130	89.0	19	84.2	2.6842	1.3245	0.1343	0.2024
Eriogonum umbellatum	113	2	diploid	41	36.5	18	66.7	2.1111	1.3732	0.1645	0.1954
Lupinus argenteus	115	6	diploid	120	112.5	23	100%	2.8261	1.4972	0.1872	0.2722
Stipa comata	134	3	allotetraploid	58	55.5	44	20.4	1.2273	1.1520	0.0075	0.0784
Vicia americana	119	2	diploid	81	81.0	24	54.2	1.7083	1.1774	0.0754	0.1190

Table 7. Genetic differentiation and gene flow in species used in this study. Project is an NFGEL identification number. Wrights F statistics include differentiation of individuals within populations (Fis), individuals within total study (Fit), and populations within total study (Fst). Nm = calculated gene flow. GI = Nei's unbiased genetic identity among populations. Ave. = average, Max. = maximum, and Min. = minimum.

Species	Project	Fis	Fit	Fst	Nm	Ave. GI	Max GI	Min GI
Atriplex canescens – 2X	132	0.1979	0.4262	0.2846	0.6283	0.9448	0.9868	0.9070
Crepis acuminata	108	0.2004	0.4215	0.2764	0.6544	0.9442	0.9976	0.8785
Erigeron pumilus	106	0.1722	0.4357	0.3182	0.5356	0.9002	0.9842	0.8571
Eriogonum umbellatum	113	0.1100	0.1612	0.0575	4.0993	0.9759	0.9759	0.9759
Lupinus argenteus	115	0.0773	0.3670	0.3140	0.5462	0.8823	0.9335	0.8096
Stipa comata	134	0.5500	0.9031	0.7847	0.0686	0.9071	0.9148	0.9031
Vicia americana	119	0.0867	0.3598	0.2990	0.5860	0.9244	0.9244	0.9244

*Note: When data for *Erigeron pumilus* is limited to loci that resolved in all populations, GI averages 0.9273, range 0.8855 – 0.9851.

Table 8. Genetic identities between outlying populations and others submitted as the same species, for three species. GI = Nei's unbiased genetic identity.

Species	Pops	Odd pop.	Ave.GI	Max. GI	Min. GI
Eriogonum umbellatum	3	Inkom, ID, #2714	0.5724	0.5812	0.5636
Lupinus argenteus	7	Eureka, UT, #2703	0.7661	0.7364	0.8094
Vicia americana	3	Egan Mts., NV, #2734	0.4242	0.4012	0.4473

APPENDIX A

Allele Frequencies Suggesting That Two or More Species Were Submitted as One

Eriogonum umbellatum

Table A1. Allele frequencies that provide evidence that the Inkom population (#2714) is different.

Locus	Allele	Tintic – 2, UT	Crystal Ice, ID	Inkom, ID
		2701	2716	2714
GLYDH	**A**	**1.0000**	**0.8421**	
GLYDH	**B**		**0.1579**	
GLYDH	**C**			**0.9615**
GLYDH	**D**			**0.0385**
MDH-2	A	0.9250	0.9524	
MDH-2	B	0.0750	0.0476	
MDH-2	C			0.9524
MDH-2	D			0.0476
PGM-s	**A**	**1.0000**	**1.0000**	
PGM-s	**B**			**1.0000**
TPI-f	A	1.0000	1.0000	0.0714
TPI-f	B			0.9286
UGPP	**A**	**0.7250**	**0.8095**	
UGPP	**B**	**0.2750**	**0.1905**	**0.1190**
UGPP	**D**			**0.0952**
UGPP	**E**			**0.5714**
UGPP	**F**			**0.2143**

Lupinus argenteus

Table A2. Frequencies of selected alleles in the Eureka population of *Lupinus argenteus,* compared to overall frequency in the other six populations. Note fixed heterozygosity in AAT-yf and IDH-f.

Enzyme	Locus	allele	Eureka, UT 2703	Six other populations
AAT	yf	A		0.4250
AAT	yf	B		0.0250
AAT	yf	C	0.5000	
AAT	yf	D	0.5000	0.5500
IDH	**fast**	**A**		**0.4158**
IDH	**fast**	**B**	**0.5000**	**0.5474**
IDH	**fast**	**C**		**0.0316**
IDH	**fast**	**F**	**0.5000**	**0.0053**
6PGD	**fast**	**A**	**0.7895**	**0.0769**
6PGD	**fast**	**B**		**0.9103**
6PGD	**fast**	**C**	**0.2105**	**0.0128**
PGM	fast	A	1.0000	0.1667
PGM	fast	B		0.8333
PGM	**slow**	**A**		**0.8333**
PGM	**slow**	**B**		**0.0292**
PGM	**slow**	**C**	**1.0000**	**0.8333**

Vicia americana

Table A3. Allele frequencies in 13 of the 24 loci identified in *Vicia americana* populations.

Locus	Allele	S2 W2-26	S2 W26-35 S3 W2-14	S4 W15-35 S5 W2
		Jackson, UT	Lost Peak, Southern UT	Egan Mts., NV
		2580	2609	2734
AAT-f	**A**	**1.0000**	**1.0000**	
AAT-f	**B**			**0.9250**
AAT-f	**C**			**0.0750**
AAT-m	A	1.0000	1.0000	
AAT-m	B			1.0000
ACO	**A**	**0.9750**		
ACO	**B**		**1.0000**	
ACO	**C**	**0.0250**		**1.0000**
CAT	A	0.9250	1.0000	
CAT	B	0.0750		1.0000
GLYDH	**A**	**0.9750**	**0.9762**	**0.0250**
GLYDH	**B**	**0.0250**	**0.0238**	**0.3000**
GLYDH	**C**			**0.6750**
G6PDH	A	1.0000	1.0000	
G6PDH	B			1.0000
IDH	**A**	**0.9750**	**0.7619**	
IDH	**B**	**0.0250**		
IDH	**C**		**0.2381**	
IDH	**D**			**0.9750**
IDH	**E**			**0.0250**
LAP	A	0.8500	1.0000	
LAP	B	0.1500		
LAP	D			0.4500
LAP	E			0.4750
LAP	F			0.0750
MDH-2	**A**	**1.0000**	**0.6429**	
MDH-2	**B**		**0.3571**	**0.4250**
MDH-2	**D**			**0.5000**
MDH-2	**E**			**0.0750**
MDH-3	C	1.0000	1.0000	
MDH-3	E			1.0000
PGD-s	**A**	**1.0000**	**1.0000**	
PGD-s	**B**			**0.9500**
PGD-s	**C**			**0.0500**
PGM-1	A	1.0000	0.5952	
PGM-1	B		0.4048	1.0000
PGM-2	**A**	**1.0000**	**0.9762**	
PGM-2	**B**		**0.0238**	**1.0000**

Stipa comata

Table A4. Evidence that Individual #4 (wick 5) in set 1 is not *Stipa comata*.

Enzyme	Unique allele	no difference	Ambiguous
CAT	X		
FEST			odd bright blob
GLYDH			faint
IDH	X		
LAP	X		
MDH fast	X		
MDH middle	X		
MDH slow	X		
ME			stains faintly
6PGD	X?		
PGI fast		X	
PGI slow	X		
PGM	X		
SKD	X		
SOD			no band
TPI		X	
UGPP		X	

APPENDIX B

Evidence for Polyploidy in Species Studied

Multiband patterns have 4 or more bands in dimers, 3 or more bands in monomers. Multiband patterns are indicative of polyploidy. Fixed or consistent heterozygosity is evidence of disomic inheritance. The presence of only one class of unbalanced heterozygosity and only one kind of homozygote also suggest disomic inheritance. The presence of reciprocal unbalanced heterozygotes (e.g. AAAB and ABBB), or of unbalanced heterozygotes plus two classes of homozygotes is evidence of autopolyploidy with tetrasomic inheritance.

Astragalus utahensis

Table B1. Evidence for autopolyploidy in *Astragalus utahensis*, by population. Homozygotes shown only for populations that have other evidence of polyploidy.

	Jake's Valley, NV	H2O plant, Orem, UT	Wolverine Canyon, ID	near Mt. Moriah, NV
	2572	2700	2715	2735
Multi-band patterns				
AAT-2			ABBC	ABBC
6PGD-2	AACD	BCCD, AACD, BBCD, ABDD	ABBD	ABDD
PGM		AABC	AABC	AABC?
TPI-s		AABC, ABCC		
Unbalanced hets				
AAT-2		AAAB, ABBB	AAAB, ABBB, AAAC	AAAB, BBBC
G6PDH			AAAB	
ME	AAAB	AABB		AAAB, ABBB
6PGD-2	AAAC, ACCC	BDDD, ACCC	BDDD	ADDD
PGI-s	AAAC	AAAC		AAAC
PGM		AAAB, ABBB	AAAB	AAAB, AAAC
TIP-s	AAAB		AAAB	AAAB
UGPP		AAAB	AAAB	AAAB
Homozygotes				
AAT-2		A	A	A
ME	A, B	B	B	A, B
PGI		A	A, B	A, B
PGM		A	A, B	A, B
TPI-s				A
UGPP	A	A	A	A

Atriplex canescens

Table B2. Evidence for autopolyploidy in *Atriplex canescens.* Note that in each these enzymes, one allele is observed in all individuals.

Enzyme	Alleles	Homozygotes?	fixed / consistent heterozygosity?	unbalanced heterozygotes?	Multiband patterns?
AAT-f	1, 2	1	no	1112	
AAT-s	1, 2, 3	1	no	1112, 1222, 1113, 1115	
FEST	1, 2	1, 2	no	1112	
G6PDH	1, 2, 3, 4	1, 3	no	1114, 1333, 1333, 3444	M
LAP	1, 2	1, 2	no	1112	
6PGD	1, 2, 3	1	no	1113, 1333	
PGM	1, 2	1, 2	no	1112, 1222	
TPI-slow	1, 2, 3	1, 2, 3	no	1112, 1222	
TPI-fast	1, 2	1	no	1112	

Bromus carinatus

Table B3. Evidence for polyploidy in *Bromus carinatus.* Note that in each these enzymes, one allele is observed in all individuals.

Enzyme	Alleles	Homozygotes?	fixed / consistent heterozygosity?	unbalanced heterozygotes?	Multiband patterns?
DIA	A, B	A		AAAB, ABBB	
SKD-slow	A, B	A		AAAB, ABBB	
PGI-slow	1,2		yes	maybe	
TPI	1,2	1		1112	

Crepis acuminata

Table B4. Evidence for polyploidy in *Crepis acuminata.* Found in few populations.

Enzyme	Alleles	Homozygotes?	fixed / consistent heterozygosity?	unbalanced heterozygotes?	Multiband patterns?
CAT				V	
LAP				X	X?
PGI		1 kind	X	X (1 kind)	
UGPP			X	V	

Vicia americana

Table B5. Evidence for polyploidy (or a gene duplication in LAP) in the Eureka population of *Vicia*.

Enzyme	Set, wick	evidence
LAP	Set 4/5	3-band patterns in this monomer, unbalanced hets.
GLYDH	Set 4	Possible BCCC hets.

Erigeron pumilus

Table B6. Evidence for polyploidy in *Erigeron pumilus.* Set 6 = Topaz Mountain, UT, #2705. Set 8 = Crystal Ice, ID, #2717. Set 9 = Welcome, Elko Co., NV, #2719

Enzyme	Set (S) and Wick (W)	Evidence
AAT slow	Set 6, W26, and Set 8, W9	possible unbalanced heterozygotes
CAT	Set 9	strange 2-banded heterozygotes
FDP	Set 8 & Set 9	Possible unbalanced heterozygotes
FEST	Set 6 & 8	4-banded patterns in all nearly all individuals
FEST	Set 9	unbalanced 2 – 3 banded patterns
GDH	Set 9	unbalanced heterozygotes
GDH	Set 8	possible unbalanced heterozygotes
ME	Set 8	either fixed heterozygosity or blurred bands
PGI	Set 8, 1st & 2nd 3rds	Both balanced and unbalanced heterozygotes
PGI	Set 6, W6	Unbalanced heterozygote
PGM	Set 8	possible unbalanced heterozygotes and/or shadow bands.

Eriogonum umbellatum

Table B7. Evidence for polyploidy in *Eriogonum.* Note that Set 7 seems to be a different species. Evidence of polyploidy in *E. umbellatum* itself is very weak.

Enzyme	Set, wick	evidence
GLYDH	Set 2, W3,9,11	Unbalanced hets? (If so, W22 is a balanced het.)
PGI	Set 6	Unbalanced hets of 2 kinds, and balanced het. Gene duplication?
IDH	Sets 6 & 7	Multiband patterns & unbalanced hets, but resolves badly
MDH slow	Set 7	1 individual with multiband pattern? But it's faint.

Stipa comata

Table B8. Evidence for allopolyploidy in *Hesperostipa comata*.

Enzyme	Alleles	Homozygotes?	fixed / consistent heterozygosity?	unbalanced heterozygotes?	Multiband patterns?
FEST slow	(A) + (A or B)	yes	no	one	no
ME	(A) + (A or B)	no	fixed in some pops	yes (1 class only)	no
PGI slow	(A or C) + (B) + (C)	no	consistent	no	yes
SKD	(A + B) + (B)	no	fixed	uncertain	no
TPI fast	(A) + (A or B)	yes (A only)	no	yes (1 class only)	no
UGPP fast	(A) + (B)	no	fixed	no	no

Lupinus argenteus

Table B9. Evidence for polyploidy in *Lupinus argenteus*.

Enzyme	Alleles	Homozygotes?	fixed / consistent heterozygosity?	unbalanced heterozygotes?	Multiband patterns?
FEST	1,2,3,4	11, 22, 33	no	X (2 classes)	X
G:YDH	A, B, C, D, E, F		yes		
IDH-fast	A, B, C	AA, BB	in some pops	X (2 classes)	X
PGI-slow	1,2,3	11, 22, 33	no	X (2 classes)	X
PGM-slow	A, B, C	AA, BB	no	X (2 classes)	X

Table B10. *Lupinus argenteus* populations in which evidence for polyploidy was observed:

Enzyme	S1 W2-23	S2 W1-23	S, W26-35, S4 W26-35	S5 W14-35	S6 W2-23	S7 W2-11, S6 W26-35	S8 W14-35
	Jackson Ridge	Salt Cave Hollow	Lost Peak	Eureka	Crystal Ice	Oak Springs	Jake's Valley
	UT	UT	UT	UT	ID	NV	NV
	2581	2586	2608	2703	2718	2720	2733
Multiband patterns							
FEST		1233, 1123	1233	1334	1223		
IDH-fast					ABBC	ABBC	
PGI-slow	1123		1123		1123		
PGM-slow					AABC, ABBC	AABC	
Unbalanced hets							
FEST		2333, 1333, 1113, 1112	1333, 1113	1114	2223, 1222	1112, 2333, 2223	1113, 1222
IDH-fast			ABBB		AAAB, AAAC	AAAB, ABBB, BBBC	
PGI-slow		1112, 1222	1113, 1333	1112	1112		
PGM-slow	AAAC				AAAC, ACCC, BCCC		
Homozygotes?							
FEST	1	1, 2	2	2, 3	2	2, 3	1, 1
IDH-fast		B	B		A	B	
PGI-slow	1	1, 2	1, 3	1	1	1, 2	1, 3
PGM-slow	A	A, C	A	C	A, C	A	A
Fixed hets							
AAT-yf				CD			
IDH-fast	AB			FB			

GENETIC DISTRIBUTION OF TREMBLING ASPEN (*Populus tremuloides*) CLONES IN THE CENTRAL SIERRA NEVADA, CALIFORNIA

Final Report

to

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PROJECT OBJECTIVES

1. How genetically variable are aspens on the Eldorado National Forest?

As is usual for the species, aspens on the Eldorado National Forest are highly diverse genetically (82% polymorphic loci, 3.1 alleles/locus). Stands are genetically different from each other, with 46.5% of all genetic variation measured being the differences among stands. Clonal diversity was 30% -- 198 of the 663 individuals sampled are unique clonal genotypes.

2. What is the clonal structure of aspen on the Eldorado National Forest?

44% of the stands studied are monoclonal (containing only one clone). Therefore, 56% of the stands studied contain two or more clones. Monoclonal stands are usually small in size, and vary from 0.1 to 5 acres, averaging 0.8 acres. 8% of clusters (groups of stands located near each other) are monoclonal. All watersheds contain more than one clone (the 8 watersheds studied contain between 2 and 44 different clones). Individual stand structure is indicated on Location Maps in the Appendix of this report.

3. What is the genetic relationship of stands within a particular watershed and within adjacent drainages?

Genetic similarity among stands within a watershed/drainage varies by watershed/drainage (see Figures 2, 3 and 4; Table 6). The Sayles Canyon watershed stands are genetically similar to each other. The Strawberry Creek watershed contains genetically similar stands which are located linearly along the creek. The southeastern South Fork American River-Forni Creek watershed stands share some similarity with the northwest edge of the adjacent Sayles Canyon watershed. The South Fork Silver Creek-Junction Reservoir watershed shares similarity with the western edge of the Lower Jones Fork Silver Creek watershed. These relationships can be used to delineate tentative breeding populations and to prioritize gene conservation strategies.

4. What is the genetic relationship of stands that are in close proximity to other stands (i.e., stands that are clustered)?

In ten cases, a single clone extended across what are now classified as two or more stands (see Table 3).

Although there are some general trends of increased genetic similarity with geographic proximity (see Figures 2, 3 and 4), stands that are in close proximity are not necessarily genetically similar, and can be, in fact, quite genetically distinct.

5. What is the genetic relationship of isolated stands to their nearest neighboring stands?

The genetic relationship of isolated stands to their nearest neighbor varies by stand pairs. The Wrights Lake Outliers (PA-SF stands) are in the same genetic group as their western neighbor (PA-IH-30). The Info Center Outlier (PA-IH-30) is in a different genetic group than it's western neighbor (PA-IH-20). The Buck Pasture Outlier (PL-SB-44) is quite distinct from anything in it's geographic proximity. Sayles Canyon (PL-ST) outliers share similarity to other Sayles Canyon stands. The Strawberry Outlier (PL-SB-13) is more similar to other Strawberry Creek watershed stands than it is to any South Fork American River-Forni Creek stands.

INTRODUCTION

Quaking Aspen *(Populus tremuloides* Michx.) spreads extensively by rhizomes. Previous studies in the Rocky Mountains have found that an aspen stand may consist of a single clone, and that one clone may cover as much as 43 hectares (Kemperman and Barnes 1976). It has been hypothesized that in the west individual clones may be 10,000 years old (Kemperman and Barns 1976) and that aspen establishment by seed has been rare for millennia (Einspahr and Winton 1976, Mitton and Grant 1996), although stand regeneration from seed has been observed (Ellison 1943; Barnes 1966; Romme et al. 1997).

The Eldorado National Forest, located along the western slope of the central Sierra Nevada, has been conducting aspen stand inventories to determine the distribution and condition of quaking aspen stands across the Forest. On-site surveys have been conducted in 230 of the 276 sites located to date. Aspen stands ranging in size from an individual stem up to 30 acres have been inventoried, some in clusters of as many as 17 stands and others located miles from the nearest known adjacent stand. Stands have been found in 14 different drainages of the South Fork of the American River and four drainages of the Rubicon River. Nearly all the stands in the inventory are located on soils related to glacial moraines, outwashes, or alluvial flows. The 1999-2000 field survey results for aspen stands in the Eldorado National Forest have been summarized (Burton 2000). This summary indicates that a significant number of stands are declining in condition and lack evidence of significant regeneration.

An important aim of conservation biology is the preservation of the evolutionary potential of species by maintaining or enhancing natural levels of adaptive genetic diversity (Hamrick et al. 1991). This begins with conducting a reliable genetic inventory of natural populations. Genetic inventories can serve many purposes, including: (a) forming a baseline for evaluating the effects of management practices on biodiversity, (b) reflecting environmental changes across the landscape, and (c) helping to describe and classify ecological units for management and protection. Understanding quaking aspen clone structure is crucial to preserving aspen genetic diversity.

Resource managers have become increasingly aware and concerned over the apparent decline of aspen throughout the western United States. Several factors appear to be leading to these vegetative changes in aspen populations, including fire suppression, livestock grazing, wild ungulate browsing, conifer succession, and perhaps climate change. High elevation and boreal species are more likely to be adversely affected by a warming and drying climate. Drought, thaw-freeze events, insect defoliation, fungal pathogens, and wood-boring insects together have probably contributed to aspen dieback and mortality in western Canada (Hogg et al. 2002).

Management efforts used to preserve aspen stands in the western U.S. include constructing grazing exclosures, transplanting root suckers and/or nursery stock to augment natural regeneration, and establishing off-site clone banks. Each of these approaches is expensive in terms of time or money, and resources for managing aspens are limited. Therefore, these procedures must be applied efficiently. If an aspen stand is a single clone, preserving a small part of it will preserve its diversity. If a stand contains many clones, more extensive efforts may be required to preserve existing diversity. If genetic diversity is associated with elevation (temperature and moisture gradients), then climate change could reduce the evolutionary potential of aspen.

This study uses an allozyme analysis to identify clones, determine the number of clones in each stand, determine whether stands are most similar to other nearby stands, detect

geographic patterns of variation, and assess the genetic diversity of aspen in the central Sierra Nevada, California.

METHODS

Utility of Allozymes. The measurement of allozyme variation in plants helps in the interpretation of genetic diversity in natural populations. Allozymes are specific enzymes with discrete Mendelian inheritance. Since they are also codominant and free of environmental effects in their expression, they are used to directly calculate allele and genotype frequencies.

While not directly associated with adaptive traits, this laboratory analysis provides quick, inexpensive quantitative measures of genetic structure (amount and pattern of variation among and within populations), genetic diversity (heterozygosity), and mating systems (outcrossing rate). In addition, these parameters can be directly compared across species and can be related to species life-history traits to interpret genetic systems, survival "strategies", and historical lineages of different species. Allozyme results are especially useful when combined with the genetic structure and inheritance of adaptive traits derived from "common garden" trials.

Sampling. Leaf tissue was collected from 663 *Populus tremuloides* individuals (saplings, mature trees, or root suckers) in the spring of 2001 by David Burton, USDA Forest Service, Region 5 Aspen Deliniation Project. Samples were collected from 82 stands located on the Pacific, Placerville, and Amador Ranger Districts, Eldorado National Forest, Eldorado County, California (Table 1). Samples were taken at roughly even intervals throughout stands, as well as collected from isolated trees in or near stands. Sample/stand location maps were drawn for each site by D. Burton. Leaf tissue was transported on ice to the USDA Forest Service National Forest Genetics Laboratory (NFGEL), Placerville, California.

Enzyme Electrophoresis. Two 7 mm discs were punched from each leaf and placed together in a microtiter plate well containing 150 µl of a Tris buffer pH 7.5 (Gottlieb 1981). Samples were stored at -70ºC. On the morning on the electrophoretic run, samples were thawed, macerated, and the extract absorbed onto wicks made from Whatman 3MM Chromatography paper.

Methods of sample preparation and electrophoresis are outlined in USDA Forest Service (2000), and follow the general methodology of Conkle et al. (1982) except that most enzyme stains are somewhat modified. A lithium borate electrode buffer (pH 8.3) was used with a Tris citrate gel buffer, pH 8.3 (Conkle et al. 1982), to resolve leucine aminopeptidase (LAP), malic enzyme (ME7), phosphoglucose isomerase (PGI), and phosphoglucomutase (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 glutamate-oxaloacetate transaminase (GOT), catalase (CAT), and uridine diphosphoglucose pyrophosphorylase (UGPP). A morpholine citrate electrode and gel buffer, pH 8.0 (USDA Forest Service 2000), was used to resolve diaphorase (DIA), phosphogluconate dehydrogenase (6PGD), shikimate dehydrogenase (SKD), and malate dehydrogenase (MDH). All enzymes were resolved on 11% starch gels. Enzyme stain recipes follow USDA Forest Service (2000).

Two loci were scored for LAP, PGM, 6PGD, and UGPP, and three for MDH, for a total of 17 loci. Two people independently scored each gel. When they disagreed, a third person resolved the conflict. For further quality control, 10% of the individuals were run and scored twice. Isozymes are inherited in a single-gene Mendelian manner in *Populus tremuloides*, and are apparently not linked (Hyun et al. 1987b, Liu and Furnier 1993b). 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). Alleles were numbered in order of their discovery in the species, not their speed of migration.

Data Analysis. Genetic diversity analyses was performed on a data set that included one sample per multilocus genotype per stand. Therefore, statistics are based on 210 genotypes. Within a stand, all samples with the same multilocus genotype were considered ramets of the same clone. Incomplete genotypes that matched a complete or another incomplete genotype within the same stand were considered ramets of that clone. The probability of occurrence of matching genotypes among stands was calculated as the product of the frequencies of all alleles in that genotype. Identical allozyme genotypes were assumed to be different clones when geographic separation among genotypes was relatively great and when probability of the same genotypes occurring by chance recombination (based on allele frequencies) was relatively small. A total of 198 genotypes exist in the study when genotypes shared between stands are removed from the dataset.

Allozyme analysis was used to: (a) define genotypes to infer clonal composition of stands, and (b) quantify the amount and describe the pattern of geographic variation. Results were analyzed using Popgene version 1.21 (Yeh et al. 1997). A locus was considered polymorphic if an alternate allele occurred even once. Statistics calculated included unbiased genetic distance (Nei 1978), effective number of alleles per locus (Kimura and Crow 1964), expected heterozygosity (Nei 1973), and gene flow (Slatkin and Barton 1989). Multilocus genotype frequencies were generated by multiplying the allele frequencies (based on genets) for each locus, assuming Hardy-Wienberg equilibrium. Fixation indices (F statistics) were calculated in Popgene by the method of Weir (1990).

To determine if a significant geographic pattern existed in allozyme loci of aspen across its range on the Eldorado National Forest, a canonical correlation analysis (CAA) was used. The objective was to build a model that would best represent the geographic pattern of allozyme genotypes in aspen. The 198 unique allozyme genotypes distributed across the Eldorado NF formed the base for the CCA between allozymes (genes) and their geographic locations (latitude, longitude, and elevation). Due to missing data, 129 clones from 73 stands were used to build the model. CCA is the multivariate equivalent of multiple regression, but with more than one dependent variable (e.g. many allozymes). The first step in the statistical process was to transform allele presence or absence in the diploid genotypes to an additive score (Smouse and Williams 1982) to achieve normality for analyses. The three geographic variables were expressed as a 2^{nd} order polynomial, creating nine geographic terms. In developing the CCA model, allozymes that contributed negligibly were dropped from the model (Westfall and Conkle 1992). Canonical scores for the first three highly significant vectors were regressed on the nine geographic terms to create predicted scores. Predicted stand scores for each of the three vectors were subdivided in halves (above and below the mean) to create eight genetic classes (A to H). Minimum differences between multi-locus genotypes were predicted to be within classes. Maximum differences were predicted between A and H (differ in all three vectors). Sequential pairs (e.g. A vs B, C vs D, E vs F, G vs H) differ only in the 3^{rd} vector. A + C vs B + D and E + G vs F + H differ only in the 2^{nd} vector. A+B+C+D vs E+F+G+H differ only in the 1^{st} vector.

RESULTS

Clonal Identification. The 663 aspen samples analyzed were located in 82 stands from eight watersheds on the Eldorado National Forest. Stand area ranged from 0.1 to 28 acres, with

an average stand size of 2.0 acres (s.d. = 3.8). Stands in geographic proximity were also identified as to a cluster location (Table 1). The number of stems sampled within a stand ranged from 1 to 22 (average = 8.1, s.d. = 5.1). Four stands consist of only a single sampled individual (Table 2).

A total of 198 multilocus genotypes (clones, or genetic individuals) were identified among the 663 sampled individuals (Table 2). The number of clones ranged from 1 to 11 within stand (average = 2.6), and from 1 to 29 within cluster (average = 8.4). Forty (49%) of the 82 stands are monoclonal (containing only one multilocus genotype). However, only one individual was sampled in each of four stands (PA-WL 28, PL-AA 21, PL-CS 18A, and PL-ST 23). When these stands are excluded, 44% of stands are monoclonal. Three clusters (Info Center, Strawberry Outlier, and Upper Sayles Outlier) are monoclonal, although only one individual was sampled in the Upper Sayles Outlier cluster (containing the PL-ST 23 stand). Watersheds contain between 2 to 44 clones (average = 24.8), and therefore, no watershed is monoclonal. The extent of clones in each stand was mapped (Appendix).

Ten genotypes are duplicated in adjacent stands within single watersheds (Table 3). These duplications were treated as clones that extended between stands. Most duplicated clones are separated by no more than 3,000 feet. The average probability of occurrence for a multilocus genotype within this study is 1.4×10^{-5} (s.d. 4.8×10^{-5}), with a range of 5.0×10^{-4} to 1.2×10^{-14}. Of these genotype pairs repeated within the same watersheds, the probabilities of these matches occurring by chance vary between 1.3×10^{-4} and 1.2×10^{-14}.

Stands that consist of a single clone vary in size from 0.1 to 5 acres (Table 1). Monoclonal stands averaged smaller in size than stands with two or more clones (0.8 and 3.1 acres, respectively) (Figure 1).

Genetic Diversity. As a group, the aspens are genetically variable, with 82.4% of loci polymorphic, an average of 3.1 alleles per locus, and expected heterozygosity of 0.279. Individual stands, which consist of only one to 11 genetic individuals, are much less variable, averaging 33.3% polymorphic loci with 1.4 alleles per locus. Watersheds, on average, contain more diversity than do individual stands (He = 0.261 and 0.229, respectively) (Table 4).

Of the total variation measured, 46.5% was found among stands, indicating that stands are very differentiated, or genetically different from each other. Watersheds are much less differentiated (Fst = 10%; Table 5). Genetic similarities among aspen stands were highly variable and often low, averaging 85.8% (similarities range from 100% to 61.8%). Similarities among watersheds average 95.8%. Genetic similarities of outlying stands to the cluster they were considered outliers of averaged 85.1%, and genetic similarity of stands within watersheds averaged 89.8%.

Geographic Patterning. The best fitting model to explain geographic patterning of the data related a subset of 27 allozymes from 12 loci with all nine geographic variables. These allozymes in combination varied in frequency in a geographic pattern. Geographic patterning was relatively strong and complex. In the 1st canonical vector, the geographic model accounted for 64% (adjusted=54%, unbaised=44%) of the new allozyme variable. Elevation and its interaction terms were primary components of the 1st vector and longitude was secondary. The 2nd vector was composed of latitude and latitude-longitude interaction. The 1st three vectors were highly significant and accounted for 72% of the original allozyme variation. These three vectors were used to form genetic-geographic classes having similar predicted gene (allozyme) frequencies (Table 6). These classes are shown on maps in Figures 2, 3, and 4.

DISCUSSION

Clonal Structure and Genetic Diversity. Clone structure of *Populus tremuloides* stands varies geographically. In northeast North America, aspen clones are often small and stands contain more than one clone (Kemperman and Barnes 1976), and establishment from seed is common (Mitton and Grant 1996). Monoclonal stands tend to be small in size, varying in size from 0.001 to 0.9 ha in northern Michigan (Bertenshaw 1965; Zahner and Crawford 1965; Barnes 1966), and from 0.006 to 1.5 ha in Manitoba (Kemperman and Barnes 1976). In the intermountain west, clones are often large, stands are often considered monoclonal, although stands may contain more than one clone (Kemperman and Barnes 1976), and establishment of new stands from seed may be rare (Mitton and Grant 1996). Aspen stands in the central Sierra Nevada combine traits of aspens studied elsewhere. As in the Rocky Mountains, an important proportion (44%) of stands appeared to consist of only one clone. As in northeast North America, the monoclonal Sierra Nevada stands observed in this study are often small (averaging 0.8 ha).

Although the extent and diversity of clones within aspen stands varies geographically, all studies of aspen genetic diversity, including this one, agree that *Populus tremuloides* harbors unusually great genetic diversity. The 82% polymorphic loci observed in this study is similar to the values observed elsewhere (Table 7). Watersheds contain similar levels of genetic diversity (Table 4). Clusters vary in their overall diversity levels. Buck Pasture, Convict Meadow, Loon Lake Trail, Middle Sayles, Upper Sayles, and Wrights Lake tend to be the more genetically diverse clusters, while the Airstrip and Outlier clusters are the least diverse. The low percent polymorphic loci within stands (Table 7) reflects the monoclonal nature of nearly half the stands sampled. The monoclonal nature of many of the stands, together with the spread of some clones between stands, suggests a previously broader range of aspen than its current size.

Levels of genetic diversity per cluster generally increase as the number of clones increase. This is reflected in several diversity measures (percent polymorphic loci, R^2=0.776 and alleles per locus, R^2=0.837). Percent polymorphic loci per stand is lower in this Project than in NFGEL Projects 84 and 68 in northeast Oregon in part due to a real difference in the proportion of monoclonal stands, but also in part due to a different definition of stand, which is more comparable to cluster in this Sierra Nevada study.

Adaptation and Geographic Variation. The distribution of species, populations, and genotypes of individual plants (i.e. genetic variation) across the landscape is often associated with geography (i.e. latitude, longitude, and elevation). Geographic patterns associated with climatic variables (e.g. temperature and moisture) suggest natural selection for adaptation of plant populations to different climates. However, recent changes in climate may cause current populations to be sub-optimal ("adaptational-lag"). Also, migration (gene flow) routes and/or genetic drift (in small, reproductively-isolated populations) may also influence genetic-geographic variation patterns and delay adaptation to local environments.

Although single-locus correlations with geography have been found for allozymes, adaptation of an individual is likely controlled by alleles (genes) at many loci, with small effects. Some genes convey a selective advantage or disadvantage to the individual, depending on the adaptive traits and the environment involved. For example, highly competitive environments may induce ("turn-on") specific genes and exert strong selective pressure for growth (e.g. rate and phenology) traits. In contrast, highly stressful environments may induce a different set of genes and exert strong pressure for defensive (e.g. stress-tolerance, injury-repair) traits.

The cumulative effects of small differences in allelic frequencies, when summed across multiple loci can increase detection of differentiation among populations and allow reliable grouping based on genetic similarity of multi-locus genotypes (Smouse et al 1982). Multi-variate statistical analyses have been successfully employed to reveal geographic patterns in trees (Guries 1984; Yeh et al 1985). Therefore, multi-locus allozyme variation based on multi-variate analytical methods may detect underlying adaptive variation patterns.

Geographic structuring exists among the aspen stands on the Eldorado National Forest (Table 6, Figures 2, 3 and 4). West of the 120.24 longitude and ranging from 5200 to 6800 ft elevation, stands share a high degree of genetic similarity. Most of these stands belong to genetic class 'C' and 'D'. East of 120.12 longitude and ranging from 6400 to 7700 ft elevation, stands belong to classes 'G' and 'H'. Stands along Sayles Canyon (PL-ST) share genetic similarity and all 15 belong to class 'H'. Strawberry Creek (PL-SB) is comprised entirely of genetic class 'G'. Stands along Caples Creek divide into two elevation groups: low (<6500ft) belong to two classes: 'A' and 'B'; and high (>7500ft) belong to 'E' and 'G'. The 'E' genetic group is one of the more geographically diverse groups and extends from Caples Creek at 7800 ft north into Lyons Creek (PA-LC) at ca 7500 ft, and encompasses half of the PA-WL stands near Wright's Lake. The most genetically diverse classes include 'B' (the most diverse class), 'H', 'C', and 'G'. The least diverse classes are 'A', 'E', 'D', and 'F' (the least diverse cluster in number of clones and levels of expected heterozygosity) (Table 6). This genetic-geographic classification may be used as a guide for delineating tentative gene management units, for prioritizing gene conservation strategies, and for further genecological study (e.g. previous migration routes, adaptive gene-environment relationships).

Management Implications. Allozyme analysis of aspen stands is useful for conservation efforts in three ways. First, the clone maps (Appendix) generated by this study permit efficient collection of ramets from different clones, and allow for effective management at the stand level. Phenotypic variation alone had not revealed the extent of many of these clones. Second, the high Fst values reveal that stands are genetically different from each other. Therefore, conservation efforts will be successful at preserving local aspen genetic resources only if those efforts are directed at preserving many stands and their component clones. Allozyme analysis can help prioritize aspen stands and clones for preservation. From a genetic standpoint, high priority stands might include the most diverse stands or remnants of ancient stands. Of course, ecological as well as genetic issues are involved in choosing priority stands for conservation. Finally, a geographic patterning of genetic variation was detected by the allozyme data. Based on genetic classifications, tentative gene management units can be delineated and conservation efforts concentrated within groupings. For example, if restoration objectives for monoclonal stands require additional clonal diversity, priorities for clonal introductions from other stands may follow transfers within genetic classes or between similar genetic classes, while also considering clonal and gene diversity.

The clonal nature of aspens makes the plants very long-lived and therefore provides many -- but not infinite -- opportunities to preserve the species' genetic variation. However, this clonal structure mandates preservation efforts at a large spatial scale and across the full elevational gradient. In addition to genetics, preservation plans must consider the ecological role of aspen stands, which increase faunal diversity in areas dominated by pines or sagebrush.

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Table 1. *Populus tremuloides* stands sampled from the Eldorado National Forest, central Sierra Nevada, California. Stand area is in acres. UTM coordinates were established using 1927 North American datum (NAD 27) and are located in Zone 10S.

Cluster ID	Watershed	Stand Code	Stand #	Stand Area	UTME	UTMN
Convict Meadow	Caples Creek	AM-AA	31	6	748344	4287659
Convict Meadow	Caples Creek	AM-AA	32	0.3	748921	4287823
Convict Meadow	Caples Creek	AM-AA	33	0.1	747940	4287400
Convict Meadow	Caples Creek	AM-AA	34	1.5	749278	4288118
Ice House	South Fork Silver Creek-Junction Reservoir	PA-IH	10A	0.1	727522	4299083
Ice House	South Fork Silver Creek-Junction Reservoir	PA-IH	10B	0.4	727483	4298970
Ice House	South Fork Silver Creek-Junction Reservoir	PA-IH	10C	0.5	727587	4298963
Ice House	South Fork Silver Creek-Junction Reservoir	PA-IH	10D	1.2	727685	4298850
Info Center	Lower Jones Fork Silver Creek	PA-IH	20	1.5	727680	4302020
Info Center Outlier	Lower Jones Fork Silver Creek	PA-IH	30	0.5	735760	2402550
Lyons Creek	South Fork Silver Creek-Wrights Lake	PA-LC	18	0.3	743050	4300250
Lyons Creek	South Fork Silver Creek-Wrights Lake	PA-LC	46	28	742150	4300398
Lyons Creek	South Fork Silver Creek-Wrights Lake	PA-LC	48	0.15	742820	4300180
Lyons Creek	South Fork Silver Creek-Wrights Lake	PA-LC	49	13	742546	4300194
Lyons Creek	South Fork Silver Creek-Wrights Lake	PA-LC	51	5	742770	4300521
Wrights Lake Outlier	South Fork Silver Creek-Wrights Lake	PA-SF	10	0.2	739940	4300840
Wrights Lake Outlier	South Fork Silver Creek-Wrights Lake	PA-SF	11	0.2	740020	4301100
Airstrip	Upper South Fork Rubicon River	PA-TC	14	1	733831	4315283
Airstrip	Upper South Fork Rubicon River	PA-TC	15	1	733689	4315385
Airstrip	Upper South Fork Rubicon River	PA-TC	16	0.6	733560	4315395
Airstrip	Upper South Fork Rubicon River	PA-TC	17	1.4	733463	4315400
Loon Lake Trail	Upper South Fork Rubicon River	PA-TC	18	5	733303	4315446
Airstrip	Upper South Fork Rubicon River	PA-TC	18A	0.3	733617	4315518
Stone Cellar Trail	Upper South Fork Rubicon River	PA-TC	21A	0.75	731912	4314823
Stone Cellar Trail	Upper South Fork Rubicon River	PA-TC	21C	0.5	731703	4314802
Loon Lake Trail	Upper South Fork Rubicon River	PA-TC	36	4	732968	4315205

Cluster ID	Watershed	Stand Code	Stand #	Stand Area	UTME	UTMN
Stone Cellar Trail	Upper South Fork Rubicon River	PA-TC	41	3	731350	4315150
Stone Cellar Trail	Upper South Fork Rubicon River	PA-TC	42	11	731495	4315189
Wrights Lake	South Fork Silver Creek-Wrights Lake	PA-WL	15	0.5	740803	4303550
Wrights Lake	South Fork Silver Creek-Wrights Lake	PA-WL	24	0.5	741375	4303920
Wrights Lake	South Fork Silver Creek-Wrights Lake	PA-WL	25	1	741127	4303875
Wrights Lake	South Fork Silver Creek-Wrights Lake	PA-WL	26	0.5	741037	4303759
Wrights Lake	South Fork Silver Creek-Wrights Lake	PA-WL	27	0.2	740986	4303632
Wrights Lake	South Fork Silver Creek-Wrights Lake	PA-WL	28	0.1	740848	4303578
Wrights Lake	South Fork Silver Creek-Wrights Lake	PA-WL	30	0.25	741305	4304100
Jack Schneider	Caples Creek	PL-AA	19	0.3	745672	4286900
Jack Schneider	Caples Creek	PL-AA	20	0.5	745884	4287104
Jack Schneider	Caples Creek	PL-AA	21	0.1	746073	4287260
Jack Schneider	Caples Creek	PL-AA	24	2	746205	4287210
Jack Schneider	Caples Creek	PL-AA	25	5	746350	4287466
Government Meadow	Caples Creek	PL-AA	27	1.2	747782	4288303
Government Meadow	Caples Creek	PL-AA	28	1	747852	4288196
Camp Sacramento	South Fork American River-Forni Creek	PL-CS	13	2.6	750303	4298443
Lovers Leap	South Fork American River-Forni Creek	PL-CS	14	0.3	749175	4298354
Lovers Leap	South Fork American River-Forni Creek	PL-CS	15	0.1	749310	4298728
Lovers Leap	South Fork American River-Forni Creek	PL-CS	16	0.4	749215	4298807
Camp Sacramento	South Fork American River-Forni Creek	PL-CS	17	0.2	749613	4298946
Camp Sacramento	South Fork American River-Forni Creek	PL-CS	18	0.2	749500	4298999
Camp Sacramento	South Fork American River-Forni Creek	PL-CS	18A	0.2	749500	4298999
Lovers Leap	South Fork American River-Forni Creek	PL-CS	20	6	749280	4298985
Camp Sacramento	South Fork American River-Forni Creek	PL-CS	22	0.2	749869	298689
Camp Sacramento	South Fork American River-Forni Creek	PL-CS	23	0.5	750089	4298459
Strawberry Outlier	Strawberry Creek	PL-SB	13	5	749523	4295893
Upper Strawberry	Strawberry Creek	PL-SB	22	2	751720	4292650
Upper Strawberry	Strawberry Creek	PL-SB	27	0.5	751729	4292259
Upper Strawberry	Strawberry Creek	PL-SB	27A	1	751680	4292240
Upper Strawberry	Strawberry Creek	PL-SB	28	0.75	751700	4292160
Buck Pasture	Strawberry Creek	PL-SB	37	0.75	751448	4291257
Buck Pasture	Caples Creek	PL-SB	39	0.2	751950	4290604

Cluster ID	Watershed	Stand Code	Stand #	Stand Area	UTME	UTMN
Buck Pasture	Caples Creek	PL-SB	39A	5	752130	4290510
Buck Pasture	Caples Creek	PL-SB	39B	0.2	751650	4290640
Buck Pasture	Strawberry Creek	PL-SB	42	1.5	751400	4290800
Buck Pasture	Strawberry Creek	PL-SB	42B	1.5	751400	4290800
Buck Pasture Outlier	Caples Creek	PL-SB	44	1.2	750350	4290750
Strawberry Lodge	South Fork American River-Forni Creek	PL-SB	45	0.5	747678	4297563
Strawberry Lodge	South Fork American River-Forni Creek	PL-SB	49	2	747375	4297410
Strawberry Lodge	South Fork American River-Forni Creek	PL-SB	50	0.2	747526	4297458
Lower Sayles	Sayles Canyon	PL-ST	11	0.2	751515	4297437
Lower Sayles	Sayles Canyon	PL-ST	12	0.2	751579	4297343
Lower Sayles	Sayles Canyon	PL-ST	13	0.1	751700	4297010
Lower Sayles	Sayles Canyon	PL-ST	14	0.1	751895	4297020
Lower Sayles	Sayles Canyon	PL-ST	16	1	751976	4296940
Lower Sayles	Sayles Canyon	PL-ST	16A	1	751976	4296940
Middle Sayles	Sayles Canyon	PL-ST	22	4	752709	4296589
Upper Sayles Outlier	Sayles Canyon	PL-ST	23	0.1	753426	4296179
Middle Sayles	Sayles Canyon	PL-ST	25	10	752855	4296635
Middle Sayles	Sayles Canyon	PL-ST	27	6	752791	4296733
Lower Sayles Outlier	Sayles Canyon	PL-ST	29	0.2	751250	4297790
Upper Sayles	Sayles Canyon	PL-ST	31	0.3	753605	4296123
Upper Sayles	Sayles Canyon	PL-ST	32	4	753738	4296123
Upper Sayles	Sayles Canyon	PL-ST	33	0.2	753829	4295882
Lower Sayles Outlier	Sayles Canyon	PL-ST	38	0.1	751311	4297718

Table 2. Numbers of *Populus tremuloides* clones by stand. N = number samples (stems) analyzed per stand, cluster, or watershed. G = number of multilocus genotypes per stand, cluster, or watershed.

	Name (Stand Code, Cluster, or Watershed)	Stand #	N	G
BY STAND				
	AM-AA	31	21	11
	AM-AA	32	3	1
	AM-AA	33	3	1
	AM-AA	34	12	2
	PA-IH	10A	3	1
	PA-IH	10B	10	1
	PA-IH	10C	14	1
	PA-IH	10D	12	1
	PA-IH	20	8	1
	PA-IH	30	8	2
	PA-LC	18	9	6
	PA-LC	46	22	6
	PA-LC	48	4	1
	PA-LC	49	18	10
	PA-LC	51	15	6
	PA-SF	10	4	2
	PA-SF	11	4	1
	PA-TC	14	11	2
	PA-TC	15	10	1
	PA-TC	16	5	1
	PA-TC	17	6	1
	PA-TC	18	14	9
	PA-TC	18A	2	1
	PA-TC	21A	14	6
	PA-TC	21C	7	2
	PA-TC	36	16	10
	PA-TC	41	3	1
	PA-TC	42	16	7
	PA-WL	15	5	2
	PA-WL	24	7	1
	PA-WL	25	9	4
	PA-WL	26	5	2
	PA-WL	27	4	2
	PA-WL	28	1	1
	PA-WL	30	9	1
	PL-AA	19	2	1
	PL-AA	20	5	1
	PL-AA	21	1	1
	PL-AA	24	12	1
	PL-AA	25	14	4
	PL-AA	27	13	4
	PL-AA	28	10	3
	PL-CS	13	13	3
	PL-CS	14	5	1
	PL-CS	15	5	1
	PL-CS	16	6	2
	PL-CS	17	3	1
	PL-CS	18	2	1
	PL-CS	18A	1	1
	PL-CS	20	16	4

	Name (Stand Code, Cluster, or Watershed)	Stand #	N	G
	PL-CS	22	2	1
	PL-CS	23	6	2
	PL-SB	13	15	1
	PL-SB	22	12	3
	PL-SB	27	9	3
	PL-SB	27A	8	1
	PL-SB	28	11	2
	PL-SB	37	10	5
	PL-SB	39	2	1
	PL-SB	39A	3	1
	PL-SB	39B	3	1
	PL-SB	42	13	5
	PL-SB	42B	6	2
	PL-SB	44	9	3
	PL-SB	45	6	1
	PL-SB	49	14	4
	PL-SB	50	2	1
	PL-ST	11	10	3
	PL-ST	12	4	2
	PL-ST	13	4	2
	PL-ST	14	6	2
	PL-ST	16	6	3
	PL-ST	16A	8	3
	PL-ST	22	14	1
	PL-ST	23	1	1
	PL-ST	25	14	4
	PL-ST	27	17	8
	PL-ST	29	4	1
	PL-ST	31	4	1
	PL-ST	32	12	3
	PL-ST	33	2	1
	PL-ST	38	4	1
	(Total)		663	210
BY CLUSTER				
	Airstrip		34	6
	Buck Pasture		37	14
	Buck Pasture Outlier		9	3
	Camp Sacramento		27	9
	Convict Meadow		39	14
	Government Meadow		23	7
	Ice House		39	2
	Info Center		8	1
	Info Center Outlier		8	2
	Jack Schneider		34	8
	Loon Lake Trail		30	19
	Lovers Leap		32	8
	Lower Sayles		38	13
	Lower Sayles Outlier		8	2
	Lyons Creek		68	29
	Middle Sayles		45	13
	Stone Cellar Trail		40	16
	Strawberry Lodge		22	4
	Strawberry Outlier		15	1

	Name (Stand Code, Cluster, or Watershed)	Stand #	N	G
	Upper Sayles		18	5
	Upper Sayles Outlier		1	1
	Upper Strawberry		40	9
	Wrights Lake		40	12
	Wrights Lake Outlier		8	3
BY WATERSHED				
	Caples Creek		113	35
	Lower Jones Fork Silver Creek		16	3
	Sayles Canyon		110	34
	South Fork American River-Forni Creek		81	21
	South Fork Silver Creek-Junction Reservoir		39	2
	South Fork Silver Creek-Wrights Lake		116	44
	Strawberry Creek		84	19
	Upper South Fork Rubicon River		104	40
ENTIRE STUDY			663	198

Table 3. Matching Aspen genotypes found in different stands but treated as single clones. G = the number of different genotypes in stand. R = the number of samples with the matching genotype/total number of samples in the stand. P = the probability that the genotype would occur, based on the frequency of alleles in the genotype. Separation = distance between stands, in feet. Multilocus genotypes are presented in this order: LAP1, LAP2, PGM1, PGM2, ME7, PGI2, UGPP1, UGPP2 CAT, GOT, SKD, MDH1, MDH2, MDH3, 6PGD1, 6PGD2, DIA. 0 = missing data.

Watershed	Stand Code	Stand #	G	R	P	Separation (feet)	Genotype
S. Fork American River-Forni Creek	PL-SB	45	1	6/6	2.1×10^{-8}	520 – 1,113	11 11 11 12 11 79 11 12 11 22 11 11 11 12 11 11 15
S. Fork American River-Forni Creek	PL-SB	49	4	8/14			
S. Fork American River-Forni Creek	PL-SB	50	1	2/2			
Sayles Canyon	PL-ST	14	2	2/6	1.1×10^{-6}	2,536	11 11 14 11 11 33 11 22 11 12 11 11 11 11 11 11 15
Sayles Canyon	PL-ST	16A	3	1/8			
South Fork Silver Creek-Wrights Lake	PA-WL	24	1	7/7	1.8×10^{-8}	633	11 16 11 33 11 23 11 22 11 12 11 11 11 23 12 11 15
South Fork Silver Creek-Wrights Lake	PA-WL	30	1	9/9			
South Fork Silver Creek-Junction Reservoir	PA-IH	10B	1	10/10	1.5×10^{-6}	342 - 771	11 16 11 33 11 77 11 22 11 22 11 11 11 12 12 11 15
South Fork Silver Creek-Junction Reservoir	PA-IH	10C	1	14/14			
South Fork Silver Creek-Junction Reservoir	PA-IH	10D	1	12/12			
Caples Creek	AM-AA	32	1	3/3	2.1×10^{-5}	3,504	11 00 11 11 11 33 11 12 11 00 11 11 11 12 12 11 55
Caples Creek	AM-AA	33	1	3/3			
Strawberry Creek	PL-SB	42	5	3/13	1.2×10^{-14}	0	22 47 11 11 11 77 11 12 11 13 13 11 11 12 30 00 55
Strawberry Creek	PL-SB	42B	2	5/6			
Sayles Canyon	PL-ST	16	3	3/6	3.4×10^{-10}	0	14 16 11 11 11 27 11 22 11 16 13 11 11 12 12 11 15
Sayles Canyon	PL-ST	16A	3	4/8			
Upper South Fork Rubicon River	PA-TC	18A	1	2/2	1.3×10^{-4}	1,057	11 11 11 11 11 33 11 22 11 22 11 11 11 12 11 11 11
Upper South Fork Rubicon River	PA-TC	18	9	3/14			
Strawberry Creek	PL-SB	27	3	2/9	4.9×10^{-5}	172	11 11 11 11 11 33 11 22 11 22 11 11 11 12 12 11 11
Strawberry Creek	PL-SB	27A	1	8/8			
Strawberry Creek	PL-SB	13	1	15/15	8.8×10^{-5}	12,848	11 11 11 11 11 77 11 22 11 00 11 11 11 12 11 11 55
Strawberry Creek	PL-SB	22	3	8/12			

Table 4. *Populus tremuloides* genetic diversity using one sample per clone per stand. #Std = number of stands. N = sample size. %P = percent polymorphic loci. A = mean number of alleles per locus. Ae = effective number of alleles per locus. Ho = observed heterozygosity. He = expected heterozygosity. F = fixation index: (He-Ho)/He. Fst = differentiation of stands within region. Nm = calculated gene flow among stands. Standard deviation in parentheses.

Region	#Std	N	%P	A	Ae	Ho	He	F	Fst	Nm
Entire study	82	407	82.4	3.1 (1.5)	1.5 (0.5)	0.229 (0.264)	0.279 (0.236)	0.179	0.465	0.287
Watershed (mean)	10	50	62.9	2.0	1.5	0.233	0.261	0.102	0.394	0.427
Caples Creek	15	66	72.2	2.1 (1.0)	1.5 (0.5)	0.212 (0.267)	0.267 (0.225)	0.206	0.547	0.207
Jones Fork Silver Creek-Forni Creek	2	6	52.9	1.8 (0.9)	1.5 (0.7)	0.275 (0.377)	0.267 (0.302)	-0.030	0.216	0.908
Sayles Canyon	15	69	70.6	2.3 (1.0)	1.5 (0.5)	0.231 (0.241)	0.287 (0.229)	0.195	0.464	0.288
South Fork American River-Forni Creek	13	45	70.6	2.2 (1.1)	1.5 (0.5)	0.242 (0.285)	0.251 (0.225)	0.036	0.397	0.380
South Fork Silver Creek-Junction Reservoir	4	8	50.0	1.6 (0.7)	1.4 (0.5)	0.250 (0.393)	0.232 (0.259)	-0.078	0.385	0.400
South Fork Silver Creek-Wrights Lake	14	84	66.7	2.1 (1.0)	1.5 (0.6)	0.233 (0.287)	0.258 (0.252)	0.097	0.348	0.469
Strawberry Creek	8	38	55.6	2.1 (1.2)	1.5 (0.5)	0.189 (0.281)	0.258 (0.249)	0.267	0.389	0.392
Upper South Fork Rubicon River	11	80	64.7	2.1 (1.1)	1.5 (0.6)	0.235 (0.280)	0.269 (0.246)	0.126	0.403	0.371
Cluster ID (mean)	3	17	51.7	1.7	1.4	0.227	0.244	0.064	0.317	0.718
Airstrip	5	11	52.9	1.5 (0.5)	1.3 (0.4)	0.177 (0.286)	0.187 (0.208)	0.053	0.391	0.389
Buck Pasture	6	28	58.8	2.1 (1.1)	1.6 (0.6)	0.225 (0.314)	0.291 (0.266)	0.227	0.462	0.291
Buck Pasture Outlier	1	6	35.3	1.4 (0.5)	1.3 (0.4)	0.275 (0.412)	0.212 (0.320)	-0.297	-------	-------
Camp Sacramento	6	17	70.6	1.8 (0.6)	1.4 (0.4)	0.284 (0.337)	0.255 (0.221)	-0.114	0.431	0.330
Convict Meadow	4	28	70.6	1.9 (0.7)	1.5 (0.5)	0.214 (0.314)	0.271 (0.243)	0.210	0.338	0.490
Government Meadow	2	14	58.8	1.7 (0.7)	1.4 (0.6)	0.190 (0.290)	0.237 (0.246)	0.198	0.177	1.161
Ice House	4	8	52.9	1.6 (0.7)	1.4 (0.5)	0.265 (0.400)	0.246 (0.260)	-0.077	0.385	0.400
Info Center	1	2	35.3	1.4 (0.5)	1.4 (0.5)	0.353 (0.493)	0.353 (0.493)	0.000	-------	-------
Info Center Outlier	1	4	35.3	1.5 (0.8)	1.4 (0.8)	0.235 (0.400)	0.235 (0.344)	0.000	-------	-------
Jack Schneider	5	16	52.9	1.8 (0.8)	1.4 (0.4)	0.156 (0.228)	0.213 (0.229)	0.268	0.477	0.274
Loon Lake Trail	2	38	64.7	1.9 (0.9)	1.6 (0.6)	0.251 (0.299)	0.281 (0.253)	0.107	0.124	1.775
Lovers Leap	4	16	52.9	1.8 (0.9)	1.4 (0.5)	0.184 (0.290)	0.238 (0.248)	0.227	0.333	0.501
Lower Sayles	6	30	64.7	2.1 (1.0)	1.4 (0.6)	0.231 (0.244)	0.235 (0.231)	0.017	0.194	1.037
Lower Sayles Outlier	2	4	29.4	1.3 (0.5)	1.2 (0.4)	0.177 (0.351)	0.177 (0.286)	0.000	0.333	0.500
Lyons Creek	5	56	70.6	2.1 (1.0)	1.5 (0.5)	0.241 (0.280)	0.266 (0.243)	0.094	0.293	0.603
Middle Sayles	3	23	70.6	1.9 (0.7)	1.5 (0.4)	0.242 (0.309)	0.302 (0.223)	0.199	0.434	0.326
Stone Cellar Trail	4	31	64.7	2.0 (1.0)	1.5 (0.6)	0.224 (0.277)	0.247 (0.250)	0.093	0.297	0.591
Strawberry Lodge	3	12	58.8	1.7 (0.7)	1.4 (0.5)	0.277 (0.353)	0.236 (0.248)	-0.174	0.092	2.483
Strawberry Outlier	1	2	11.8	1.1 (0.3)	1.1 (0.3)	0.118 (0.332)	0.118 (0.332)	0.000	-------	-------
Upper Sayles	3	10	58.8	1.8 (0.8)	1.5 (0.5)	0.278 (0.353)	0.290 (0.262)	0.041	0.416	0.352
Upper Sayles Outlier	1	2	23.5	1.2 (0.4)	1.2 (0.4)	0.235 (0.437)	0.235 (0.437)	0.000	-------	-------
Upper Strawberry	4	17	58.8	1.9 (0.9)	1.5 (0.6)	0.169 (0.266)	0.261 (0.259)	0.352	0.360	0.445
Wrights Lake	7	26	58.8	1.9 (1.0)	1.6 (0.7)	0.281 (0.343)	0.286 (0.271)	0.017	0.304	0.571
Wrights Lake Outlier	2	6	29.4	1.3 (0.5)	1.3 (0.5)	0.157 (0.336)	0.173 (0.276)	0.092	0.182	1.125
Stand (mean)	------	5	33.3	1.4	1.3	0.226	0.229	0.011	-------	-------
AM-AA 31	------	21	70.6	1.8 (0.7)	1.5 (0.5)	0.186 (0.288)	0.267 (0.229)	0.303	-------	-------
AM-AA 32	------	2	17.7	1.2 (0.4)	1.2 (0.4)	0.200 (0.414)	0.200 (0.414)	0.000	-------	-------
AM-AA 33	------	2	17.7	1.2 (0.4)	1.2 (0.4)	0.188 (0.403)	0.188 (0.403)	0.000	-------	-------
AM-AA 34	------	4	35.3	1.4 (0.6)	1.4 (0.5)	0.324 (0.466)	0.235 (0.334)	-0.379	-------	-------
PA-IH 10A	------	2	35.3	1.4 (0.5)	1.4 (0.5)	0.353 (0.493)	0.353 (0.493)	0.000	-------	-------
PA-IH 10B	------	2	23.5	1.2 (0.4)	1.2 (0.4)	0.235 (0.437)	0.235 (0.430)	0.000	-------	-------
PA-IH 10C	------	2	23.5	1.2 (0.4)	1.2 (0.4)	0.235 (0.437)	0.235 (0.437)	0.000	-------	-------
PA-IH 10D	------	2	23.5	1.2 (0.4)	1.2 (0.4)	0.235 (0.437)	0.235 (0.437)	0.000	-------	-------
PA-IH 20	------	2	35.3	1.4 (0.5)	1.4 (0.5)	0.353 (0.492)	0.353 (0.492)	0.000	-------	-------
PA-IH 30	------	4	35.3	1.5 (0.8)	1.4 (0.8)	0.235 (0.400)	0.235 (0.344)	0.000	-------	-------
PA-LC 18	------	12	47.1	1.6 (0.7)	1.4 (0.5)	0.333 (0.445)	0.236 (0.269)	-0.411	-------	-------
PA-LC 46	------	12	35.3	1.4 (0.6)	1.2 (0.4)	0.162 (0.252)	0.153 (0.217)	-0.059	-------	-------
PA-LC 48	------	2	11.8	1.1 (0.3)	1.1 (0.3)	0.125 (0.342)	0.125 (0.342)	0.000	-------	-------
PA-LC 49	------	20	64.7	1.9 (0.8)	1.6 (0.6)	0.274 (0.353)	0.290 (0.261)	0.055	-------	-------
PA-LC 51	------	11	53.0	1.6 (0.7)	1.4 (0.5)	0.152 (0.257)	0.230 (0.254)	0.339	-------	-------
PA-SF 10	------	4	23.5	1.2 (0.4)	1.2 (0.4)	0.118 (0.332)	0.157 (0.292)	0.248	-------	-------

Region		#Std	N	%P	A	Ae	Ho	He	F	Fst	Nm
PA-SF	11	------	2	23.5	1.2 (0.4)	1.2 (0.4)	0.235 (0.437)	0.235 (0.437)	0.000	-------	-------
PA-TC	14	------	4	35.3	1.4 (0.5)	1.3 (0.4)	0.219 (0.364)	0.219 (0.297)	0.000	-------	-------
PA-TC	15	------	2	23.5	1.2 (0.4)	1.2 (0.4)	0.235 (0.437)	0.235 (0.437)	0.000	-------	-------
PA-TC	16	------	2	29.4	1.3 (0.5)	1.3 (0.5)	0.313 (0.479)	0.313 (0.479)	0.000	-------	-------
PA-TC	17	------	2	5.9	1.1 (0.3)	1.1 (0.3)	0.067 (0.258)	0.067 (0.258)	0.000	-------	-------
PA-TC	18	------	18	52.9	1.6 (0.7)	1.4 (0.5)	0.177 (0.249)	0.202 (0.236)	0.124	-------	-------
PA-TC	18A	------	2	5.9	1.1 (0.2)	1.1 (0.2)	0.059 (0.243)	0.059 (0.243)	0.000	-------	-------
PA-TC	21A	------	12	52.9	1.6 (0.6)	1.4 (0.6)	0.216 (0.322)	0.218 (0.251)	0.009	-------	-------
PA-TC	21C	------	4	35.3	1.4 (0.6)	1.3 (0.5)	0.235 (0.359)	0.206 (0.298)	-0.141	-------	-------
PA-TC	36	------	20	58.8	1.8 (0.9)	1.6 (0.6)	0.318 (0.397)	0.300 (0.272)	-0.060	-------	-------
PA-TC	41	------	2	29.4	1.3 (0.5)	1.3 (0.5)	0.313 (0.479)	0.313 (0.479)	0.000	-------	-------
PA-TC	42	------	13	52.9	1.7 (0.8)	1.4 (0.6)	0.216 (0.268)	0.212 (0.252)	-0.019	-------	-------
PA-WL	15	------	4	35.3	1.4 (0.5)	1.3 (0.5)	0.324 (0.466)	0.226 (0.307)	-0.434	-------	-------
PA-WL	24	------	2	35.3	1.4 (0.5)	1.4 (0.5)	0.352 (0.493)	0.353 (0.493)	0.003	-------	-------
PA-WL	25	------	8	41.2	1.7 (0.9)	1.5 (0.7)	0.206 (0.309)	0.240 (0.307)	0.142	-------	-------
PA-WL	26	------	4	47.1	1.6 (0.7)	1.5 (0.6)	0.294 (0.398)	0.294 (0.336)	0.000	-------	-------
PA-WL	27	------	4	52.9	1.6 (0.7)	1.5 (0.6)	0.324 (0.393)	0.324 (0.331)	0.000	-------	-------
PA-WL	28	------	2	23.5	1.2 (0.4)	1.2 (0.4)	0.235 (0.437)	0.235 (0.437)	0.000	-------	-------
PA-WL	30	------	2	35.3	1.4 (0.5)	1.4 (0.5)	0.353 (0.493)	0.353 (0.493)	0.000	-------	-------
PL-AA	19	------	2	17.7	1.2 (0.4)	1.2 (0.4)	0.188 (0.403)	0.188 (0.403)	0.000	-------	-------
PL-AA	20	------	2	17.7	1.2 (0.4)	1.2 (0.4)	0.177 (0.393)	0.177 (0.393)	0.000	-------	-------
PL-AA	21	------	2	5.9	1.1 (0.2)	1.1 (0.2)	0.059 (0.243)	0.059 (0.243)	0.000	-------	-------
PL-AA	24	------	2	11.8	1.1 (0.3)	1.1 (0.3)	0.118 (0.332)	0.118 (0.332)	0.000	-------	-------
PL-AA	25	------	8	35.3	1.5 (0.7)	1.4 (0.5)	0.177 (0.303)	0.212 (0.282)	0.165	-------	-------
PL-AA	27	------	8	35.3	1.5 (0.7)	1.3 (0.5)	0.132 (0.267)	0.189 (0.274)	0.302	-------	-------
PL-AA	28	------	6	47.1	1.5 (0.5)	1.3 (0.4)	0.255 (0.364)	0.228 (0.264)	-0.118	-------	-------
PL-CS	13	------	6	29.4	1.4 (0.6)	1.3 (0.5)	0.225 (0.395)	0.182 (0.294)	-0.236	-------	-------
PL-CS	14	------	2	29.4	1.3 (0.5)	1.3 (0.5)	0.294 (0.470)	0.294 (0.470)	0.000	-------	-------
PL-CS	15	------	2	17.7	1.2 (0.4)	1.2 (0.4)	0.177 (0.393)	0.177 (0.393)	0.000	-------	-------
PL-CS	16	------	4	23.5	1.2 (0.4)	1.2 (0.4)	0.177 (0.393)	0.157 (0.292)	-0.127	-------	-------
PL-CS	17	------	2	35.3	1.4 (0.5)	1.4 (0.5)	0.353 (0.493)	0.353 (0.493)	0.000	-------	-------
PL-CS	18	------	2	23.5	1.2 (0.4)	1.2 (0.4)	0.235 (0.437)	0.235 (0.437)	0.000	-------	-------
PL-CS	18A	------	2	35.3	1.4 (0.5)	1.4 (0.5)	0.353 (0.493)	0.353 (0.493)	0.000	-------	-------
PL-CS	20	------	8	52.9	1.6 (0.7)	1.4 (0.4)	0.162 (0.279)	0.244 (0.248)	0.336	-------	-------
PL-CS	22	------	2	29.4	1.3 (0.5)	1.3 (0.5)	0.313 (0.479)	0.313 (0.479)	0.000	-------	-------
PL-CS	23	------	4	29.4	1.3 (0.5)	1.3 (0.4)	0.250 (0.408)	0.188 (0.291)	-0.330	-------	-------
PL-SB	13	------	2	11.8	1.1 (0.3)	1.1 (0.3)	0.118 (0.332)	0.118 (0.332)	0.000	-------	-------
PL-SB	22	------	6	41.2	1.5 (0.6)	1.3 (0.5)	0.125 (0.269)	0.208 (0.262)	0.399	-------	-------
PL-SB	27	------	6	41.2	1.5 (0.6)	1.3 (0.4)	0.118 (0.202)	0.208 (0.267)	0.433	-------	-------
PL-SB	27A	------	2	11.8	1.1 (0.3)	1.1 (0.3)	0.125 (0.342)	0.125 (0.342)	0.000	-------	-------
PL-SB	28	------	4	47.1	1.6 (0.7)	1.5 (0.6)	0.265 (0.437)	0.324 (0.361)	0.182	-------	-------
PL-SB	37	------	10	41.2	1.5 (0.6)	1.3 (0.5)	0.203 (0.373)	0.203 (0.256)	0.000	-------	-------
PL-SB	39	------	2	17.7	1.2 (0.4)	1.2 (0.4)	0.200 (0.414)	0.200 (0.414)	0.000	-------	-------
PL-SB	39A	------	2	23.5	1.3 (0.4)	1.3 (0.5)	0.250 (0.447)	0.250 (0.447)	0.000	-------	-------
PL-SB	39B	------	2	17.7	1.2 (0.4)	1.2 (0.4)	0.188 (0.403)	0.188 (0.403)	0.000	-------	-------
PL-SB	42	------	10	52.9	1.9 (1.0)	1.4 (0.5)	0.220 (0.297)	0.239 (0.267)	0.079	-------	-------
PL-SB	42B	------	4	47.1	1.6 (0.7)	1.5 (0.6)	0.281 (0.364)	0.323 (0.347)	0.130	-------	-------
PL-SB	44	------	6	35.3	1.4 (0.5)	1.3 (0.4)	0.275 (0.412)	0.212 (0.320)	-0.297	-------	-------
PL-SB	45	------	2	29.4	1.3 (0.5)	1.3 (0.5)	0.294 (0.470)	0.294 (0.470)	0.000	-------	-------
PL-SB	49	------	8	58.8	1.7 (0.7)	1.4 (0.5)	0.270 (0.325)	0.263 (0.259)	-0.027	-------	-------
PL-SB	50	------	2	29.4	1.3 (0.5)	1.3 (0.5)	0.294 (0.470)	0.294 (0.470)	0.000	-------	-------
PL-ST	11	------	6	41.2	1.5 (0.6)	1.3 (0.4)	0.157 (0.239)	0.188 (0.243)	0.165	-------	-------
PL-ST	12	------	4	35.3	1.4 (0.6)	1.3 (0.5)	0.294 (0.435)	0.226 (0.322)	-0.301	-------	-------
PL-ST	13	------	4	23.5	1.3 (0.6)	1.2 (0.5)	0.118 (0.281)	0.147 (0.282)	0.197	-------	-------
PL-ST	14	------	4	29.4	1.4 (0.6)	1.3 (0.5)	0.177 (0.303)	0.177 (0.292)	0.000	-------	-------
PL-ST	16	------	6	52.9	1.7 (0.8)	1.5 (0.6)	0.314 (0.322)	0.310 (0.315)	-0.013	-------	-------
PL-ST	16A	------	6	58.8	1.8 (0.8)	1.5 (0.5)	0.294 (0.309)	0.286 (0.279)	-0.028	-------	-------
PL-ST	22	------	2	23.5	1.2 (0.4)	1.2 (0.4)	0.235 (0.437)	0.235 (0.437)	0.000	-------	-------
PL-ST	23	------	2	23.5	1.2 (0.4)	1.2 (0.4)	0.235 (0.437)	0.235 (0.437)	0.000	-------	-------
PL-ST	25	------	8	58.8	1.6 (0.6)	1.4 (0.4)	0.235 (0.336)	0.271 (0.253)	0.133	-------	-------
PL-ST	27	------	16	41.2	1.7 (0.8)	1.3 (0.5)	0.170 (0.262)	0.188 (0.234)	0.096	-------	-------
PL-ST	29	------	2	23.5	1.3 (0.4)	1.3 (0.4)	0.250 (0.447)	0.250 (0.447)	0.000	-------	-------
PL-ST	31	------	2	23.5	1.2 (0.4)	1.2 (0.4)	0.235 (0.437)	0.235 (0.437)	0.000	-------	-------
PL-ST	32	------	6	58.8	1.6 (0.6)	1.5 (0.5)	0.324 (0.384)	0.328 (0.297)	0.012	-------	-------
PL-ST	33	------	2	11.8	1.1 (0.4)	1.1 (0.4)	0.143 (0.363)	0.143 (0.363)	0.000	-------	-------
PL-ST	38	------	2	11.8	1.1 (0.3)	1.1 (0.3)	0.118 (0.332)	0.118 (0.332)	0.000	-------	-------

Table 5. F-statistics (fixation indices) for *Populus tremuloides.*

Comparison			F
individual	within	total	0.228
stand	within	total	0.465
cluster	within	total	0.215
watershed	within	total	0.100

Table 6. Genetic-geographic variation pattern classification for aspen samples. Data plotted in Figures 2,3 and 4.

Genetic Class	Area	Stand	# Clones	Ave He
A	AM-AA PA-LC PA-WL	32,33,34 46,49,51 15,27,28	30	0.198
B	AM-AA PL-CS PL-SB PL-AA	31 15,16,17,18,18A,20,22 45,49,50 19,20,21,24,25,27,28	41	0.236
C	PA-IH PA-TC PA-SF	30 21A,21C,36,41,42 10,11	31	0.234
D	PA-TC PA-IH	16,17,18,18A 10A,10B,10C,10D,20	14	0.194
E	PA-LC PA-WL PL-SB	18,48 24,25,26,30 44	17	0.197
F	PA-TC	14,15	3	0.183
G	PL-SB	13,22,27,27A,28,37,39,39A,39B,42,42B	22	0.228
H	PL-CS PL-ST	13,14,23 11,12,13,14,16,16A,22,23,25,27,29,31,32,33,38	40	0.234

Table 7. Comparison of allozyme genetic diversity in aspens from different areas. %P = percent polymorphic loci. A = average number of alleles per locus. Ho = observed heterozygosity. He = expected heterozygosity.
* = unclear, but > 30 samples in 10 clones.

Reference	Location	# of Samples	# of Loci	%P	A	Ho	He
Isozymes: entire study							
Cheliak and Dancik 1982	Alberta	896	26	92	2.3	0.52	0.42
Cheliak and Pitel 1984	Ontario	*	16	50	1.6		
Jelinski and Cheliak 1992	Alberta	182	16	87	2.5		0.32
Liu and Furnier 1993a	MI, MN, WI	130	13	77	2.8	0.19	0.25
NFGEL: Projects 84 & 68	Oregon	789	17	88	3.9	0.16	0.23
NFGEL: this study	California	663	17	82	3.1	0.23	0.28
Isozymes: mean per population							
Cheliak and Dancik 1982	Alberta	40	26	87	2.3	0.52	0.42
Hyun et al. 1987a	Ontario	25 clones	15	79	2.7	0.13	0.24
Jelinski and Cheliak 1992	Alberta	26	16	81	2.4	0.32	0.29
Lund et al. 1992	Minnesota	39	10	91	2.6	0.22	0.22
NFGEL: Projects 84 & 68	Oregon	23	17	71	2.4	0.18	0.26
NFGEL: this study	California	17	17	33	1.4	0.23	0.23

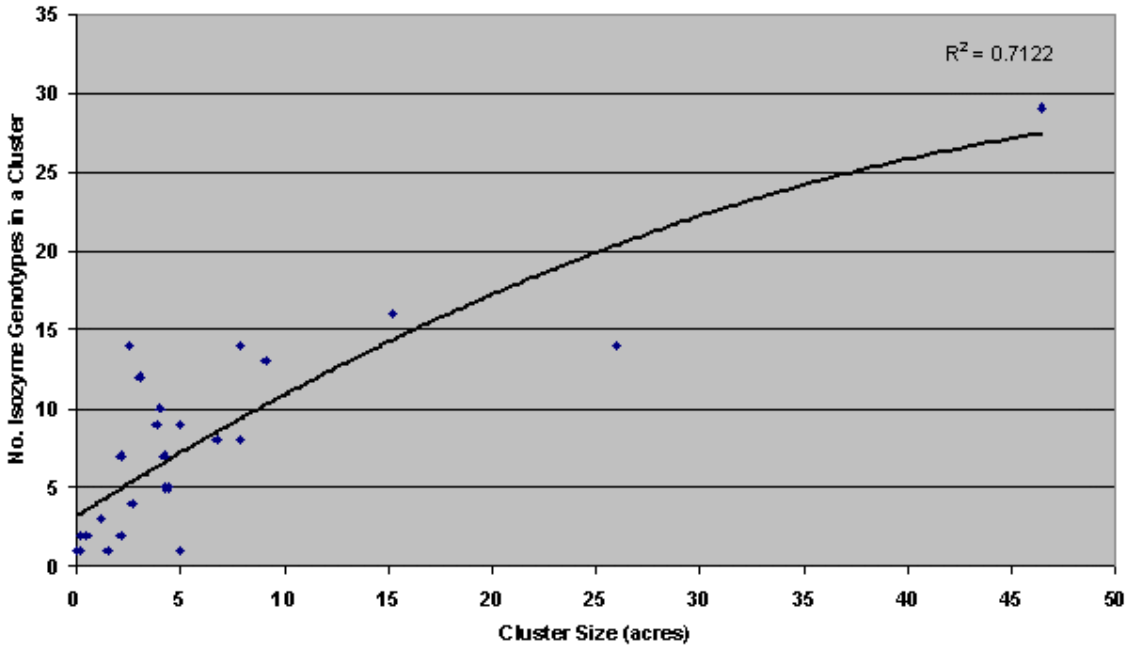


Figure 1. Genotype diversity and stand cluster size of aspen.

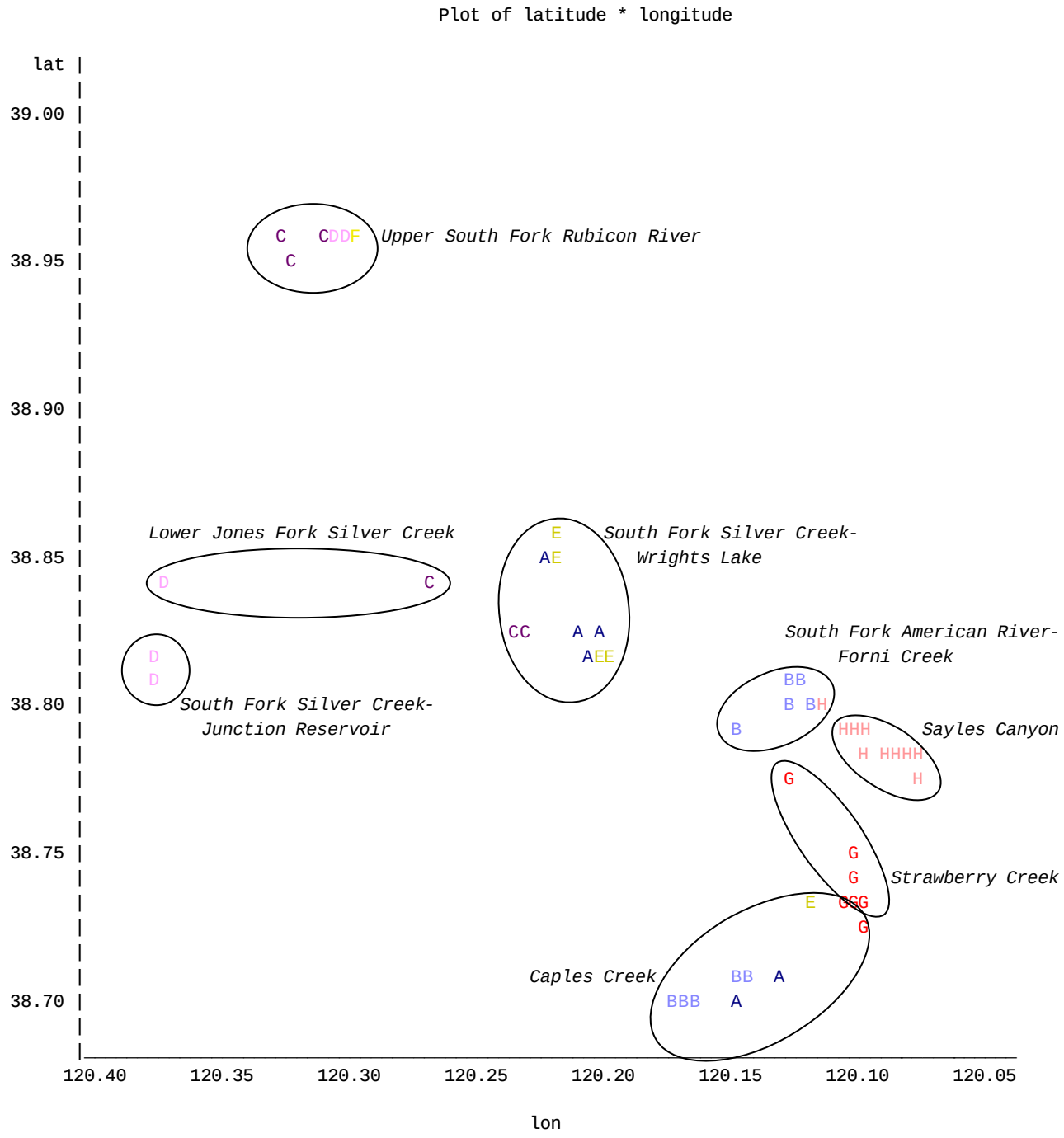


Figure 2. Geographic variation pattern for aspen clones, based on first three canonical vectors. watersheds are 'circled' in black, and named in black italics. Symbols are genetic pattern classifications found in Table 6. Note: some observations are hidden.

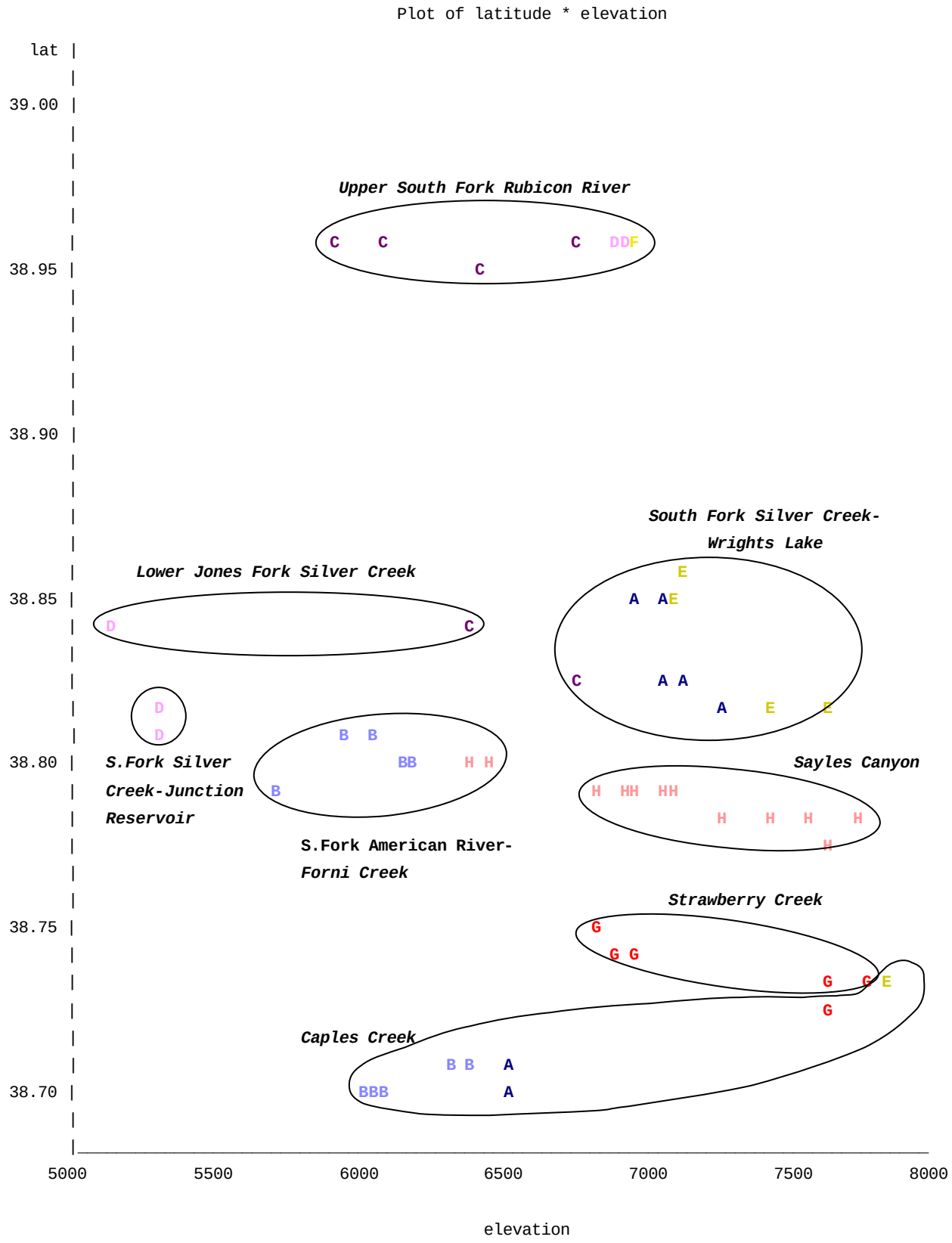

Plot of latitude * elevation
lat
39.00
38.95
38.90
38.85
38.80
38.75
38.70
Upper South Fork Rubicon River
C C C DDF C
Lower Jones Fork Silver Creek
D C
South Fork Silver Creek-
Wrights Lake
E A AE C A A A E E
D D
S.Fork Silver
Creek-Junction
Reservoir
B B BB H H B
S.Fork American River-
Forni Creek
Sayles Canyon
H HH HH H H H H H
Strawberry Creek
G G G G G E G
Caples Creek
B B A BBB A
5000
5500
6000
6500
7000
7500
8000
elevation

Figure 3. Geographic variation pattern for aspen clones, based on first three canonical vectors. Watersheds are 'circled' in black, and named in black italics. Symbols are genetic pattern classifications found in Table 6. Note: some observations are hidden.

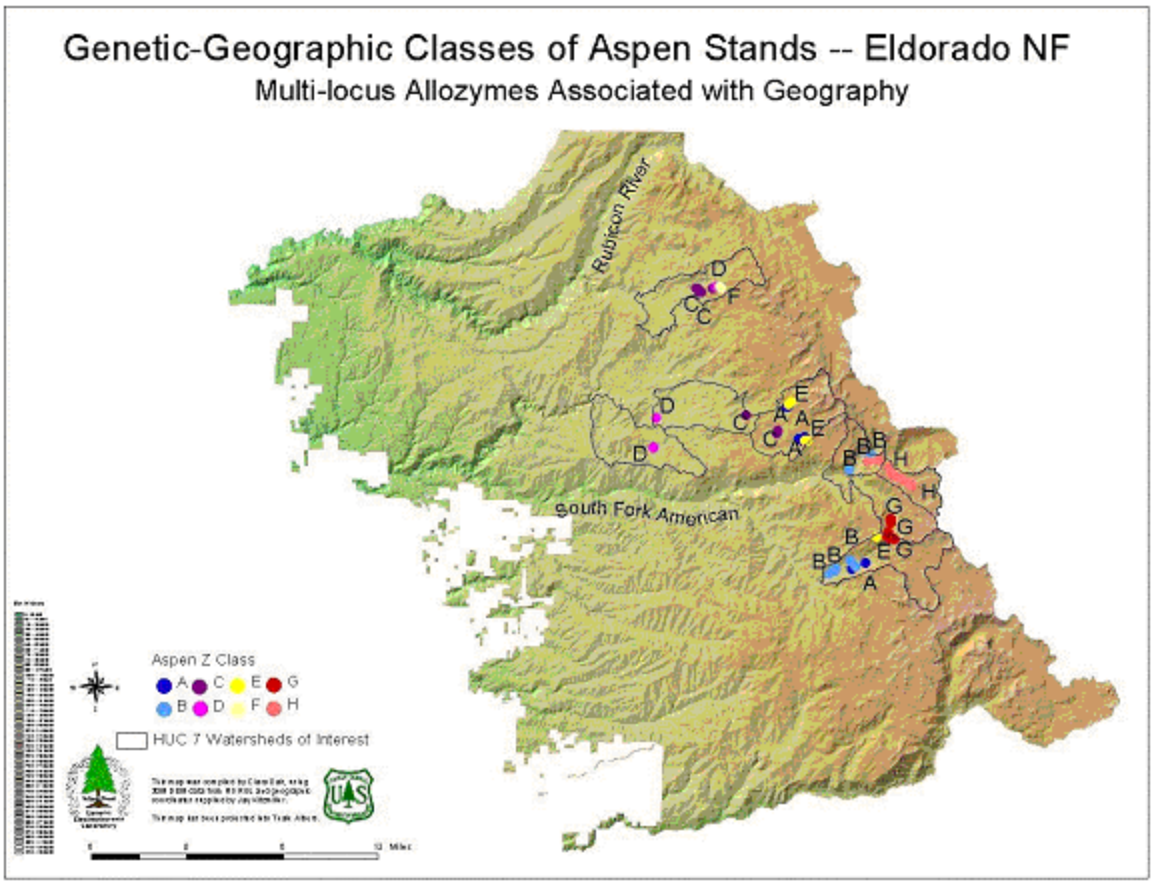
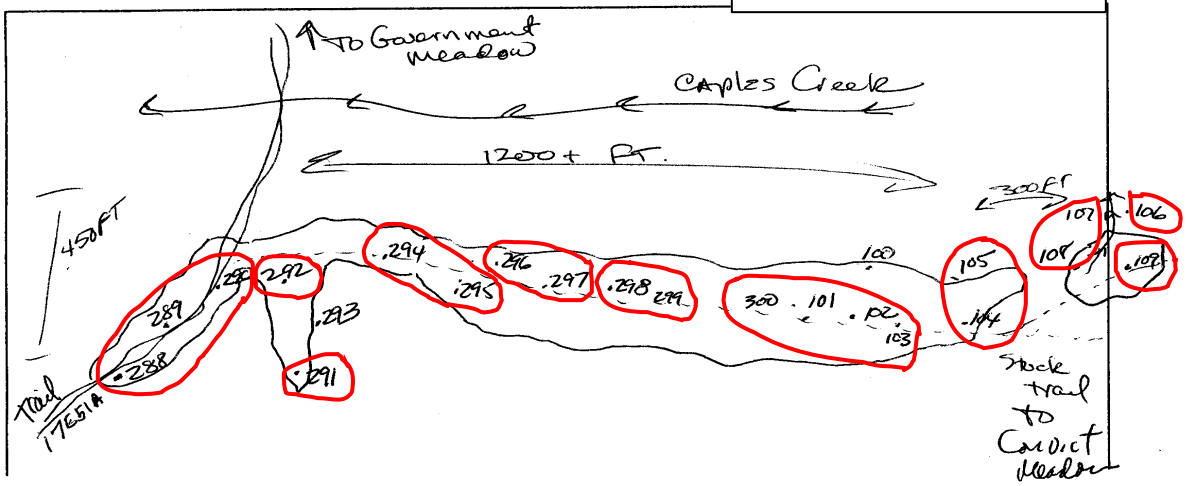


Figure 4. Genetic-Geographic classes of Aspen stands on the Eldorado National Forest. HUC 7 watershed boundaries are indicated by black lines. Symbols are genetic pattern classifications found in Table 6. Note: some observations are hidden.

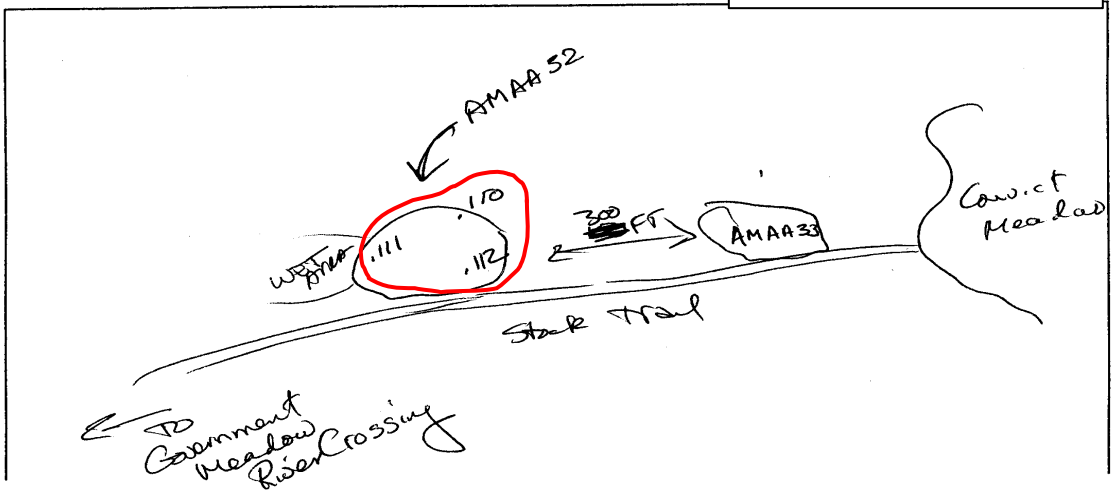
APPENDIX

Location Maps Of Aspen Samples And Clones Within Stands

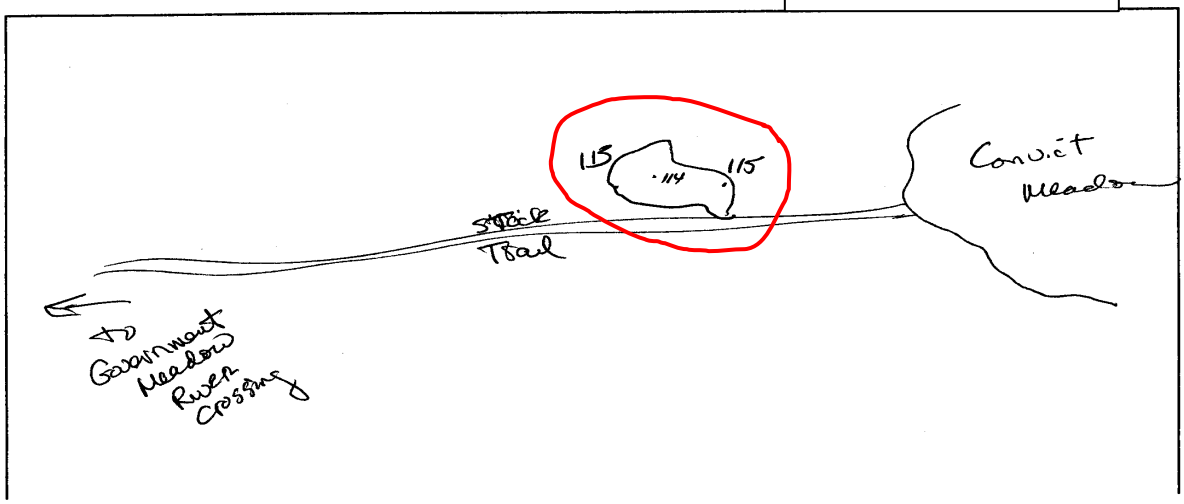
SAMPLING MAP (Attach Topo Map) AMAA 31



SAMPLING MAP (Attach Topo Map) AMAA 32

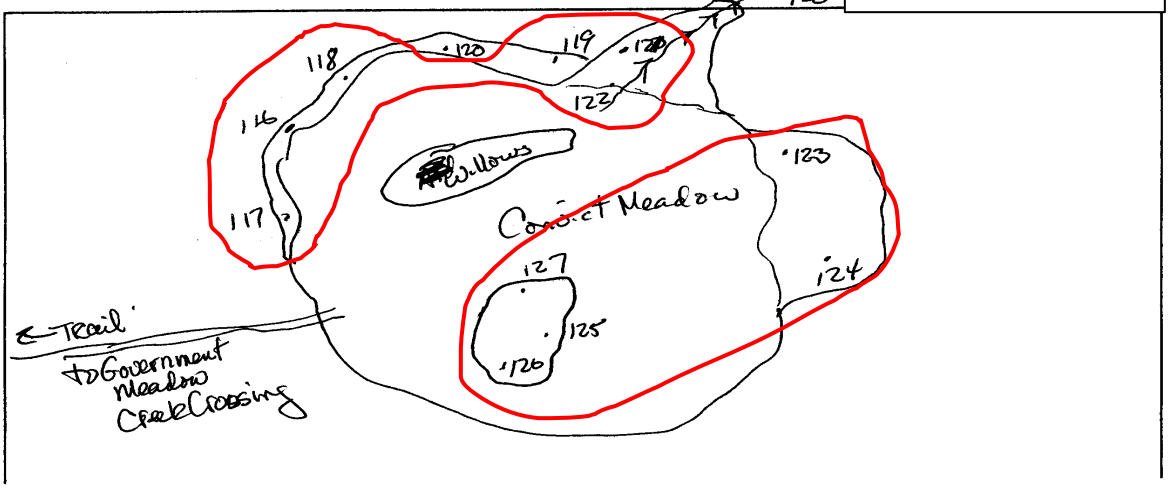


SAMPLING MAP (Attach Topo Map) AMAA 33



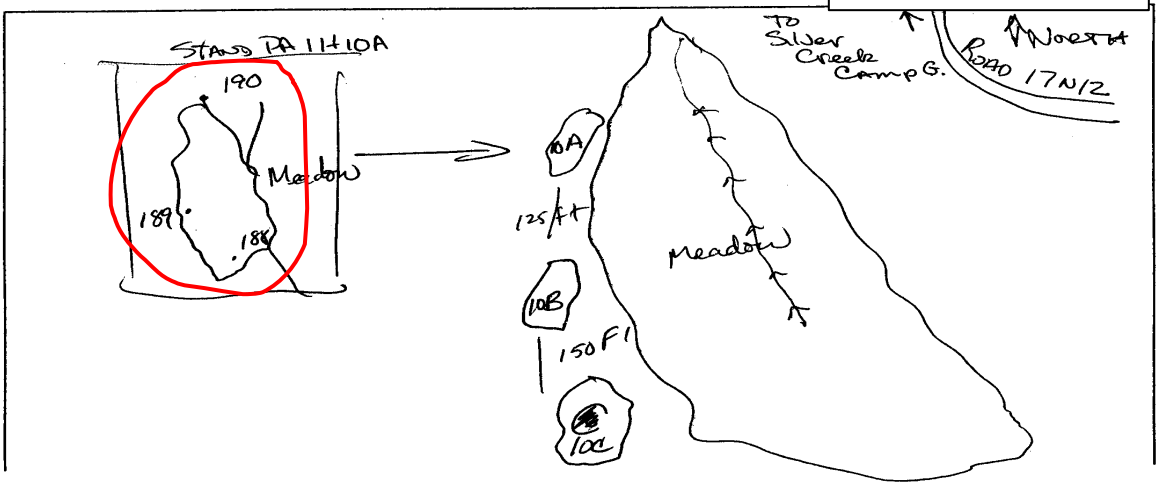
SAMPLING MAP (Attach Topo Map)

AMAA 34



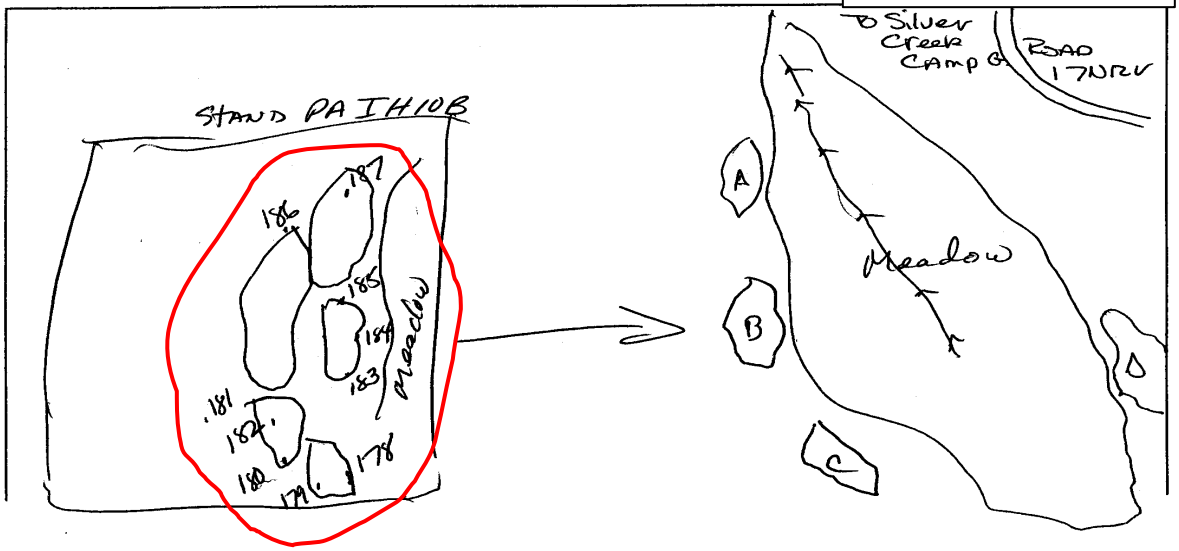
SAMPLING MAP (Attach Topo Map)

PAIH10A



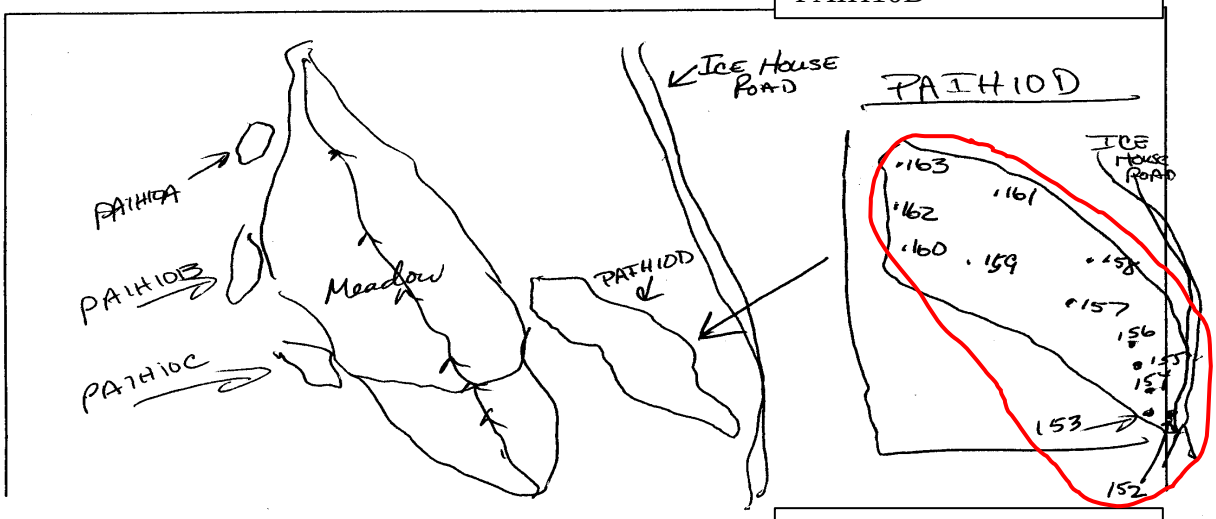
SAMPLING MAP (Attach Topo Map)

PAIH10B

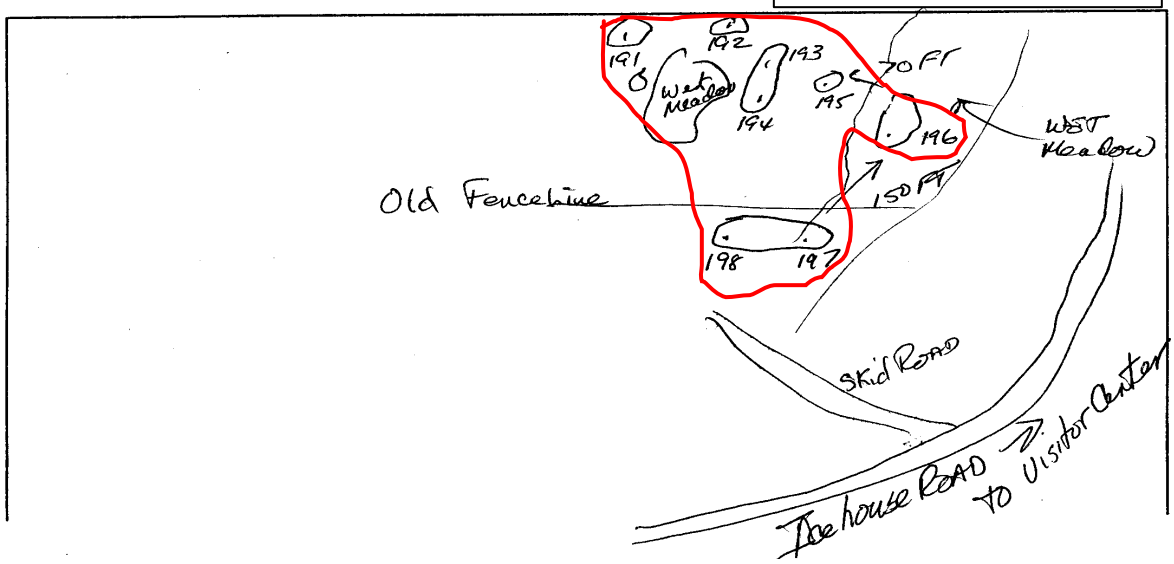


SAMPLING MAP (Attach Topo Map) PAIH10C

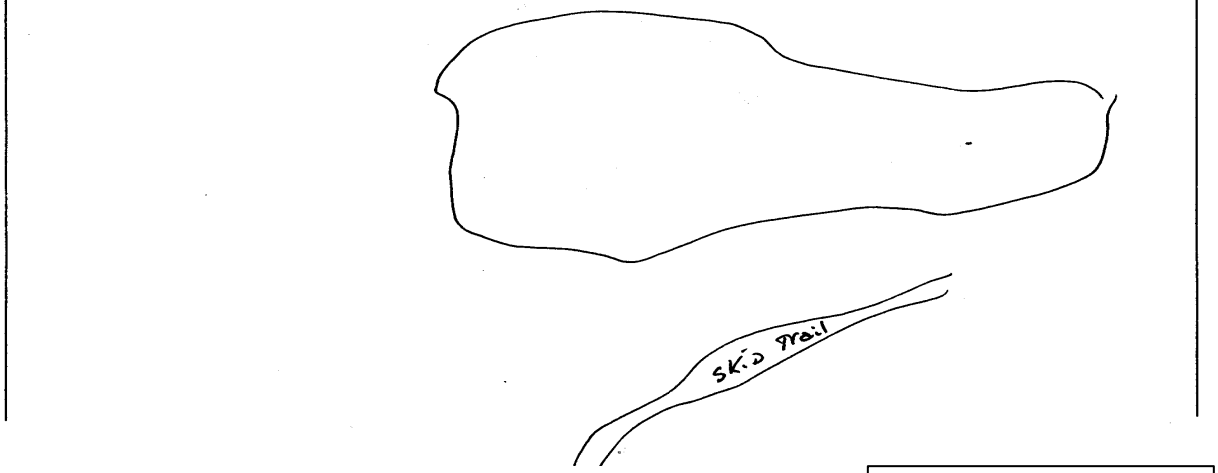
SAMPLING MAP (Attach Topo Map) PAIH10D



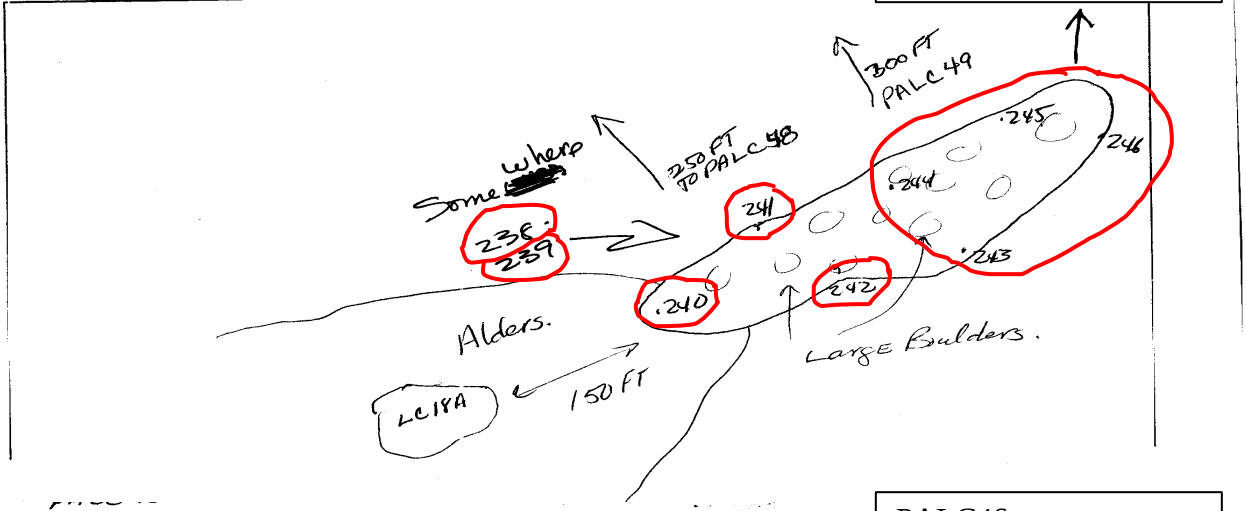
SAMPLING MAP (Attach Topo Map) PAIH20



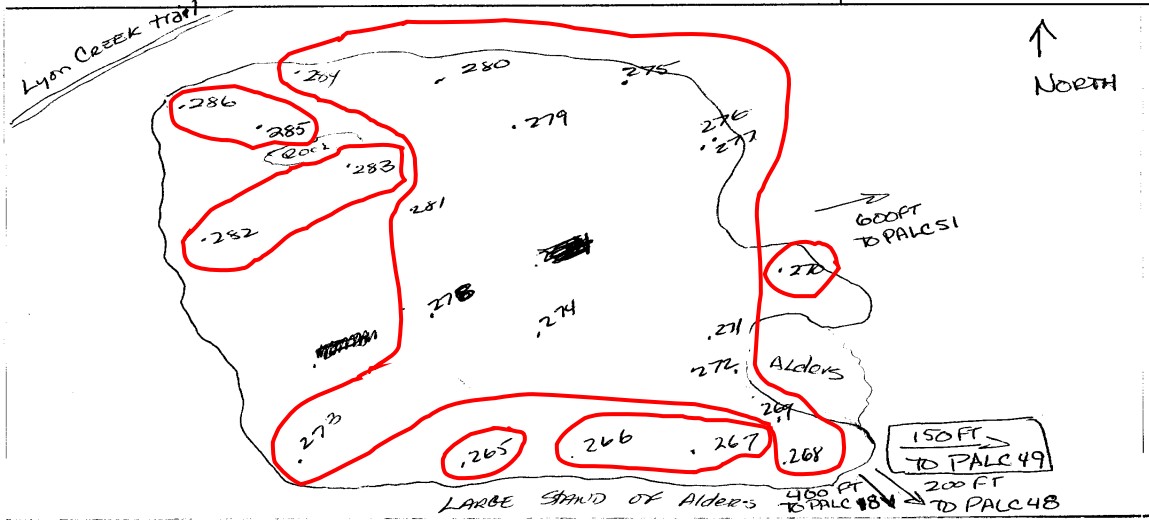
SAMPLING MAP (Attach Topo Map) PAIH30



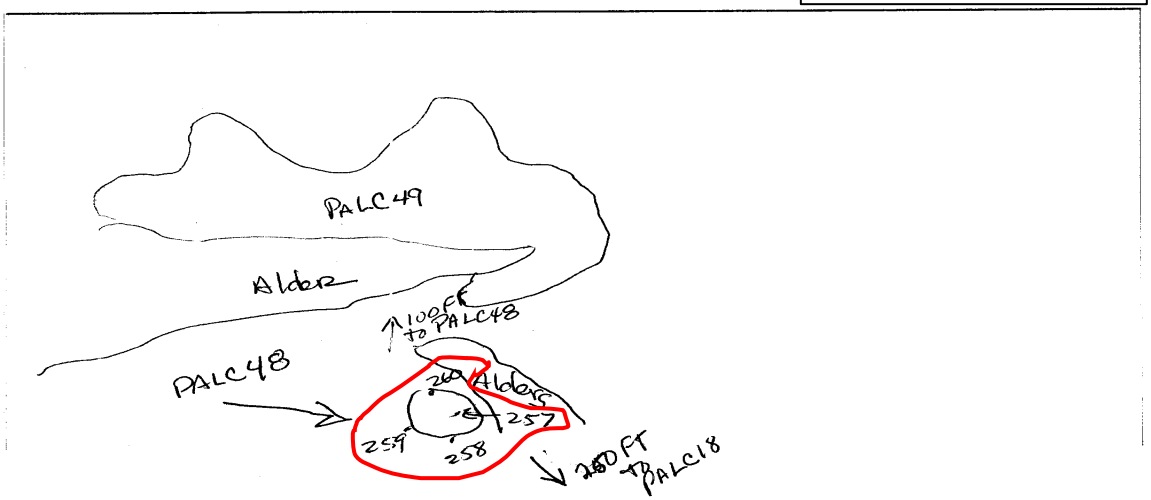
SAMPLING MAP (Attach Topo Map) PALC18



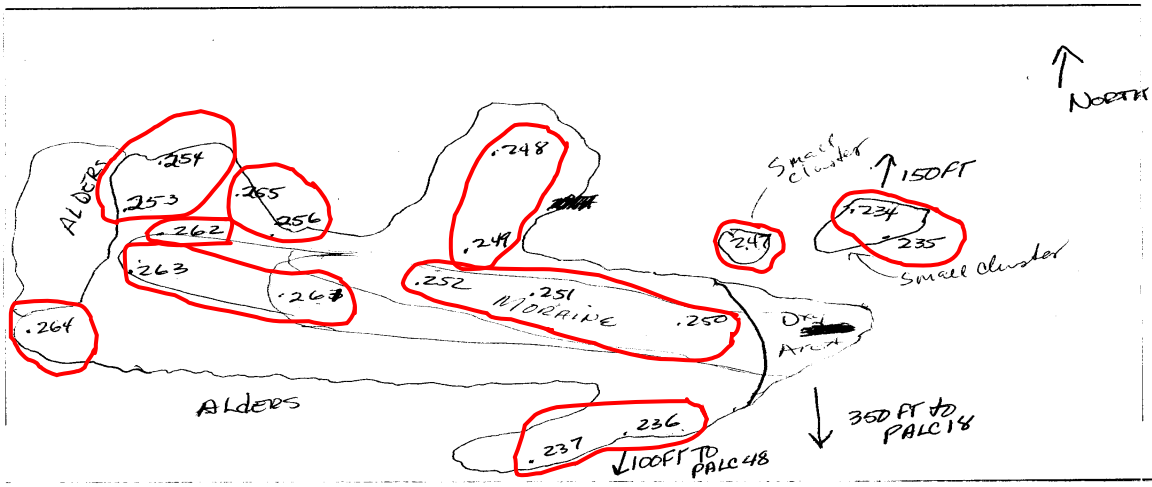
SAMPLING MAP (Attach Topo Map) PALC46



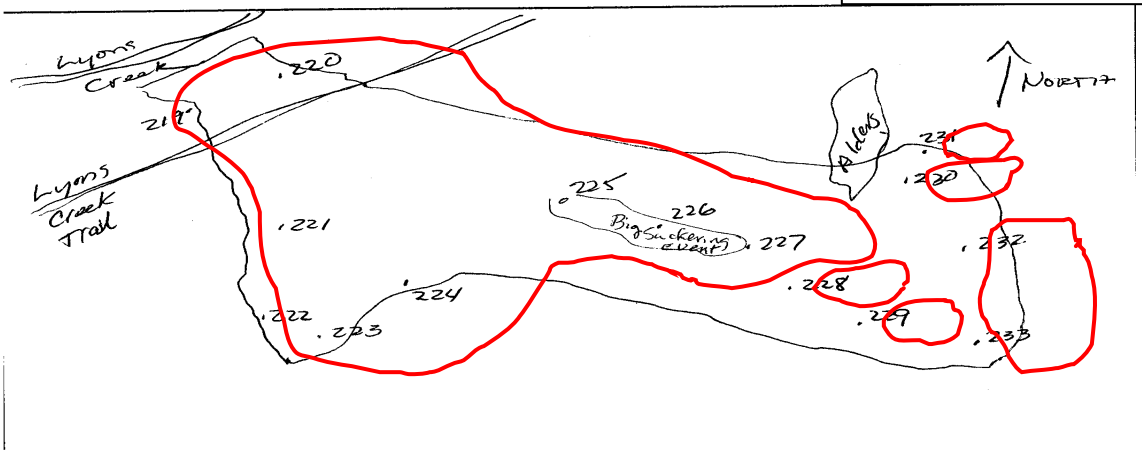
SAMPLING MAP (Attach Topo Map) PALC48



SAMPLING MAP (Attach Topo Map) PALC49

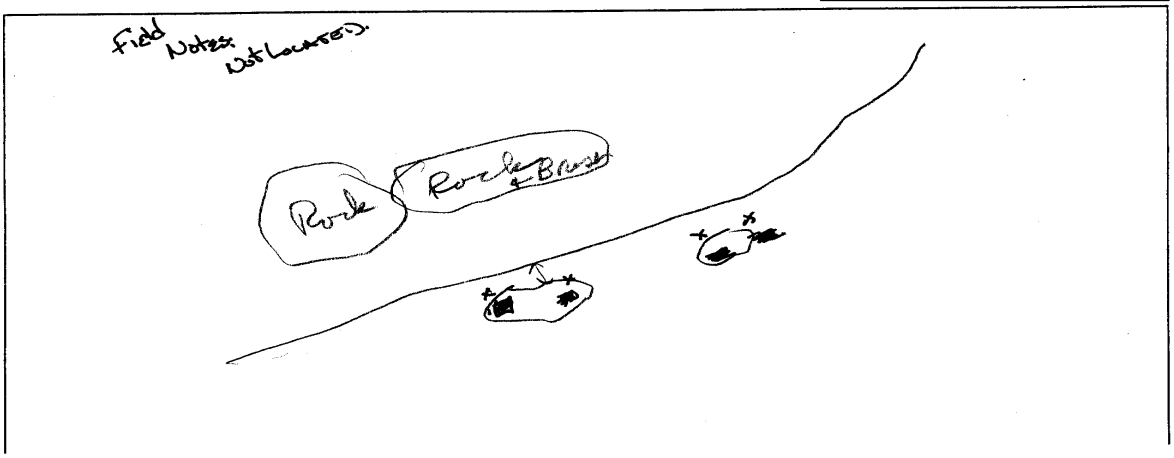


SAMPLING MAP (Attach Topo Map) PALC51



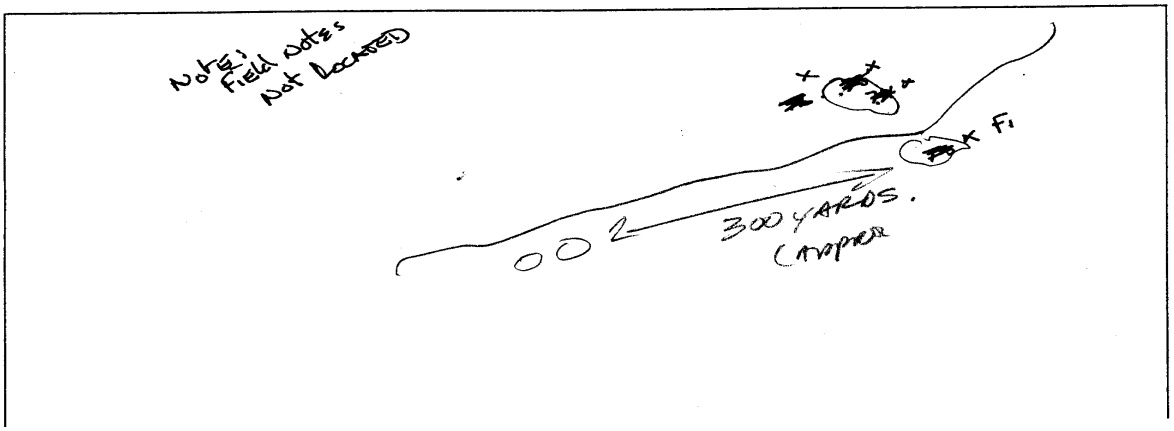
SAMPLING MAP (Attach Topo Map)

PASF10



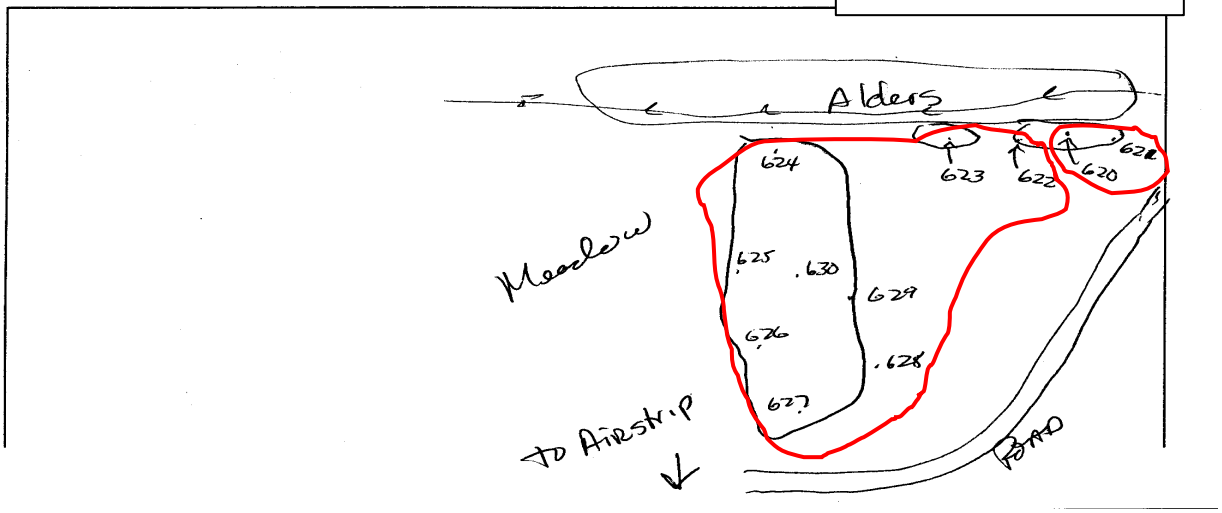
SAMPLING MAP (Attach Topo Map)

PASF11



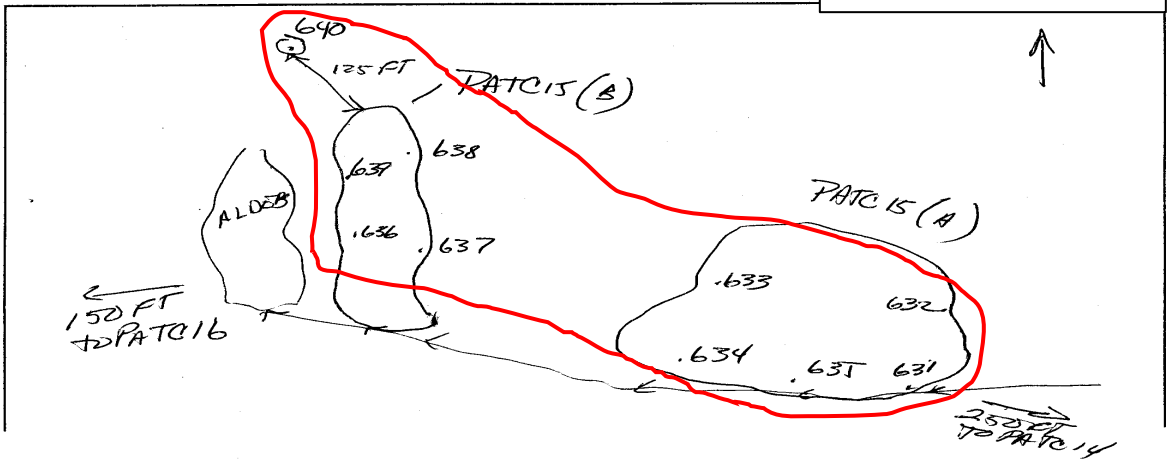
SAMPLING MAP (Attach Topo Map)

PATC14



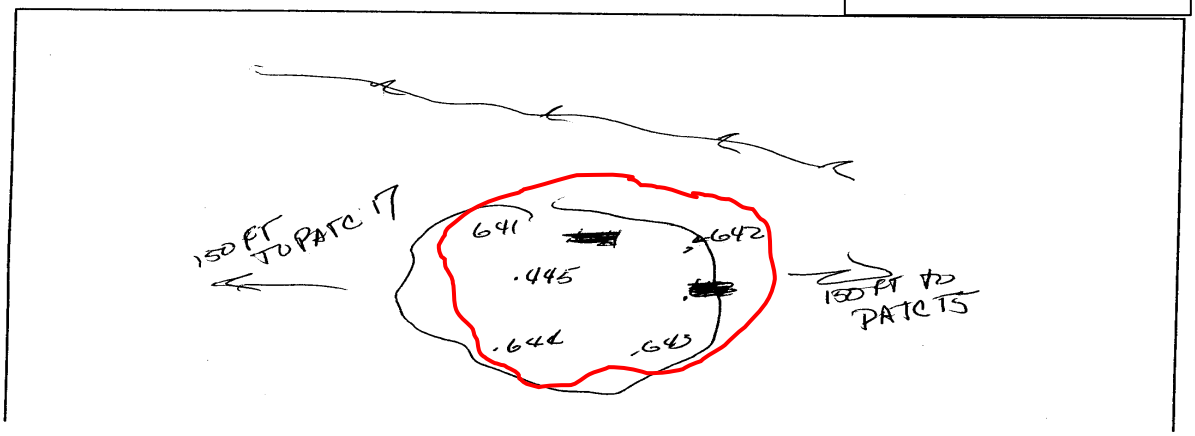
SAMPLING MAP (Attach Topo Map)

PATC15



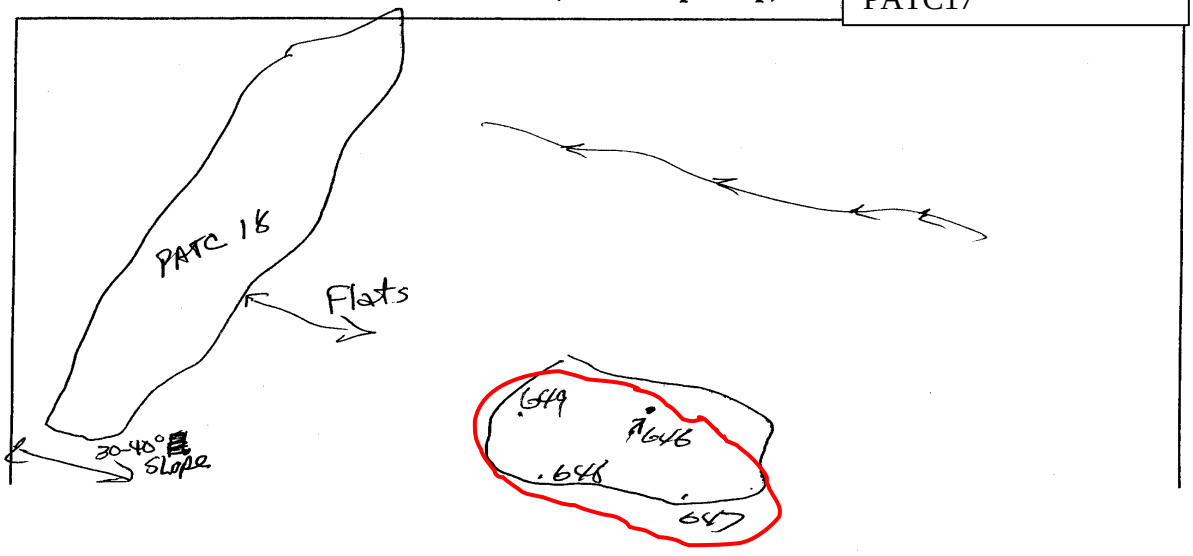
SAMPLING MAP (Attach Topo Map)

PATC16

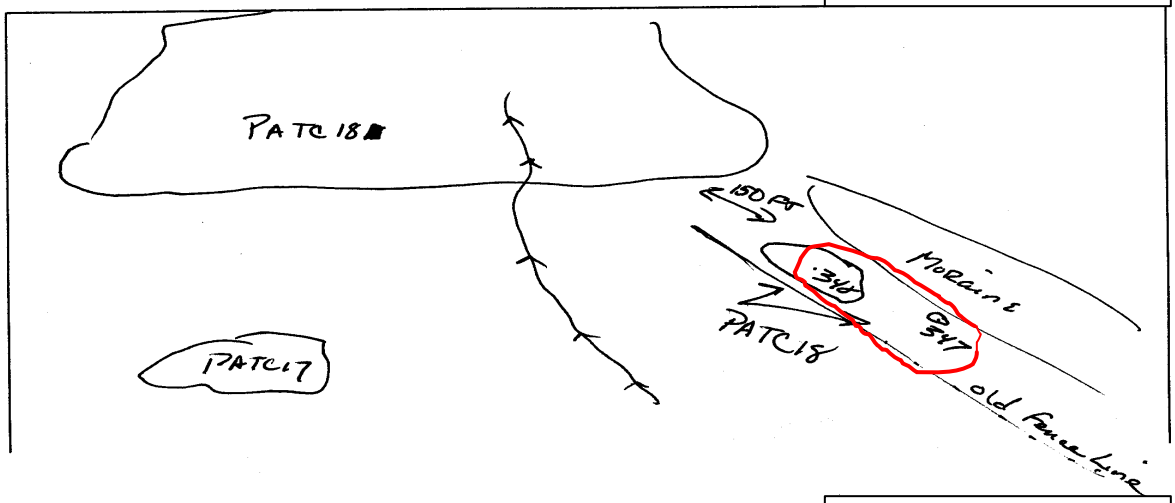


SAMPLING MAP (Attach Topo Map)

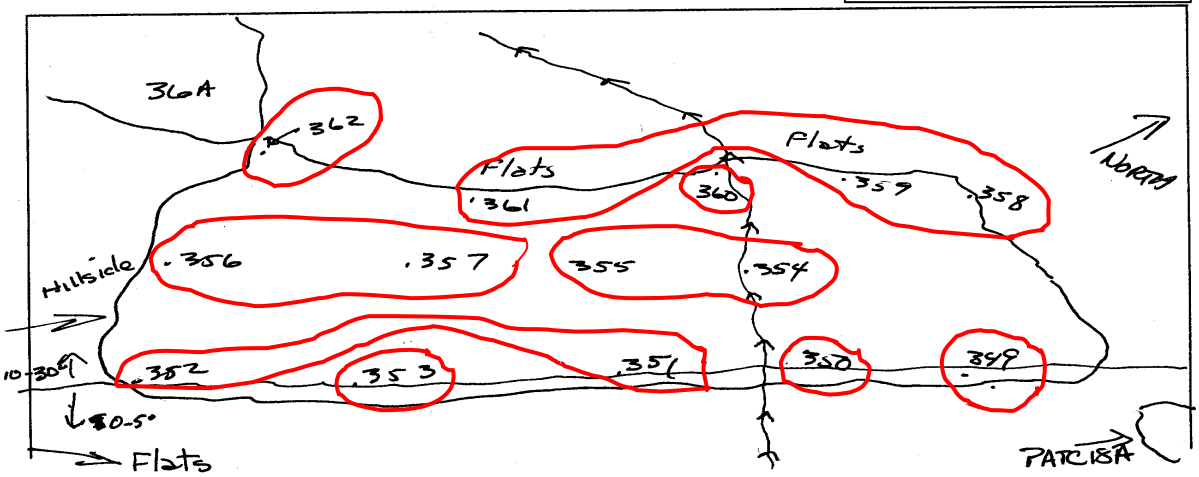
PATC17



SAMPLING MAP (Attach Topo Map) PATC18A

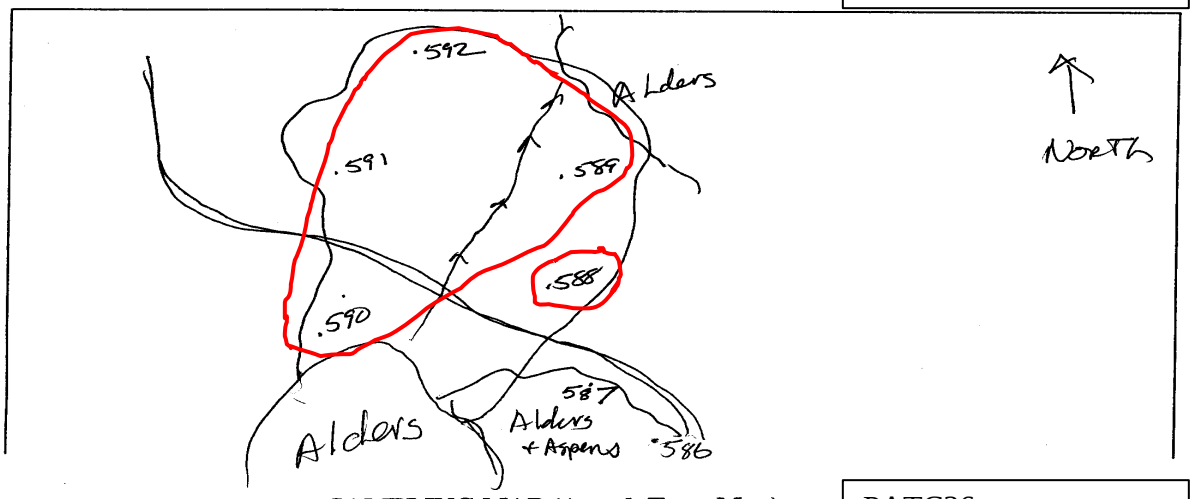


SAMPLING MAP (Attach Topo Map) PATC18

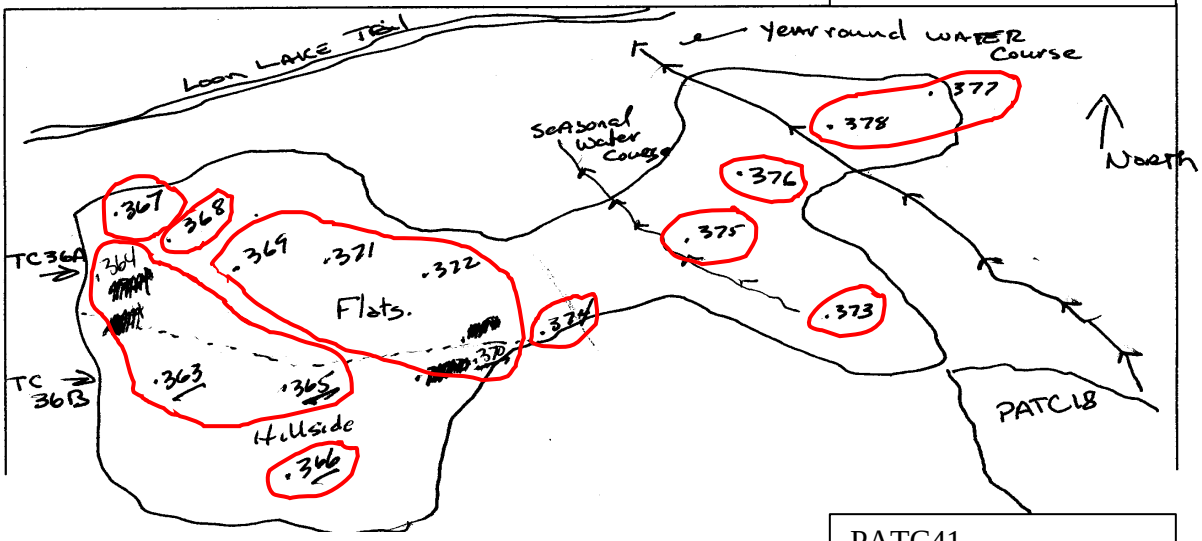


SAMPLING MAP (Attach Topo Map) PATC21A

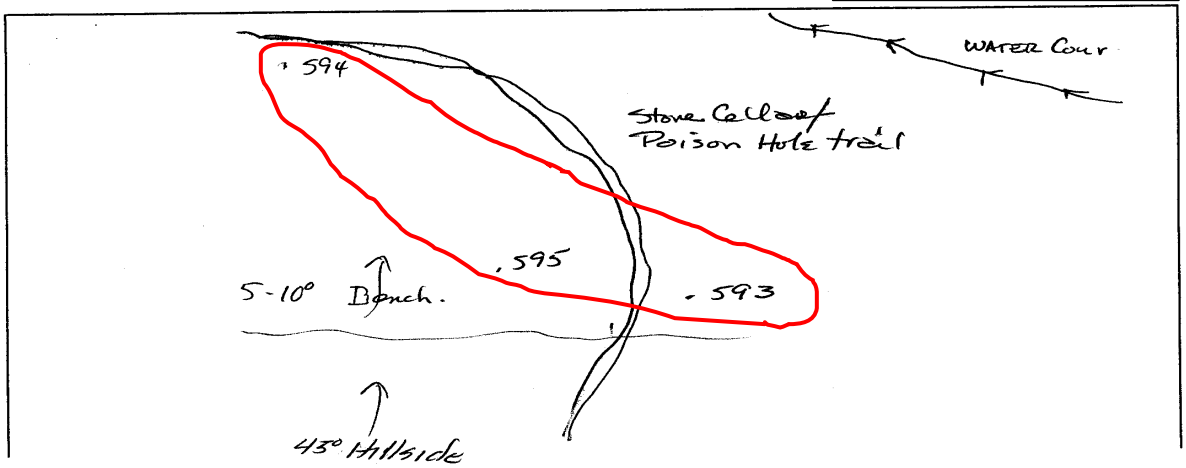
SAMPLING MAP (Attach Topo Map) PATC21C



SAMPLING MAP (Attach Topo Map) PATC36

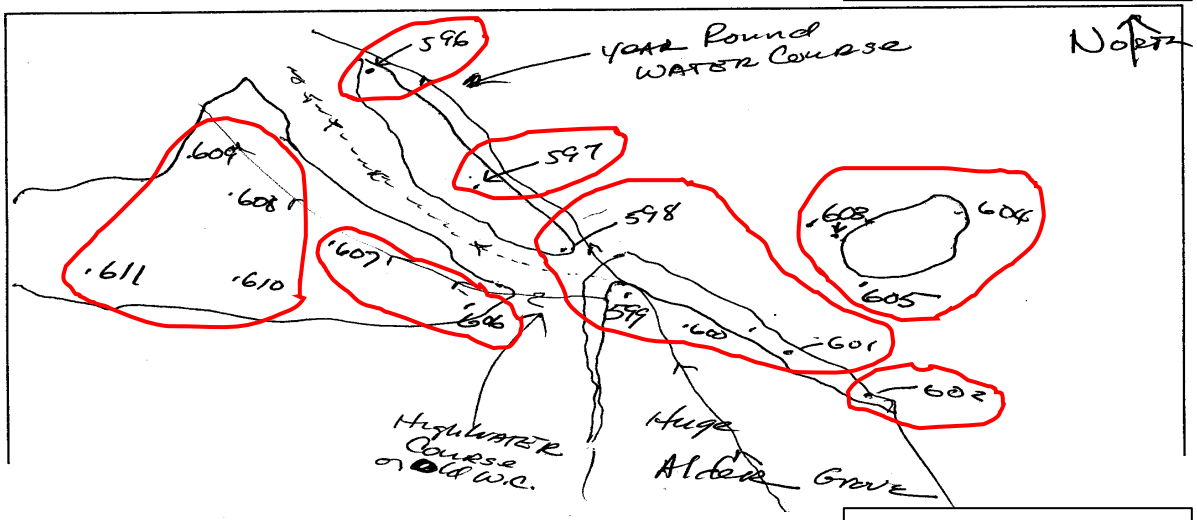


SAMPLING MAP (Attach Topo Map) PATC41



SAMPLING MAP (Attach Topo Map)

PATC42

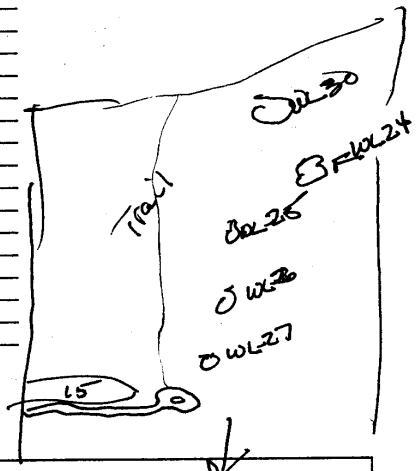


SAMPLING MAP (Attach Topo Map)

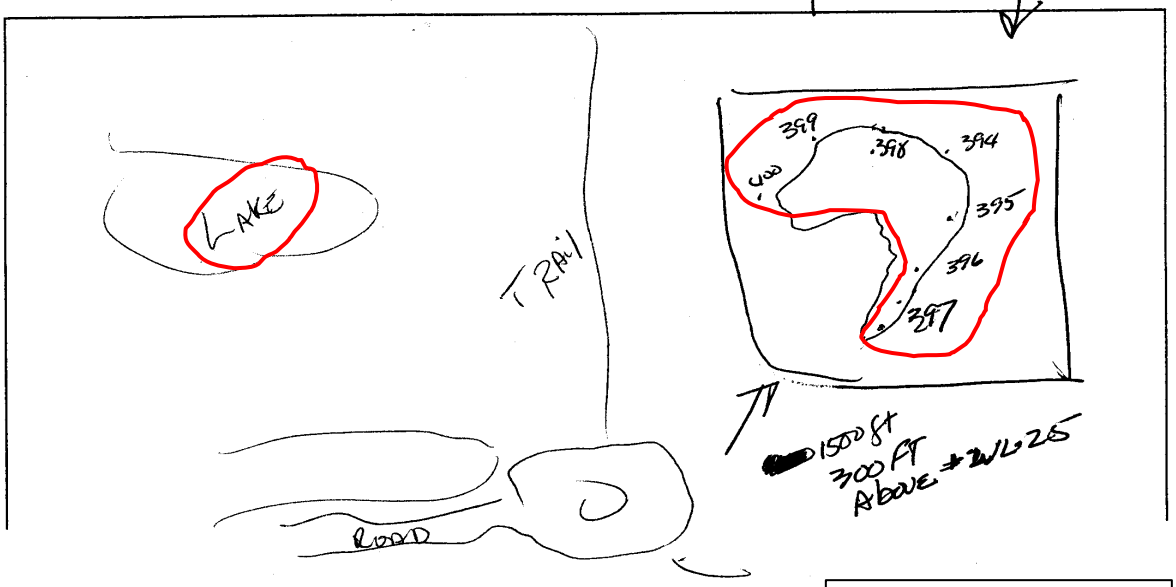
PAWL15

PAWL24

PAWL24-399	8733
PAWL24.398	8732
PAWL24.397	8755
PAWL24.396	8754
PAWL24.395	8753
PAWL24.394	8752

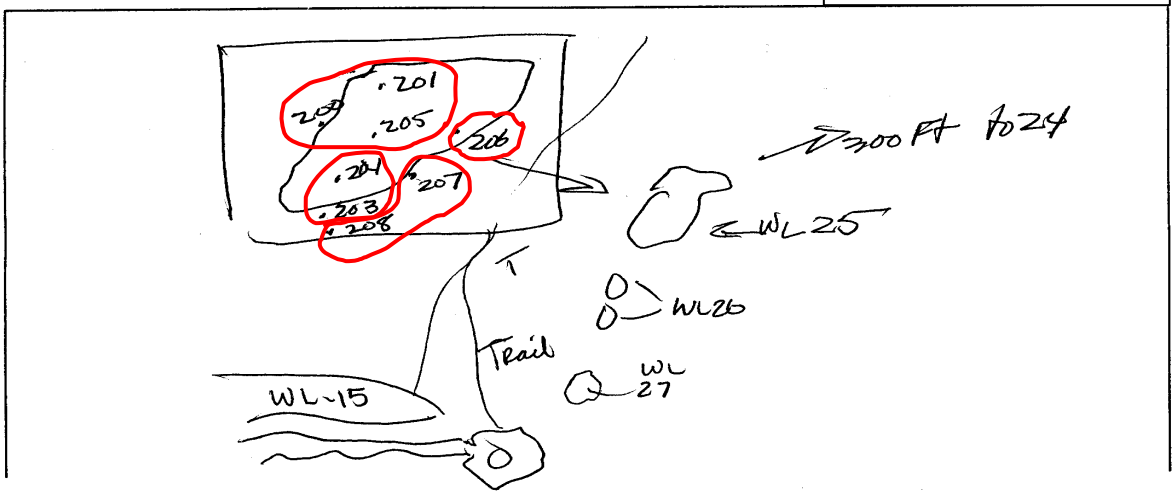


SAMPLING MAP (Attach Topo Map)

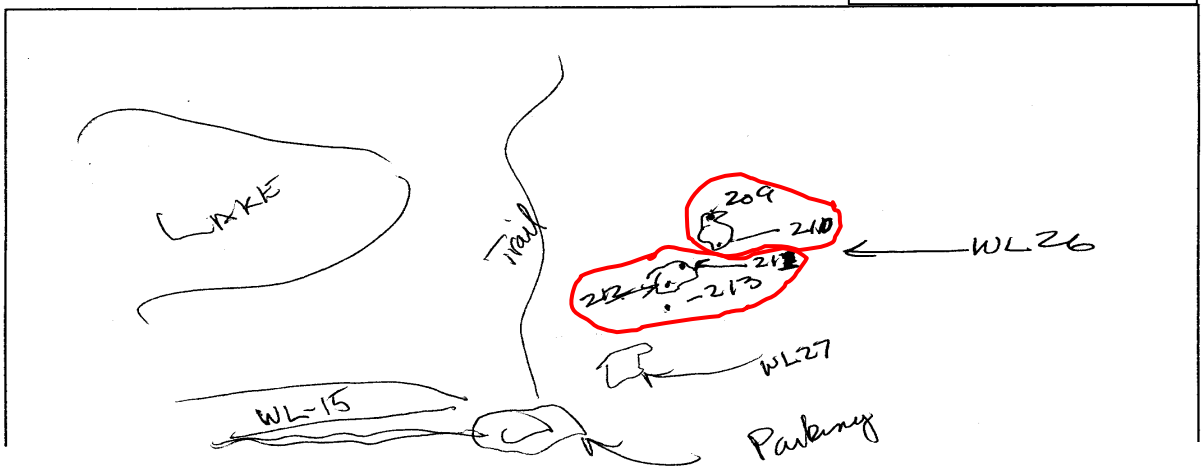


SAMPLING MAP (Attach Topo Map)

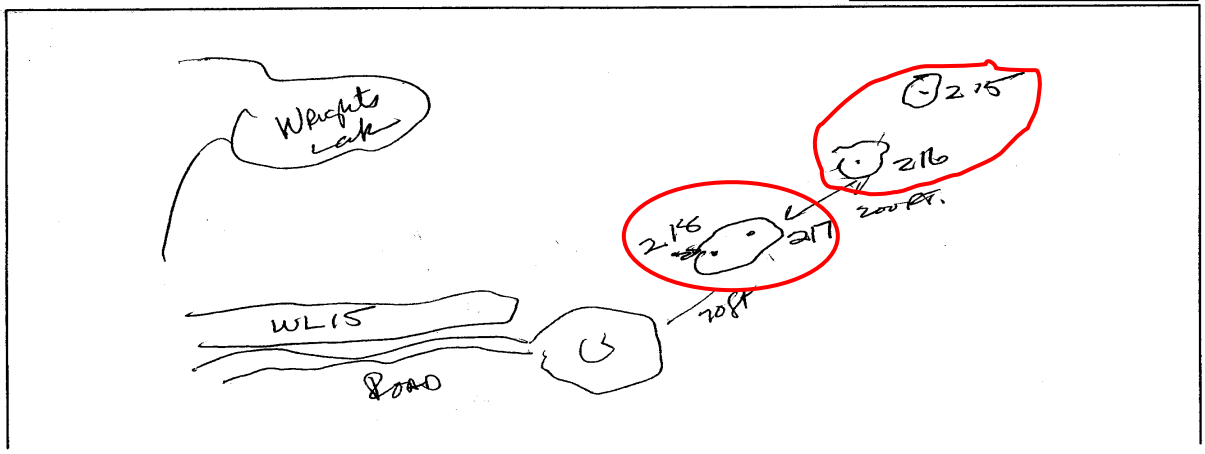
PAWL25



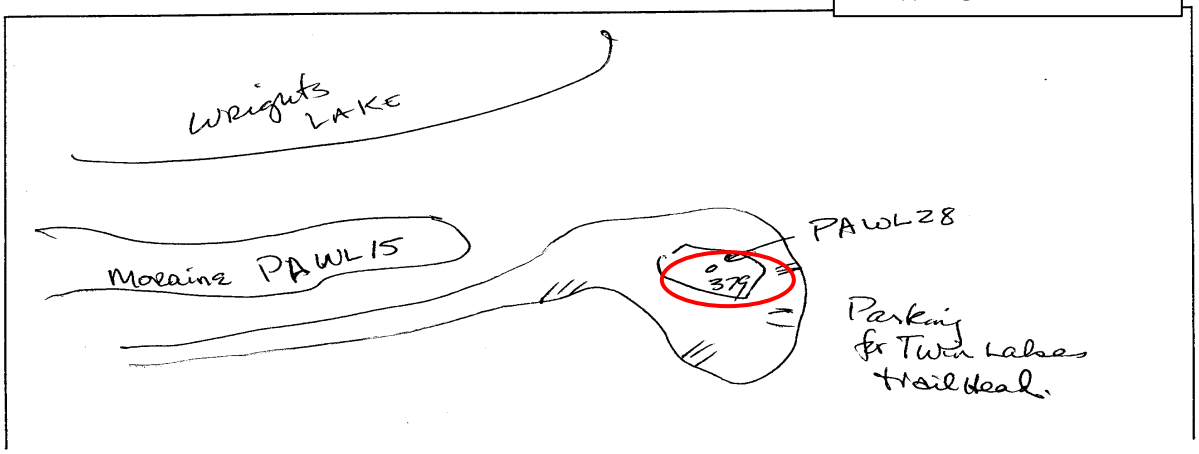
SAMPLING MAP (Attach Topo Map) PAWL26



SAMPLING MAP (Attach Topo Map) PAWL27

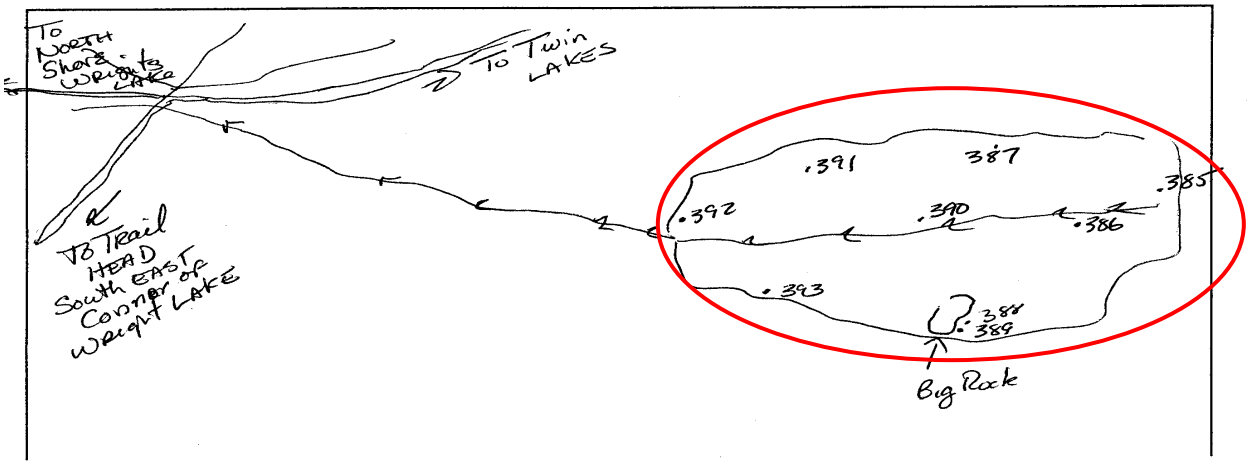


SAMPLING MAP (Attach Topo Map) PAWL28



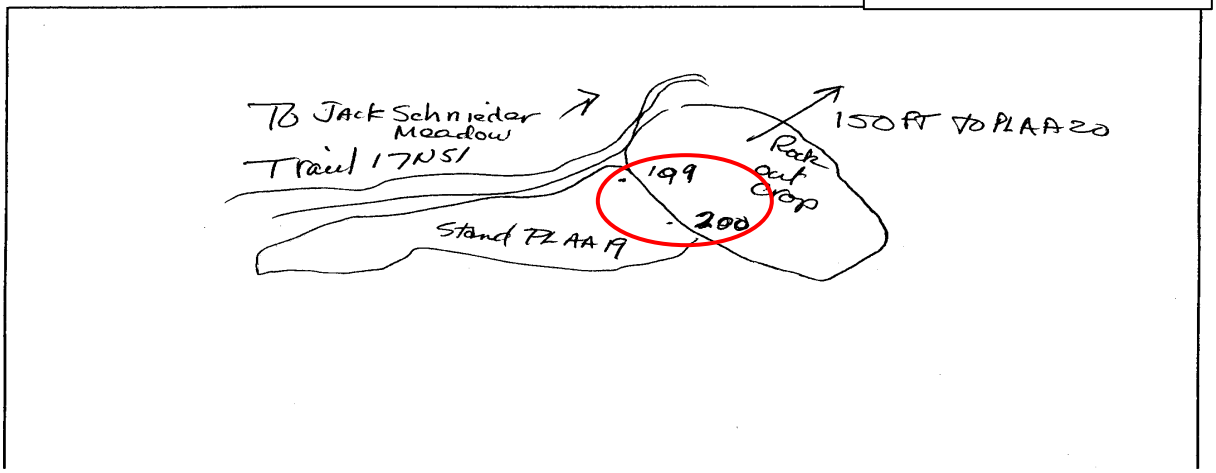
SAMPLING MAP (Attach Topo Map)

PAWL30



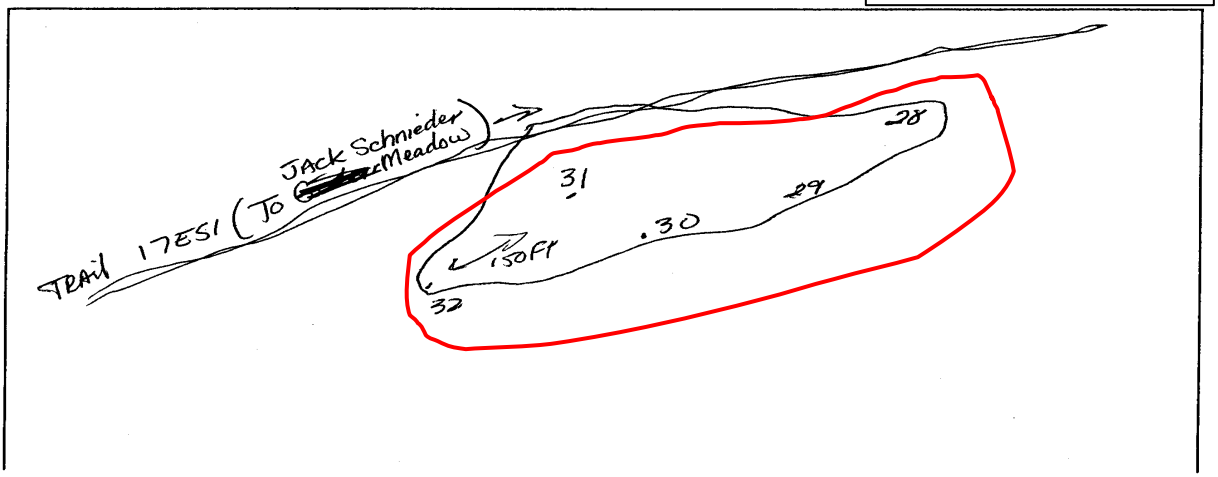
SAMPLING MAP (Attach Topo Map)

PLAA19

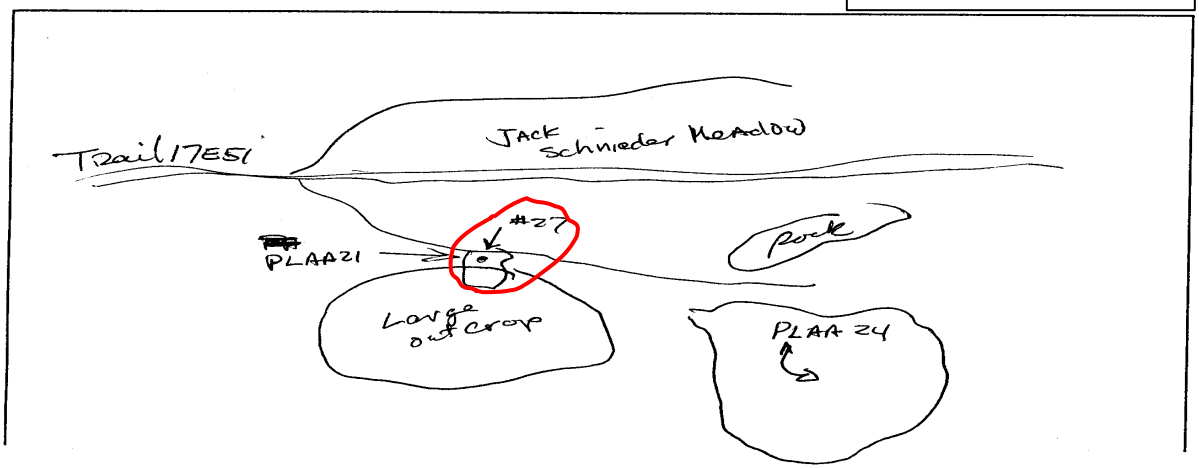


SAMPLING MAP (Attach Topo Map)

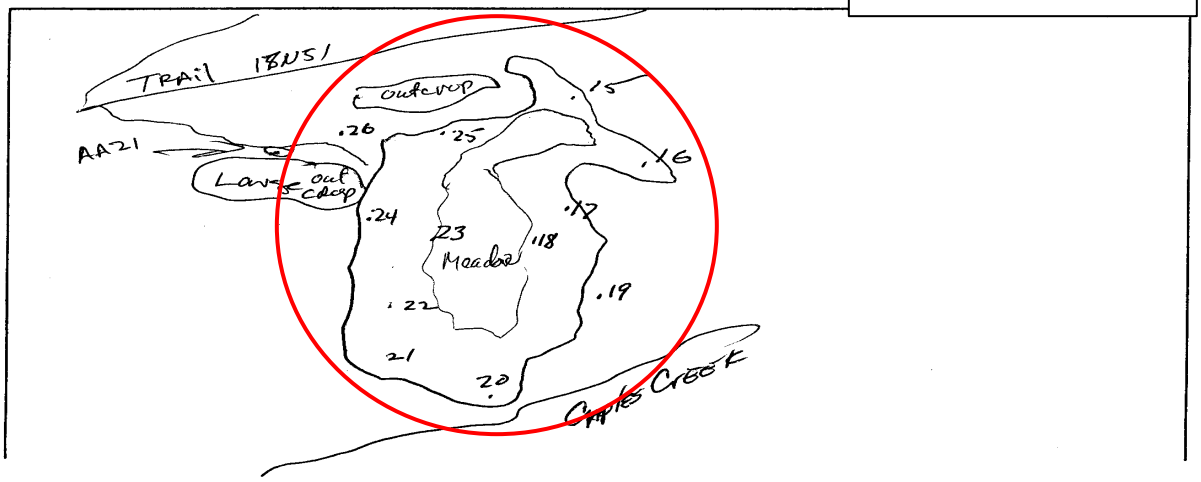
PLAA20



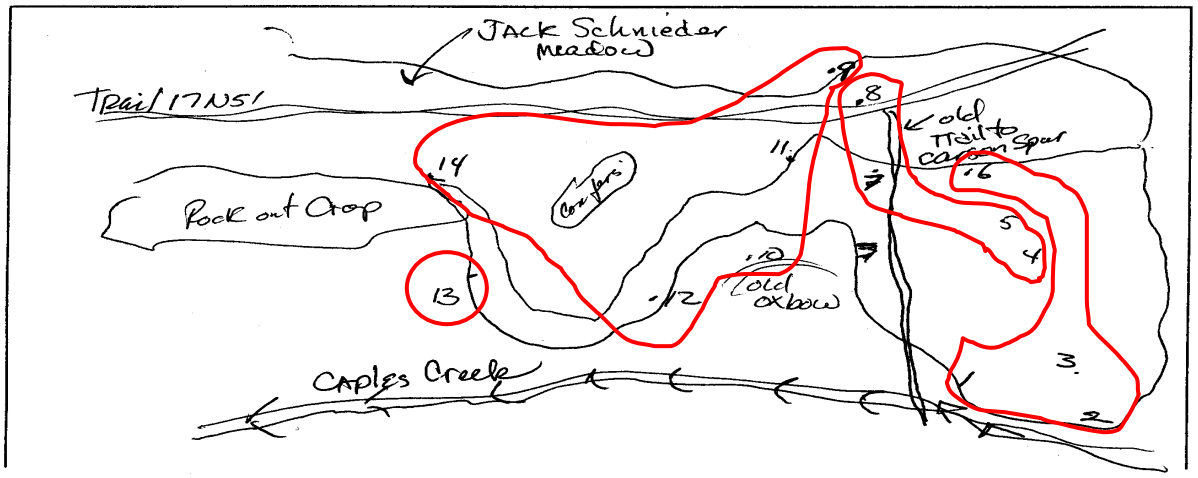
SAMPLING MAP (Attach Topo Map) PLAA21



SAMPLING MAP (Attach Topo Map) PLAA24

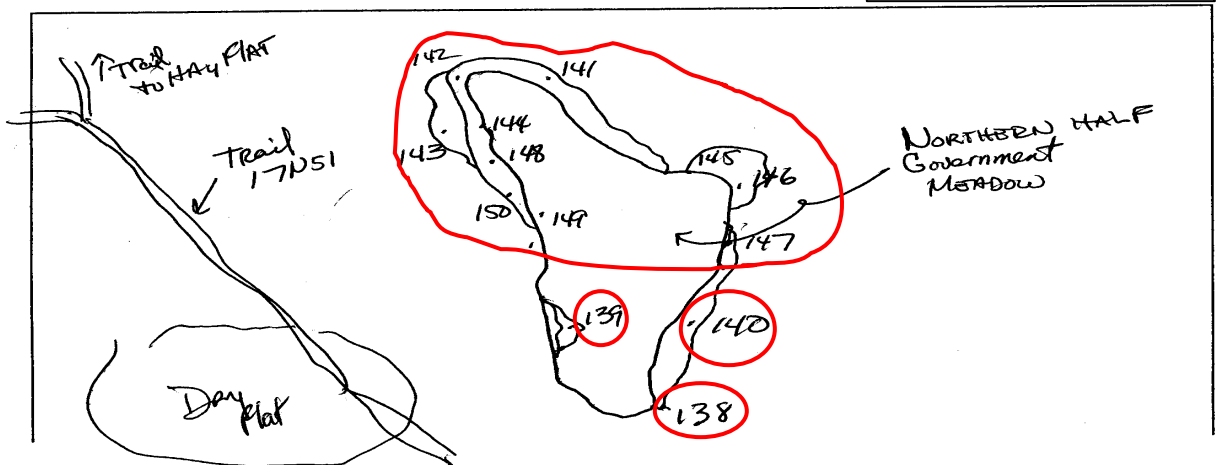


SAMPLING MAP (Attach Topo Map) PLAA25



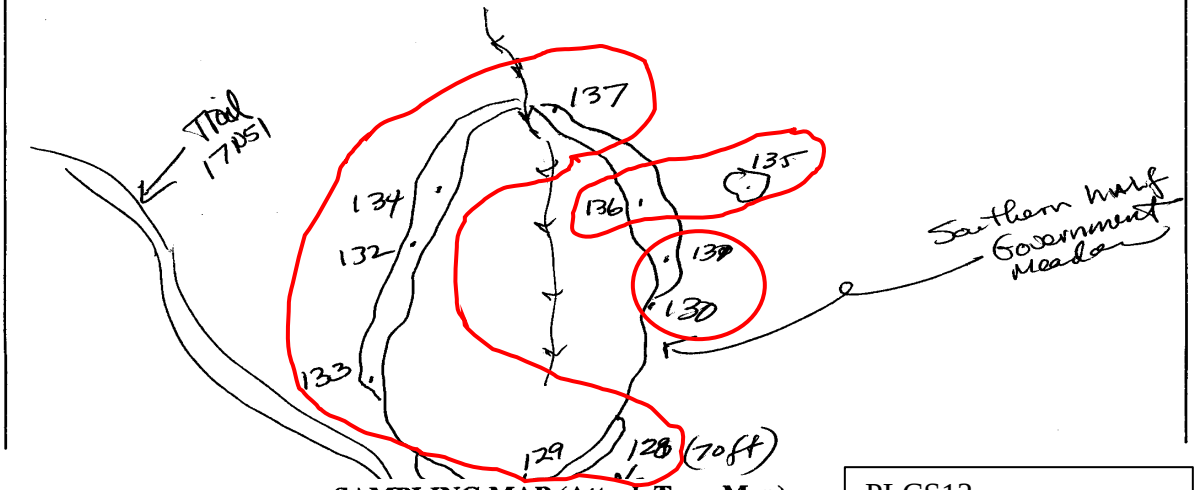
SAMPLING MAP (Attach Topo Map)

PLAA27



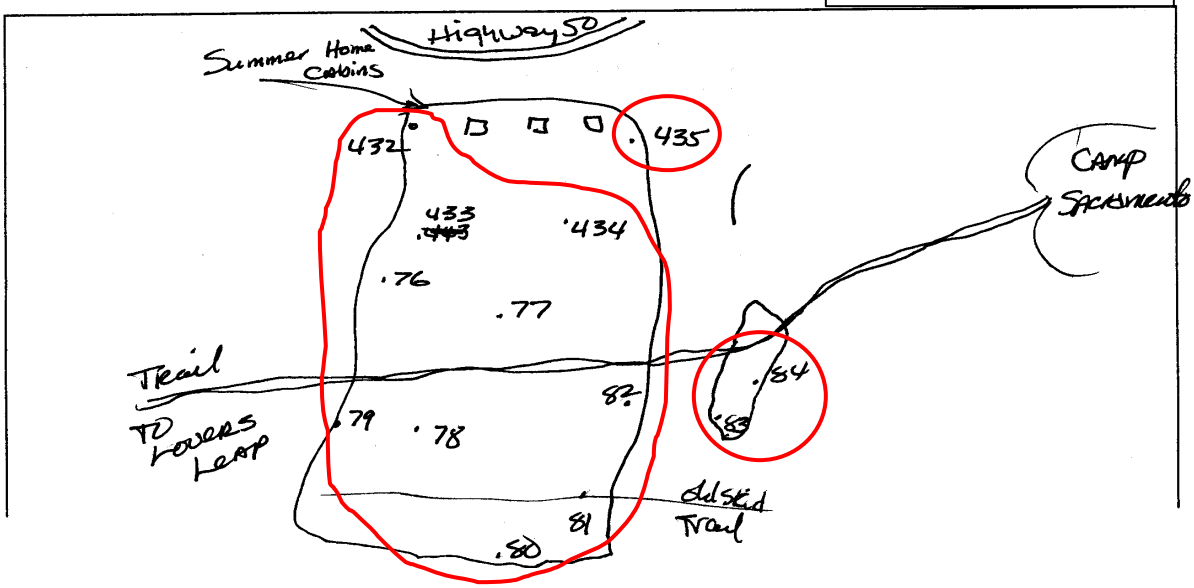
SAMPLING MAP (Attach Topo Map)

PLAA28

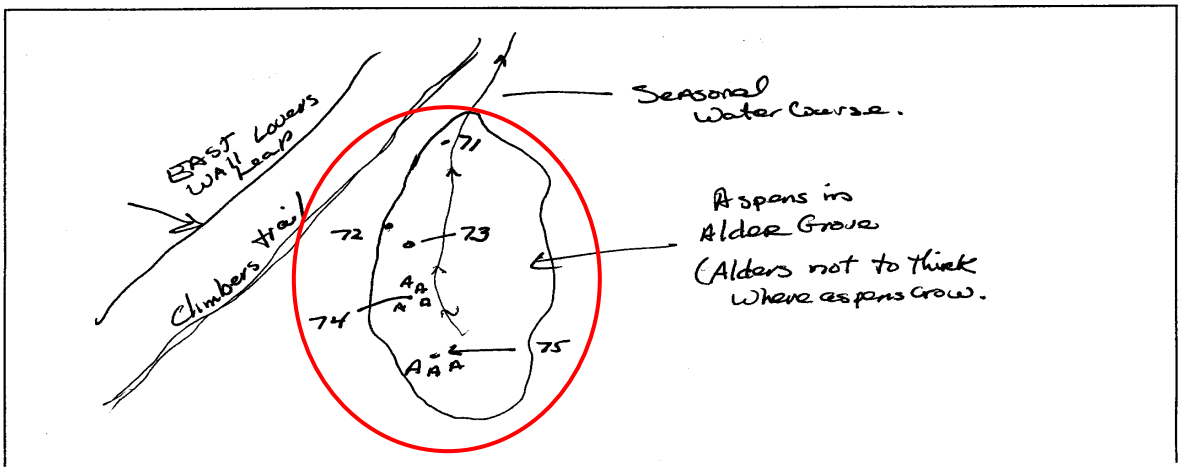


SAMPLING MAP (Attach Topo Map)

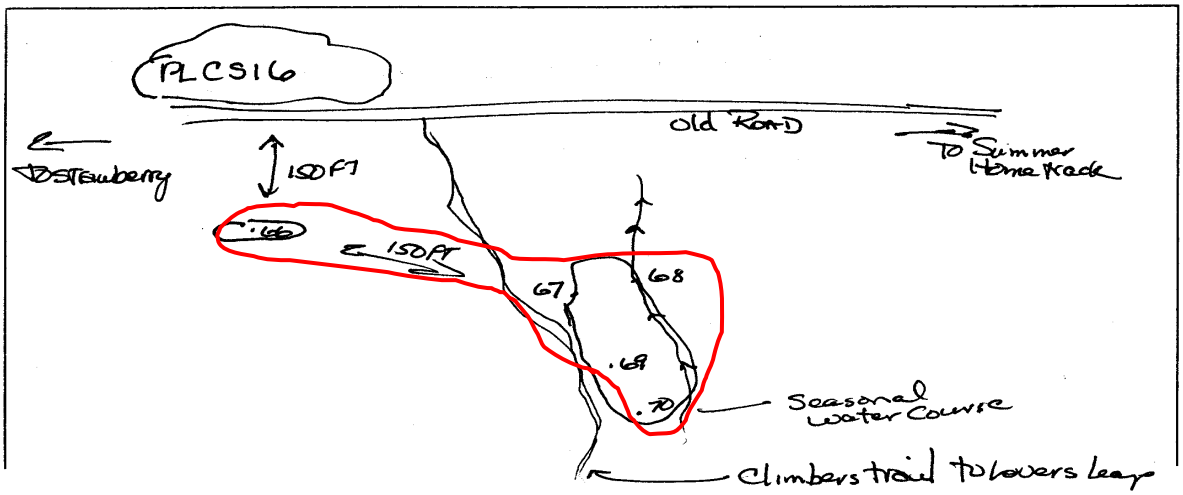
PLCS13



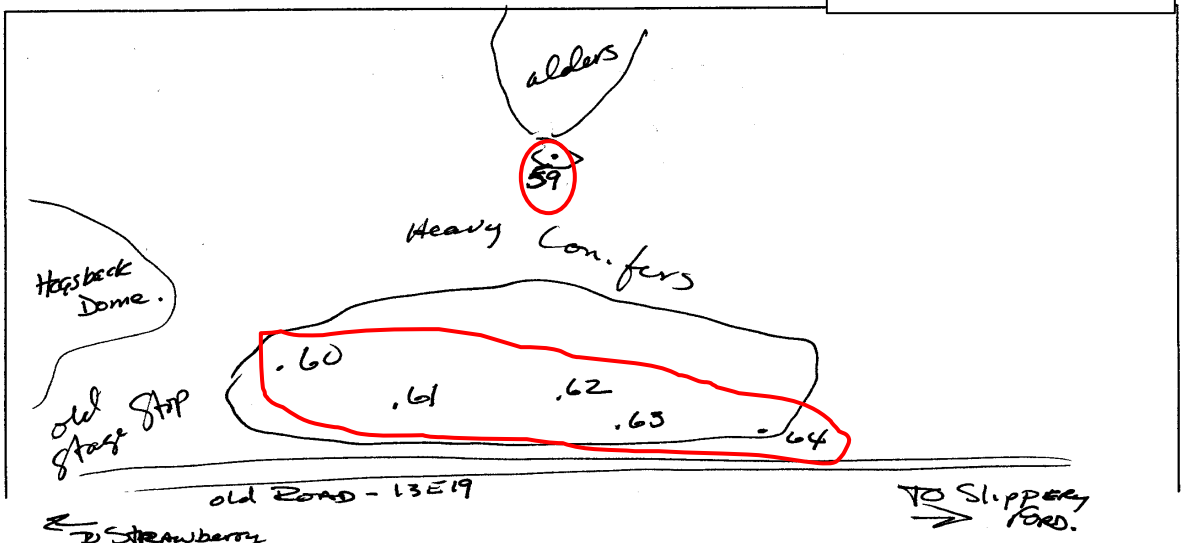
SAMPLING MAP (Attach Topo Map) PLCS14



SAMPLING MAP (Attach Topo Map) PLCS15

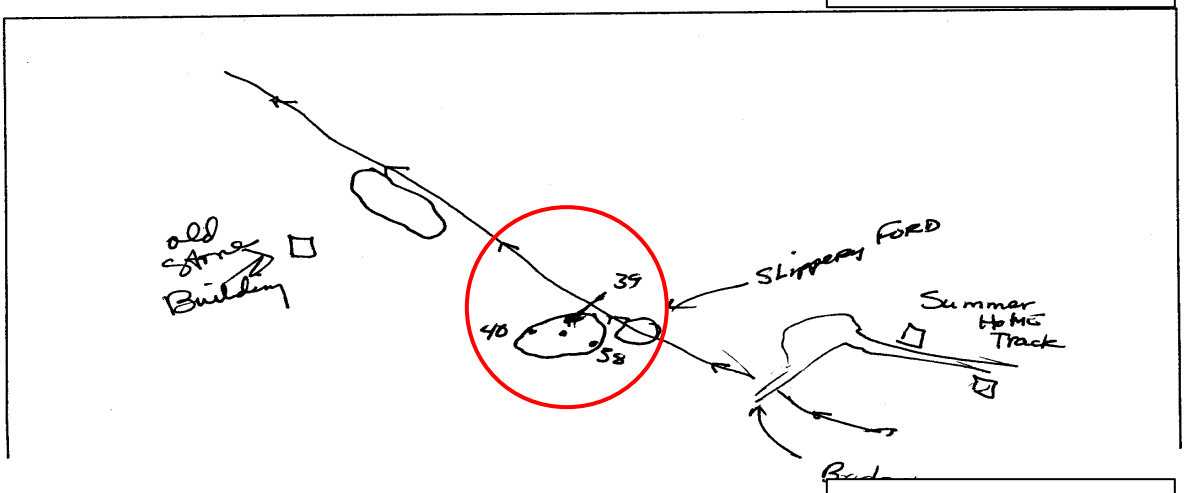


SAMPLING MAP (Attach Topo Map) PLCS16



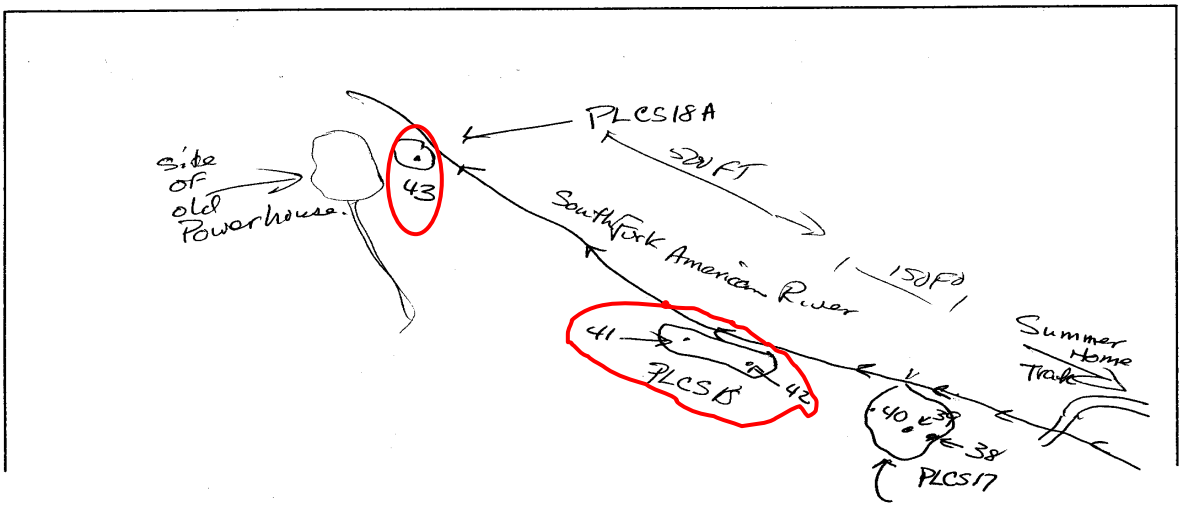
SAMPLING MAP (Attach Topo Map)

PLCS17



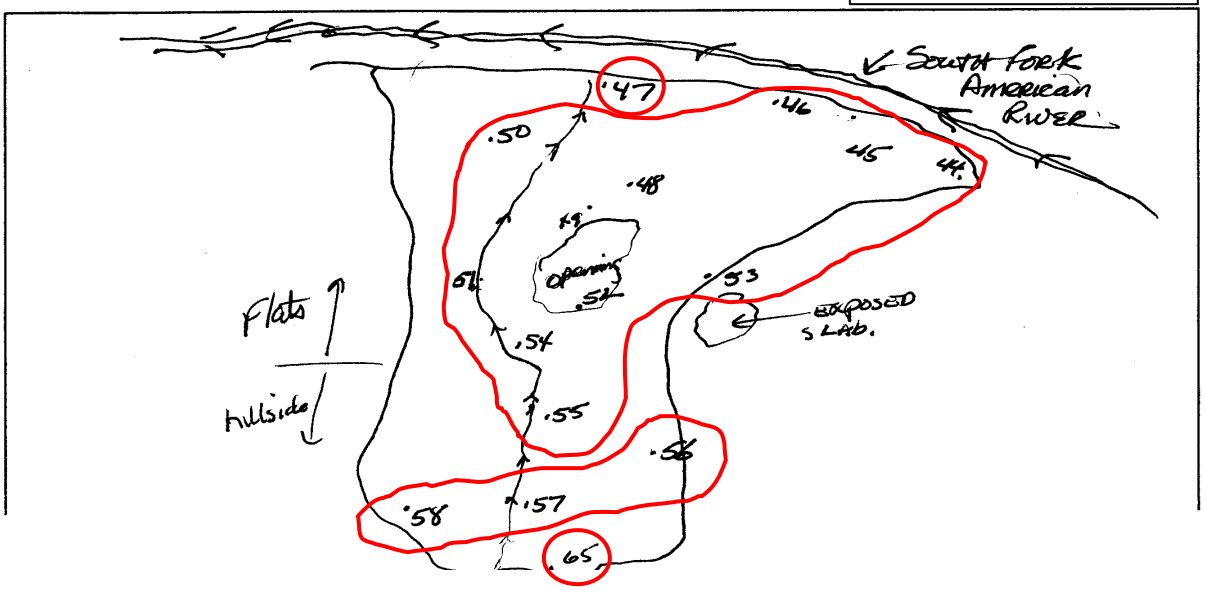
SAMPLING MAP (Attach Topo Map)

PLCS18A + PLCS18

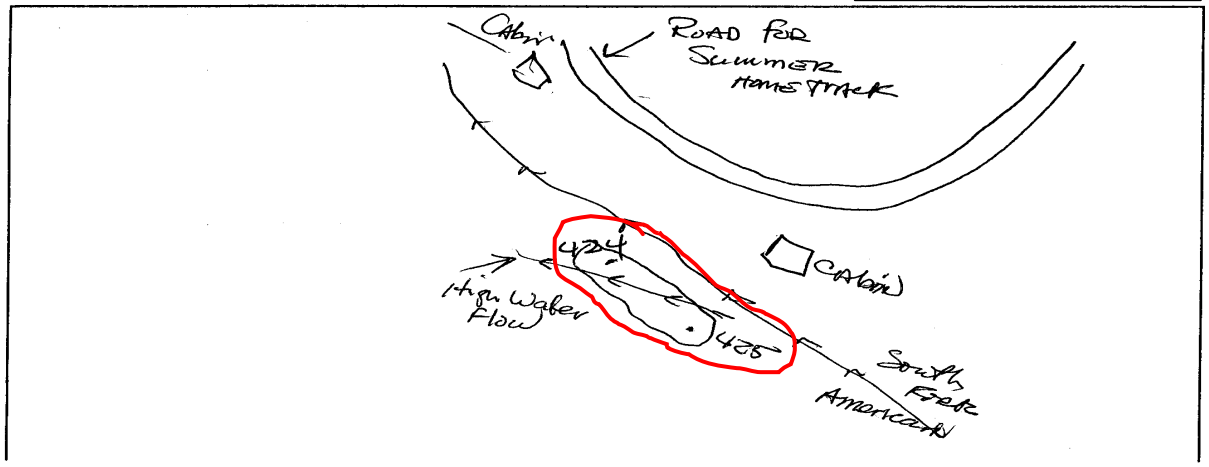


SAMPLING MAP (Attach Topo Map)

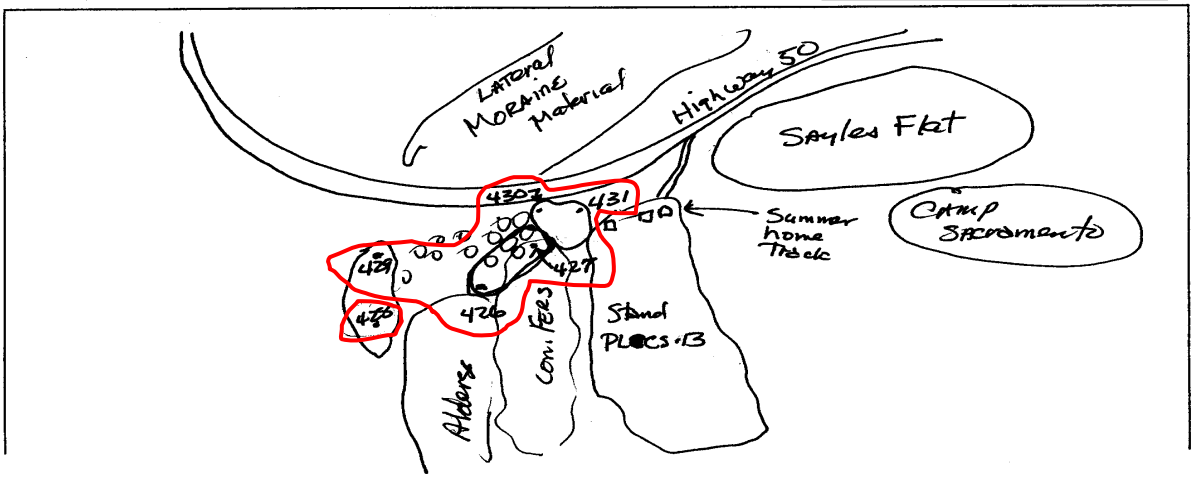
PLCS20



SAMPLING MAP (Attach Topo Map) PLCS22



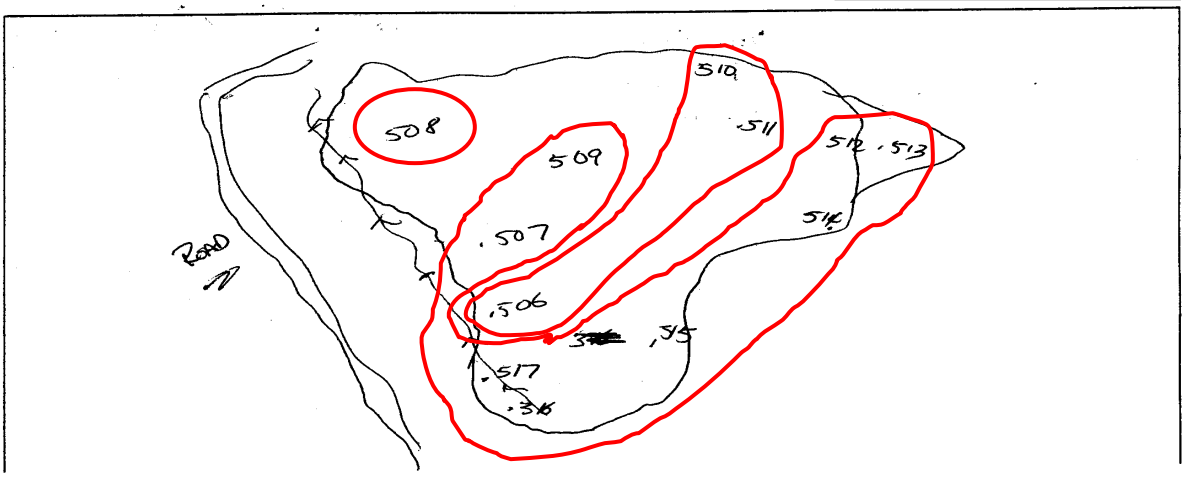
SAMPLING MAP (Attach Topo Map) PLCS23



SAMPLING MAP (Attach Topo Map) PLSB13

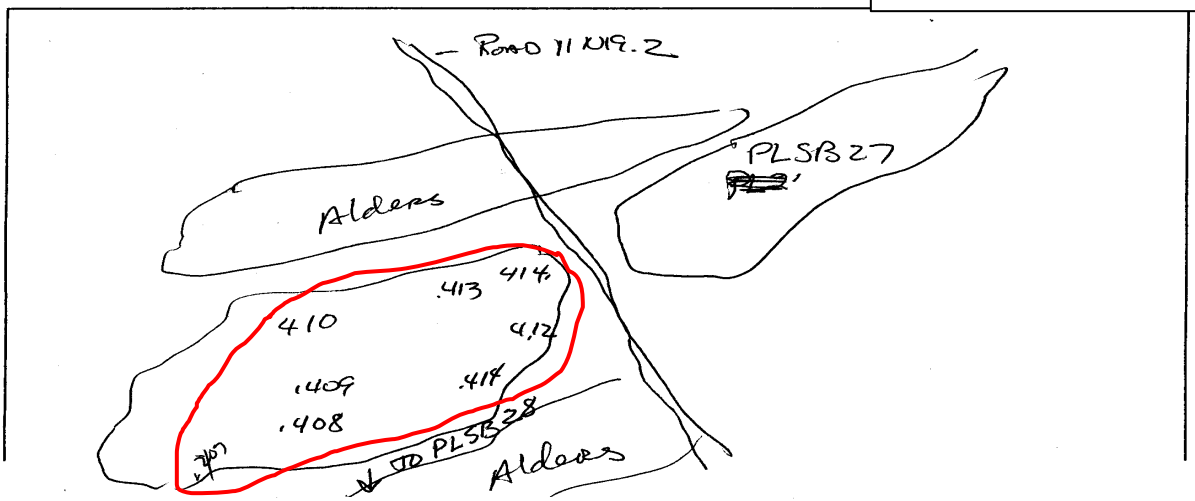
SAMPLING MAP (Attach Topo Map)

PLSB22



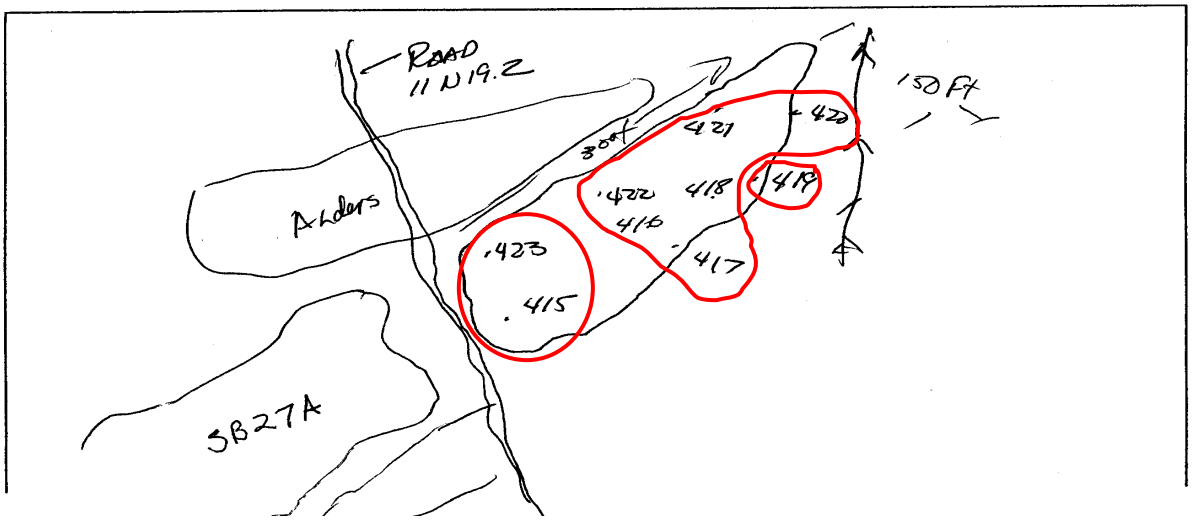
SAMPLING MAP (Attach Topo Map)

PLSB27A



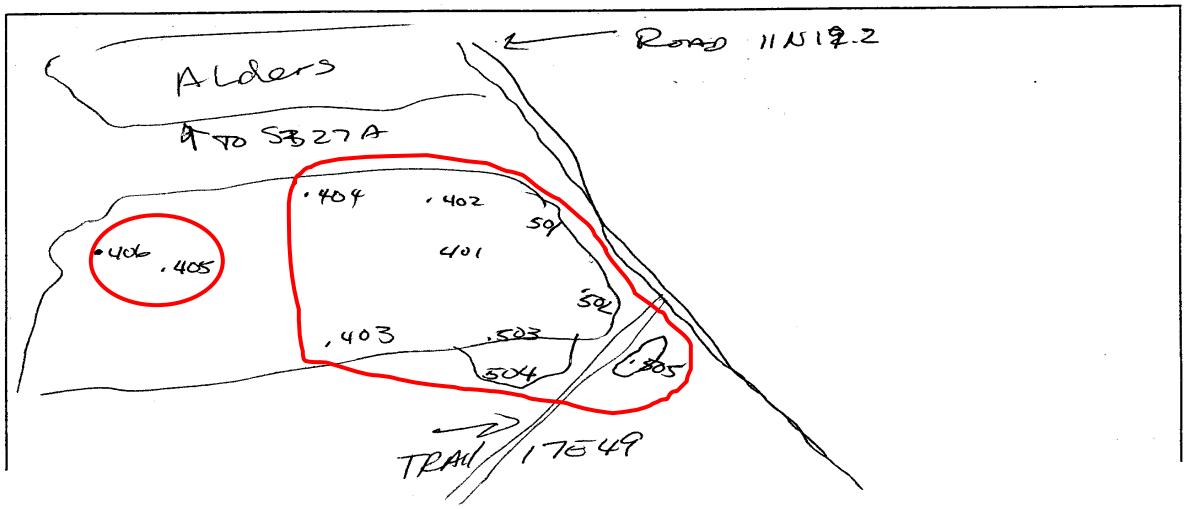
SAMPLING MAP (Attach Topo Map)

PLSB27



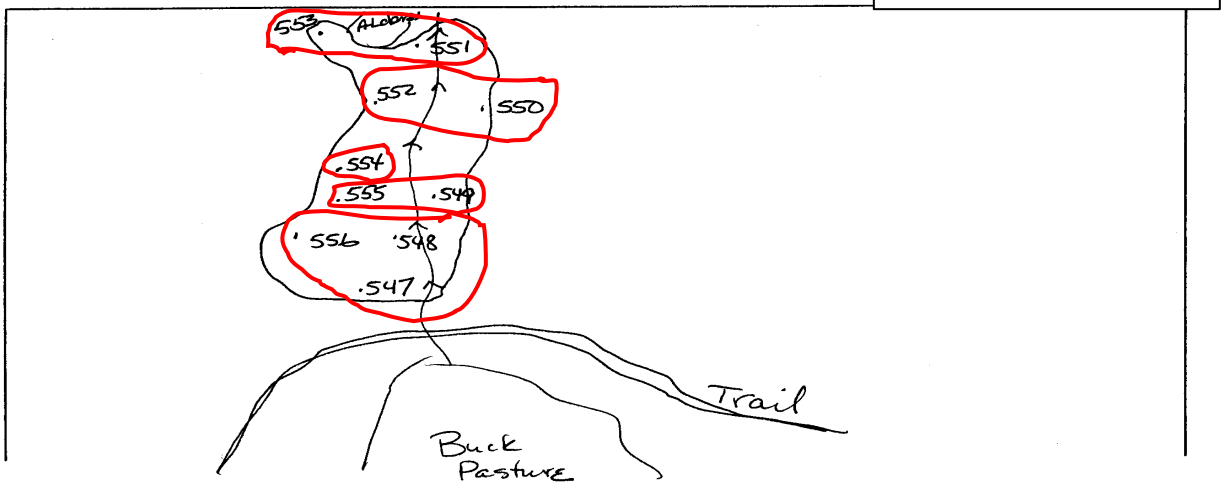
SAMPLING MAP (Attach Topo Map)

PLSB28



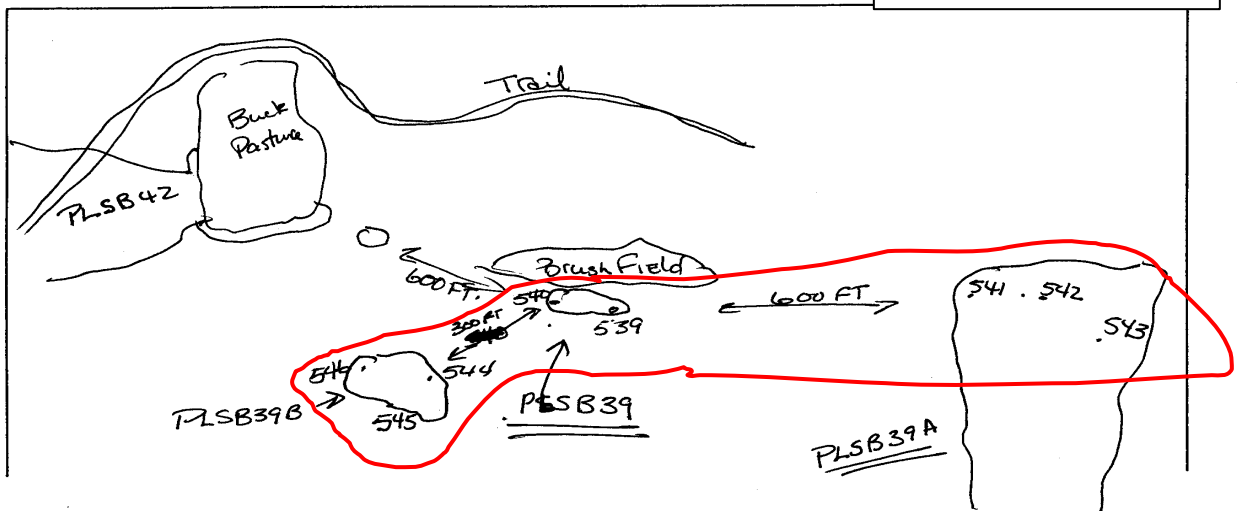
SAMPLING MAP (Attach Topo Map)

PLSB37



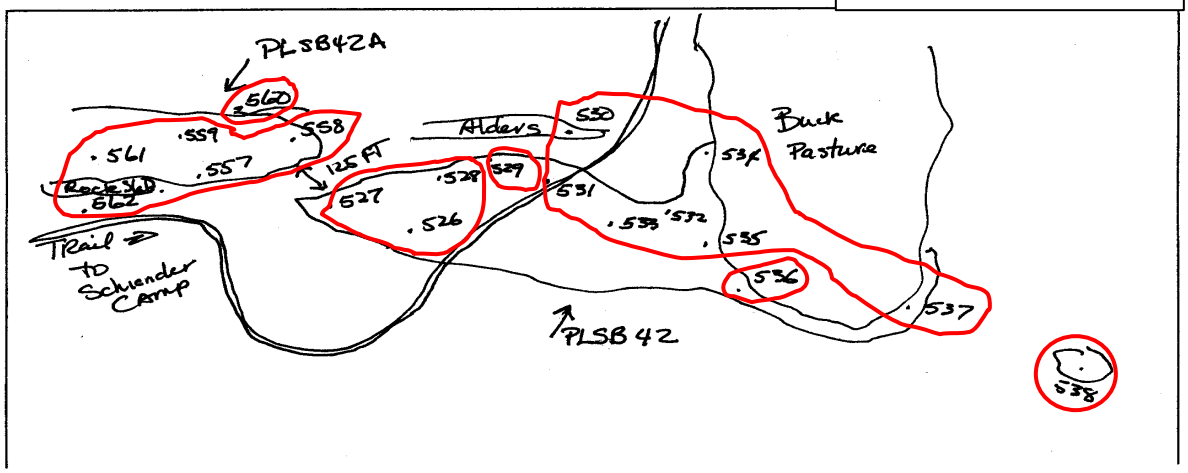
SAMPLING MAP (Attach Topo Map)

PLSB39+39A+39B



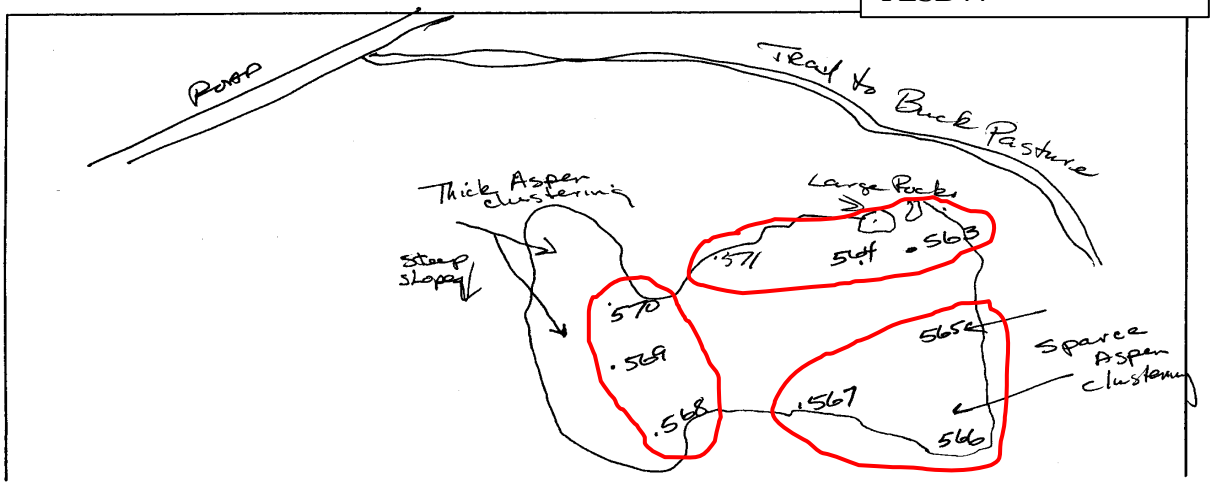
SAMPLING MAP (Attach Topo Map)

PLSB42+42A



SAMPLING MAP (Attach Topo Map)

PLSB44



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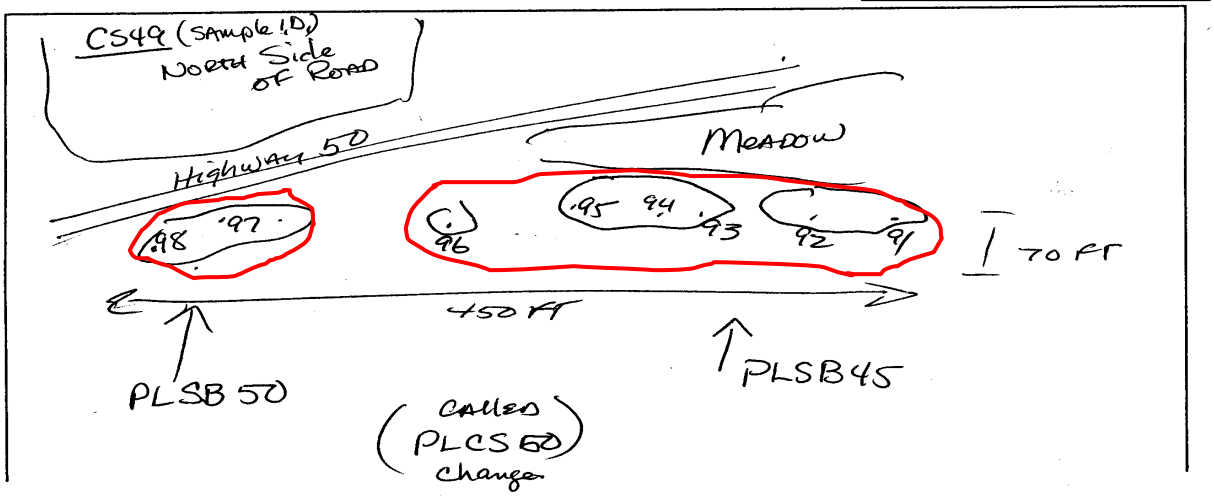

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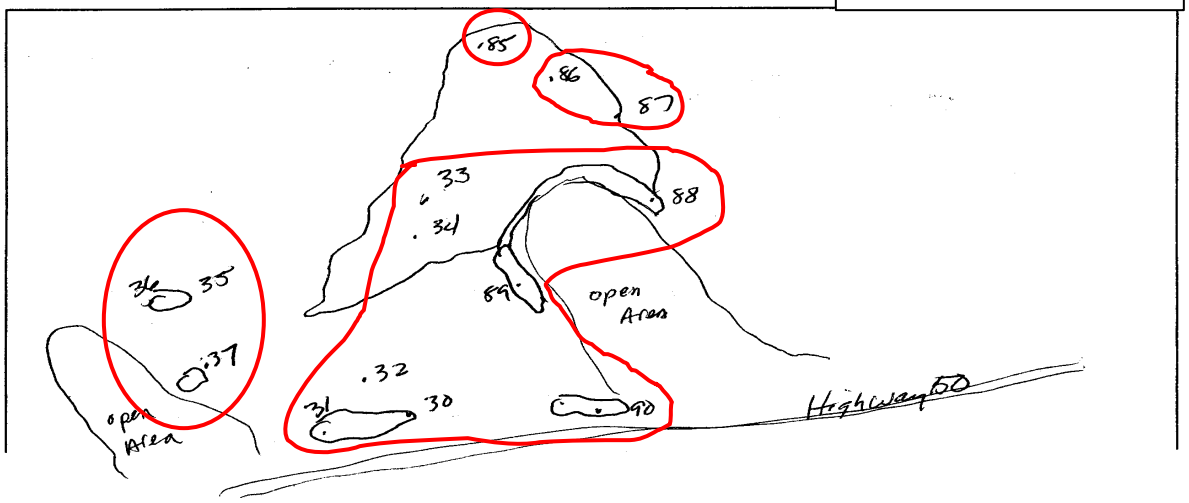

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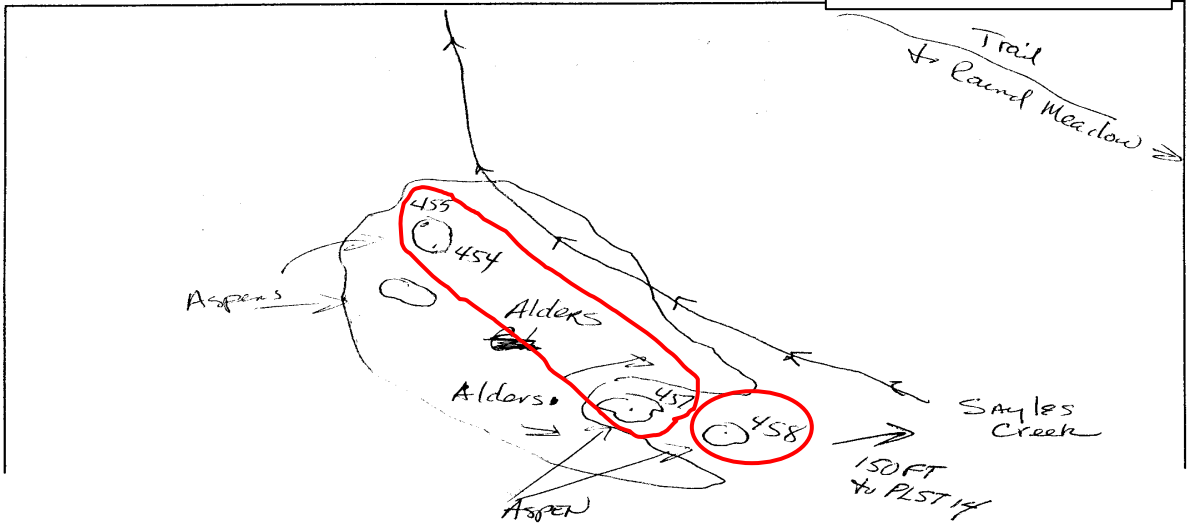

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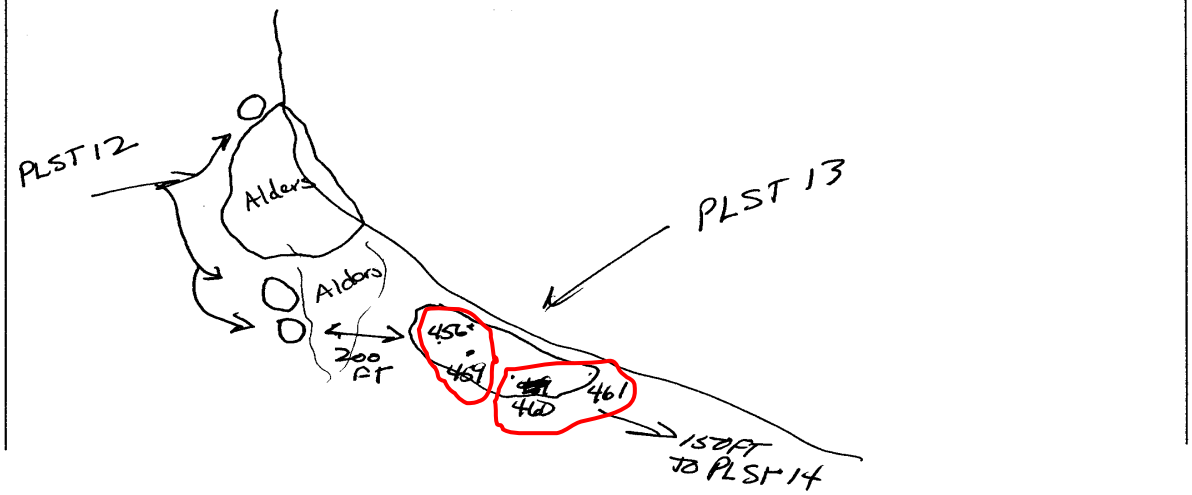

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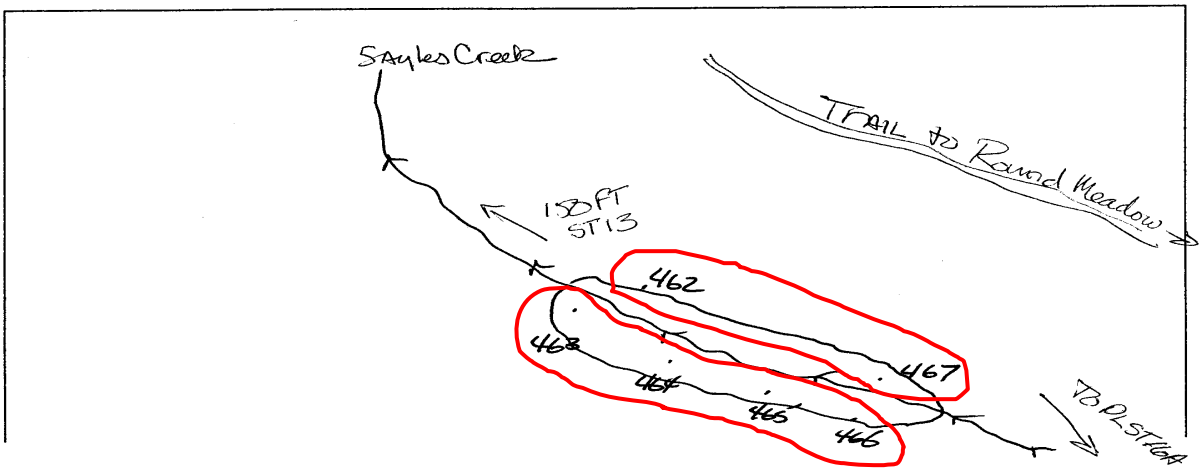

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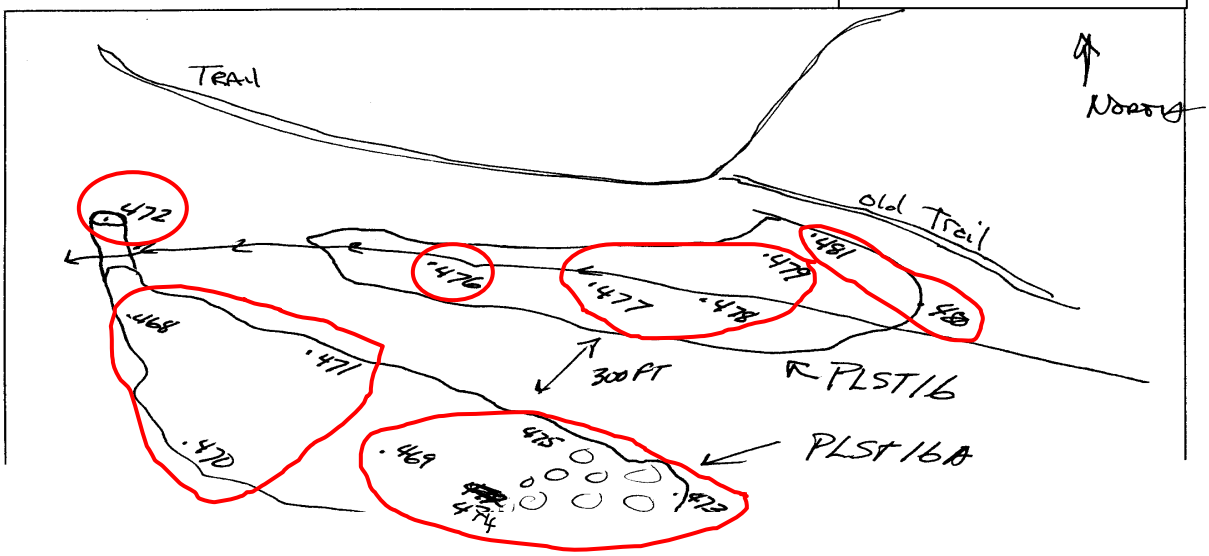

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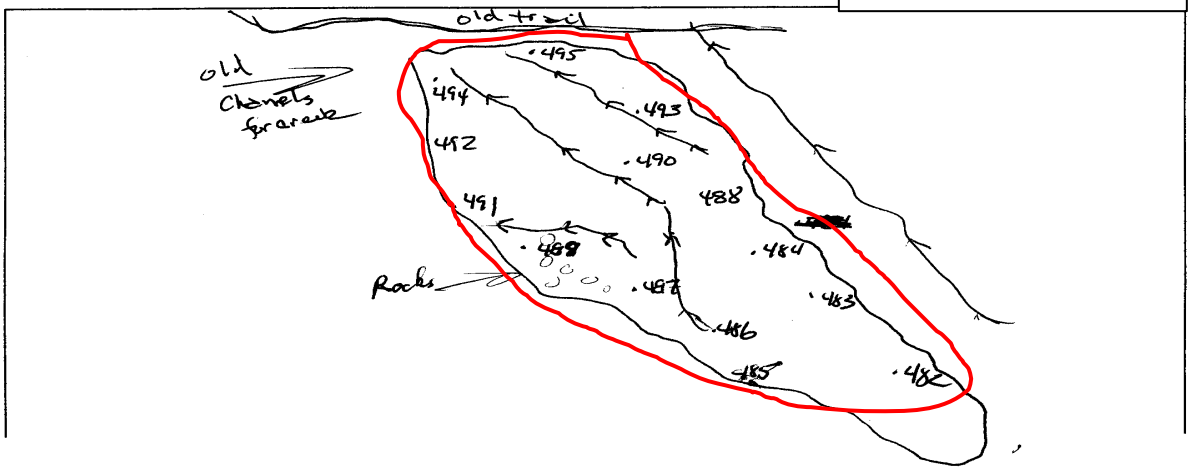

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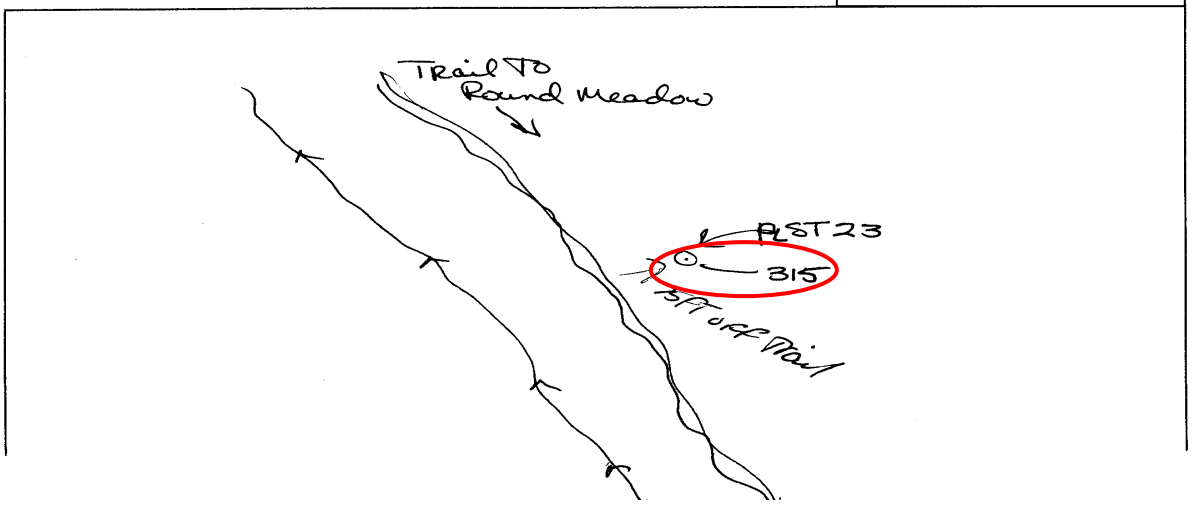

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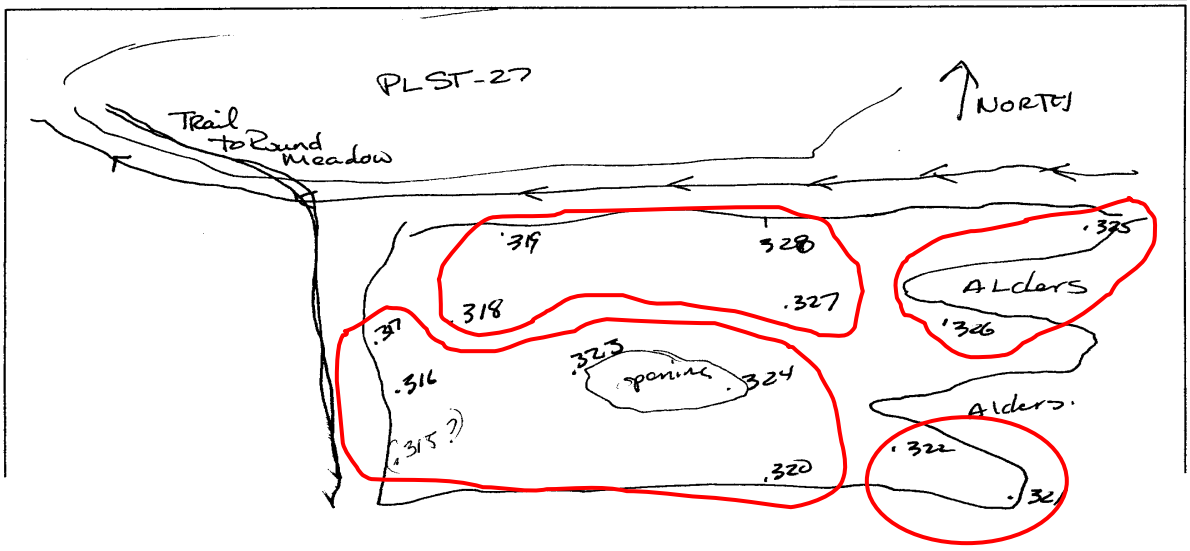

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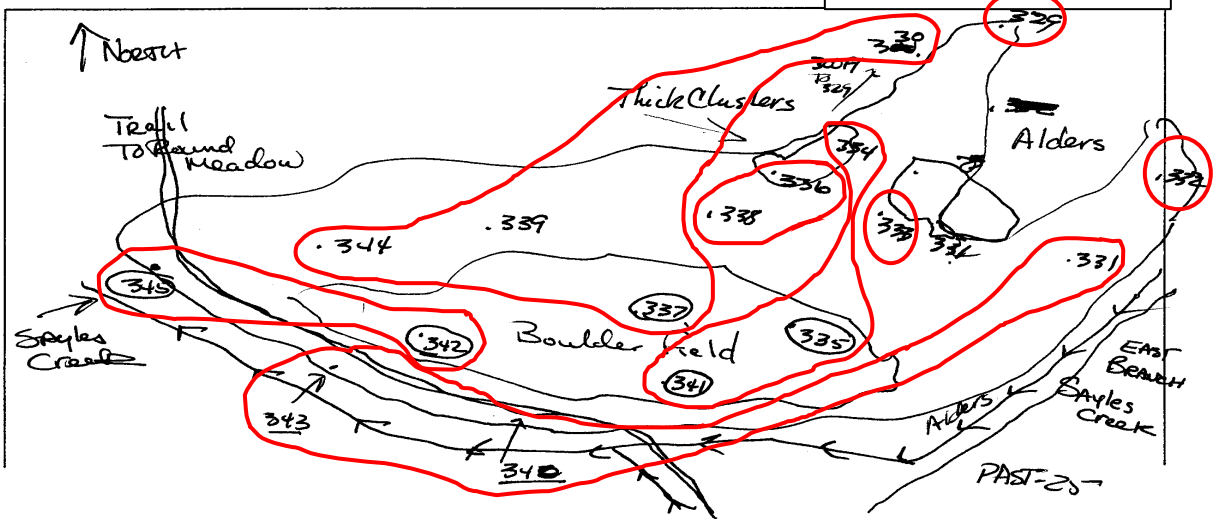

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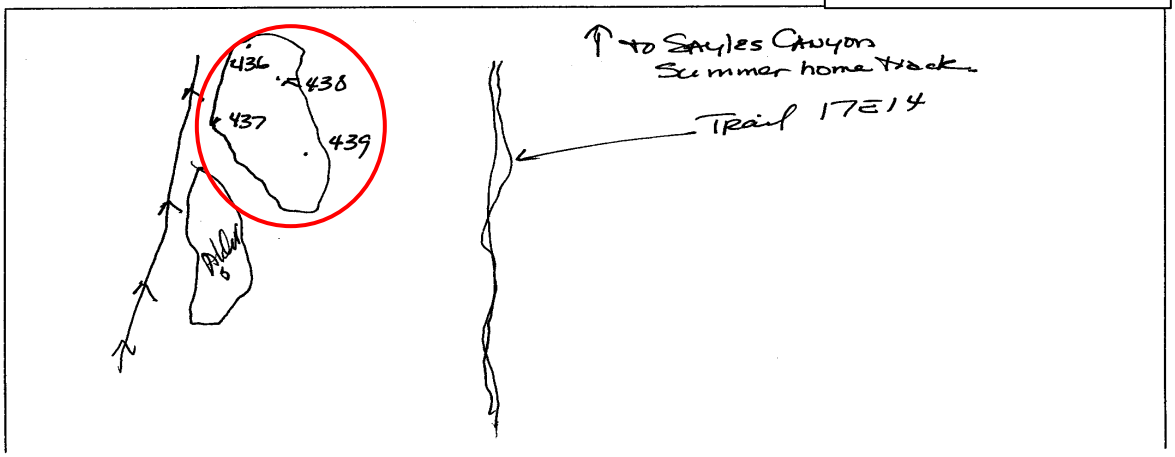

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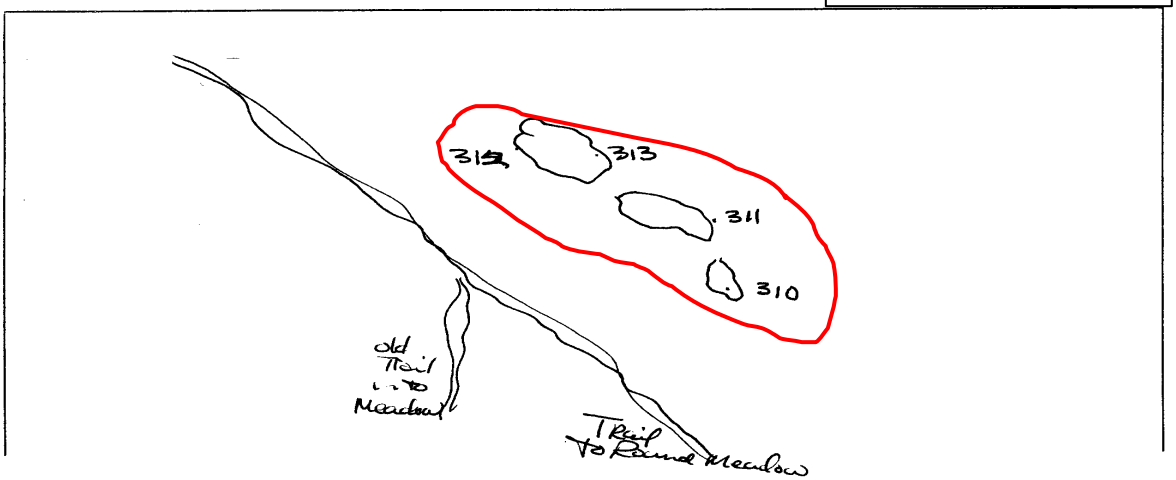

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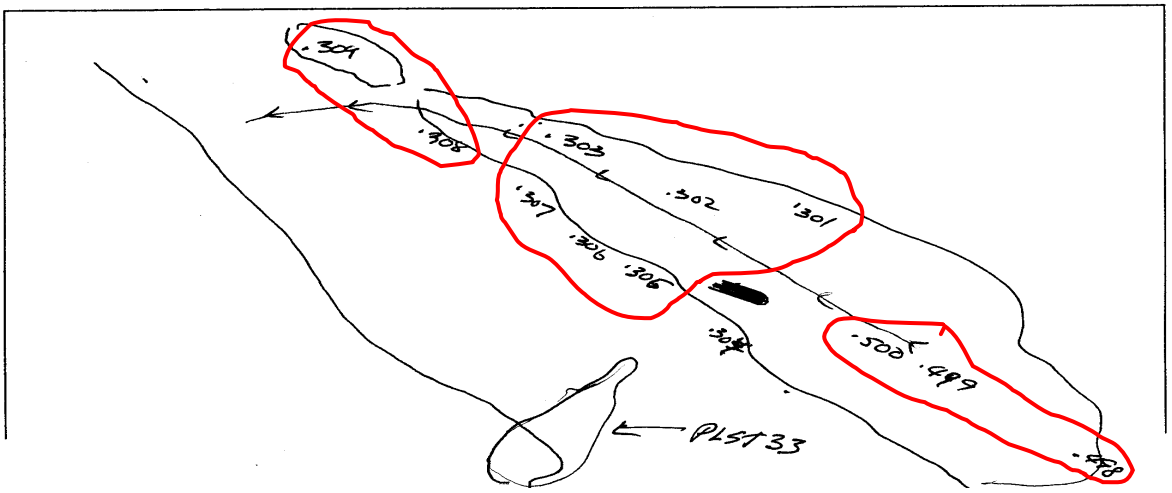

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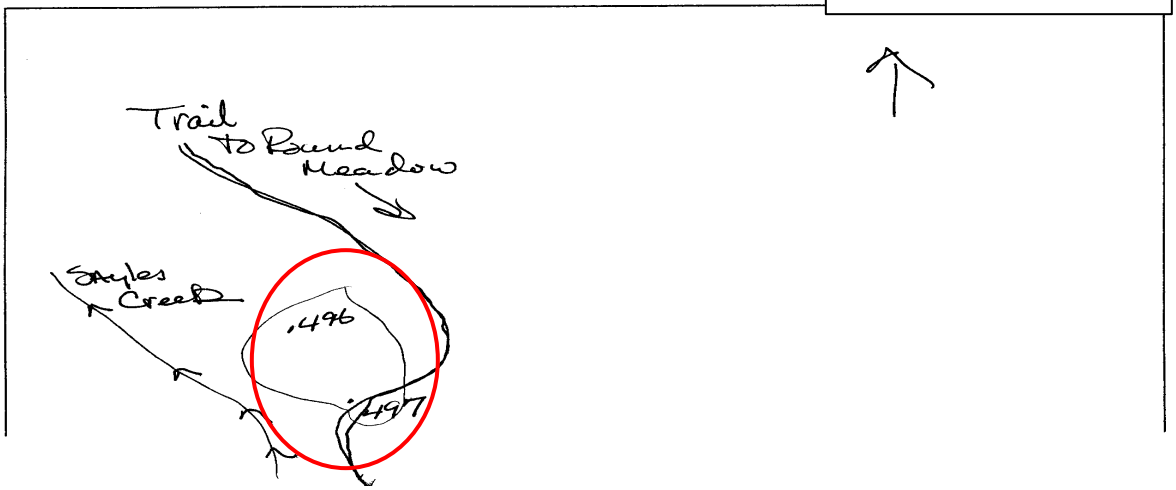

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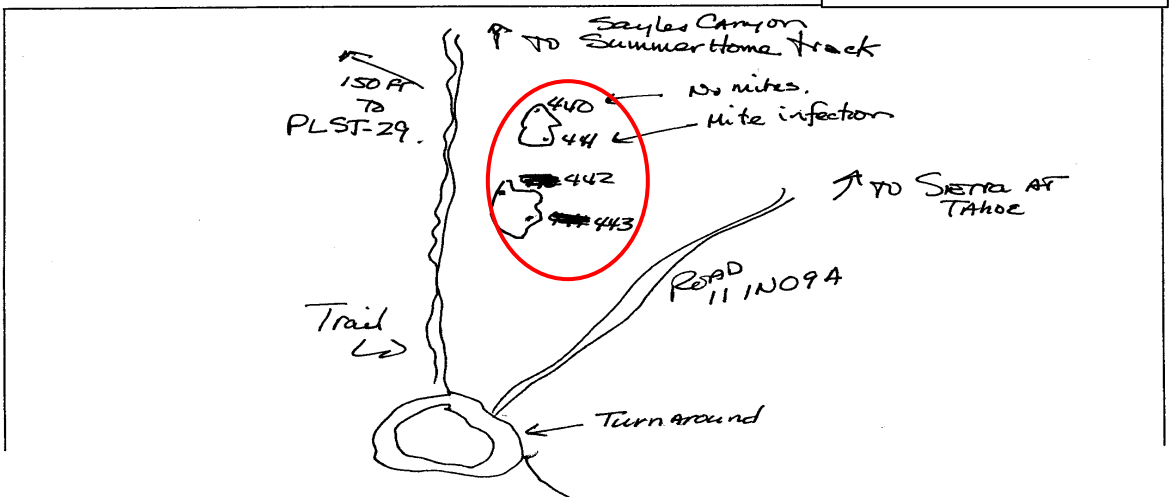

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Expanded Study of the Genetic Diversity in *Perideridia erythrorhiza*: A Rare Plant in Southern Oregon

(NFGEL Projects 78 and 136)

Center for Plant Conservation, National Collection Plant Profiles

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STUDY OBJECTIVES

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

Yes, they do differ. The three locations are about as different from each other in genetic variation as they are from the distinct species *Perideridia oregana* (genetic similarity 0.83). The Cave Junction area is the most genetically divergent of the general locations and shares the most similarity to *P. oregana*.

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

In general, isozyme variation alone is not sufficient to determine whether two sets of populations should be considered distinct species or not. However, isozyme variation can support a decision, made on other, non-isozyme evidence, to divide *Perideridia erythrorhiza* into two (east vs west) or three (by location) species. Isozyme data show that the three locations are highly divergent.

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, Pelican Barn, and Wampler) and a western group (including populations Illinois River, Leather, Roseburg, and Umpqua). East and West are different, but a more useful way to express the pattern of variation is to divide the populations into three regions; Douglas County (the Roseburg and Umpqua populations), Josephine County (the Illinois River and Leather populations), and Klamath Falls (the Howard Prairie, Mud Flat, Pelican Barn, and Wampler populations).

1d. What is the species identity of the Leather 1 population that had such divergent isozymes in the original study?

Leather 1 seems to be an unusual *Perideridia erythrorhiza* population. It may exhibit introgression resulting from past or present hybridization with *P. oregana*.

2. Does the Pelican Barn population from the Winema National Forest differ from the population at Mud Flat? Can Mud Flat be used as a seed source for increasing the Pelican Barn population?

The Pelican Barn and Mud Flat populations (Klamath County) have a genetic similarity of approximately 90%. Transfer of seed from Mud Flat to Pelican Barn population can be justified on genetic grounds. However, the Pelican Barn population actually shares more genetic similarity to the Wampler population (99.6%) and the Howard Prairie population (91.6%) than it does to the Mud Flat population. This suggests that the other Klamath Falls area populations would be a better source of seed to augment the Pelican Barn population if that becomes necessary, and seed was available.

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

Despite their great differences in size, the Pelican Barn and Mud Flat populations are surprisingly similar in measures of genetic diversity.

INTRODUCTION

Perideridia erythrorhiza (Piper) Chuang & Constance, Red-root Yampah, is a rare perennial herb that occurs only in three regions of southwest Oregon: near Klamath Falls in Klamath and Jackson Counties, near Roseburg in Douglas County, and near Cave Junction in Josephine County. The biology, history, and geographic range of the species are covered elsewhere (Chuang and Constance 1969, and Meinke 1998). *P. 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.

Seed transfer among *Perideridia erythrorhiza* populations may be necessary to maintain small, vulnerable populations in the Winema National Forest. However, inappropriate seed transfer could lead to unintended loss of genetic diversity. The Wampler and Howard Prairie sites from the Klamath Falls area (Table 1) are small and at risk due to their restricted size. 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. The largest and most vigorous population known in the entire species grows in a meadow on private land at Mud Flat. This meadow is grazed, and adjacent woodland is logged. The Mud Flat population would be an obvious source of plants to augment the smaller populations on Forest Service land if mitigation became necessary. The Mud Flat site is separated from the other Klamath County sites by 25 miles, raising the question of whether it is too genetically differentiated from the other Klamath Falls region populations for such use.

At the request of the Winema National Forest, the National Forest Genetic Electrophoresis Laboratory (NFGEL) has performed two studies of *Perideridia erythrorhiza* genetic diversity to address the question of species delimitation. The first study (NFGEL Project #78) examined differentiation of populations within and among the three areas in which the plants occur. The second study (NFGEL Project #136) extended that work to additional populations and addressed questions of species identification raised in the first project. Two species with which *P. erythrorhiza* can easily be confused, *P. gairdneri* (Hook. and Arn.) Mathias and *P. oregana* (W. Watson) Mathias, were included in the second phase of the study. Combined results from the two projects are reported here.

MATERIAL AND METHODS

Sample Collection and Seed Germination. Seed was collected from between six and 61 individuals per population (Table 2; Figure 1). Seed from each individual flower stalk was stored separately in a paper envelope. Envelopes were spread out to dry at room temperature for about a month. Three to five seed per plant were stratified and germinated. For stratification, seeds were soaked for about half an hour in tap water to which a dishwashing detergent was added (1 drop/liter) to permit seeds to sink. Seeds were drained and placed on a moist paper towel in a sealed plastic bag and held at 4°C mainly in the dark until germination, which took two to three months. Bags were opened briefly once a month to introduce fresh air. Two to three seedlings from each mother plant were planted in a single Styrofoam cup in a commercial growing medium containing peat and perlite. For most shipments, seedlings were grown at room temperature indoors under fluorescent lights set to give the seedlings 16 hours of light, but plants delivered 19 June 2000 were grown outdoors under partial shade. Seedlings were delivered to NFGEL when they had reached 5 to 10 cm in length and ceased vigorous growth, a sign that they would soon enter dormancy.

Sample Preparation. Seedlings from a total of 338 individuals were submitted for isozyme analysis using starch gel electrophoresis for Project #78, and 148 samples for Project #136. 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 (bulbil) in the preparation. Therefore, each seedling was gently dug out of the soil taking care not to break the shoot off of the bulbil. For each cluster of half siblings, if the first seedling selected lacked a bulbil on the root, it was discarded and replaced with one that had a bulbil, unless all lacked bulbils. In that case, one seedling including roots, but with no bulbil, was processed. Each seedling was washed with water and ground in a mortar using liquid nitrogen. Approximately 0.4 ml 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 at -70C 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). For Project #78, two people independently scored each gel. When they disagreed, a third person resolved the conflict. For Project #136, gels were scored from photographs, using the allele maps developed for Project #78. 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 thought to be aneuploid and approximately tetraploid (Chuang and Constance 1969). Although all loci of a tetraploid should theoretically contain four alleles, observed band patterns at some loci were not consistent with that expectation. We therefore treated the plants as if they were diploid and scored each set of potential homoeologous loci as one diploid locus.

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). Seven enzymes of the 15 loci resolved showed the unbalanced heterozygotes or three-allele patterns typical of tetraploids, but three loci appeared to be diploid. The other five loci provided no clear evidence one way or the other due to limited variation. Enzymes that gave evidence of polyploidy exhibited the alternate homozygous states and, in most cases, alternate unbalanced heterozygotes, typical of autopolyploids (Soltis and Reiseberg 1986). *Perideridia oregana* is diploid (n=8, 9, 10, 13), and *P. gairdneri* may be a high polyploid (n=19, 20, 40, 60) (Chuang and Constance 1969). In the absence of computer software capable of analyzing

autopolyploids or organisms containing both diploid and tetraploid loci, all loci were scored as diploid. All heterozygotes, balanced or not, were scored as diploid heterozygotes. This method of scoring artificially raises the observed heterozygosity compared to genetically similar diploids. Because the three MDH loci failed to resolve in Leather 1 and 2, they were omitted from analysis.

Data Analysis. Results of the combined studies were analyzed using Popgene, version 1.32 (Yeh 1997). 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

Variation in Isozyme Resolution. A preliminary study (not shown) indicated that seedlings had strong enough enzymatic activity for effective isozyme analysis only if the bulbil (small tuberous root) was included in the tissue studied. Lack of bulbils was a serious problem in 2002, when 3 (10%) individuals of Leather 2, 8 (30%) of Leather 3, and 15 (52%) of Odessa lacked bulbils. In these populations, individuals with bulbils also had poor enzyme activity, except for five individuals from the Odessa population.

Up to fifteen enzymes were resolved in this study, but enzyme activity varied greatly among shipments of seedlings and among individuals. Therefore, complete 15-locus genotypes could be determined for only 29% of seedlings in the first study and 1.4% of those in the second study. In some populations, only six loci resolved well (AAT, GDH, ME, PGI2, TPI1, TPI2).

At the ME locus, enzyme migration varied with the date on which the seedlings were delivered to NFGEL, which in turn depended on the date at which germination began. For the Illinois River and Umpqua River populations, the most common ME allele shifted from ME-1 in May 1999 to the much faster ME-6 for the 19 June 2000 delivery. Leather 1 (delivered 19 June 2000) and Leather 2 (delivered 17 May 2002) also contained the ME-6 allele (frequency = 1.0 and 0.78, respectively).

Genetic Variation. All three species of *Perideridia* examined in this study were genetically variable (Table 2). In *P. erythrorhiza*, higher levels of genetic diversity were observed in the Cave Junction area compared to the Roseburg and Klamath Falls areas. On average, the Klamath County populations contained more genetic diversity than the Douglas and Jackson County populations. The most genetically diverse *P. erythrorhiza* populations are Leather 1 and Leather 2. *P. oregana* populations are more diverse than any of the *P. erythrorhiza* or *P. gairdneri* populations. Observed heterozygosity (*Ho*) was consistently lower than expected heterozygosity (*He*), and therefore $F > 0$, particularly for the Klamath Falls and Douglas County populations (Table 2), suggesting considerable inbreeding, perhaps through self-pollination. However, this study was based on seedlings, and numerous studies have shown that adult plants are less inbred than seedlings, apparently because the more homozygous plants have lower survival rates than heterozygotes.

Distinctions Among Species. The three *Perideridia* species examined are sympatric. The Leather populations of *P. erythrorhiza* and *P. oregana* were collected less than half a mile apart, and the Wampler population of *P. erythrorhiza* was within a mile of the Odessa

population of *P. oregana*. Both populations of *P. gairdneri* were collected in the general area of the Klamath Falls area populations of *P. erythrorhiza* (Table 1; Figure 1).

The three *Perideridia* species examined share most alleles, and no fixed differences among them were detected. Important differences in allele frequencies at 6 of the 15 loci resolved help separate the species (Table 3). Genetic identities between populations of *P. gairdneri* and the other species were low (0.48 – 0.62), but genetic identities of *P. erythrorhiza* populations to each other averaged only about the same as genetic identities of *P. erythrorhiza* to *P. oregana* (0.83; Table 4).

Identity of the Leather 1 Population. Between the two projects reported here, three *Perideridia* collections were made at the Leather site in Josephine County. Leather 1 was collected for Project #78. Leather 2 and Leather 3 were collected for Project #136. Leather 2 is known to be *P. erythrorhiza* and Leather 3 is known to be *P. oregana*.

The original analysis found that the Leather 1 population, thought to be *Perideridia erythrorhiza*, was extremely divergent from the other *P. erythrorhiza* populations studied (Nei's genetic similarity averaged 0.47, standard deviation 0.042). The original interpretation of Leather 1 as extremely divergent rested on six loci (MDH1, MDH2, MDH4, ME, PGM2, and UGPP2). Because of the presence of high frequency null alleles (alleles with no enzymatic activity under the experimental conditions used) at MDH, MDH was omitted from the combined analysis.

In the expanded analysis, the Leather 1 and Leather 2 samples of *Perideridia erythrorhiza* cluster together and are similar (genetic identity 0.86) to the geographically close Illinois Valley sample and to the *P. oregana* populations (Figure 2). Even in the revised analysis, genetic identities between Leather 1 and other *P. erythrorhiza* populations outside Josephine County were low (average 0.74, standard deviation 0.05), and genetic identities between Leather 1 and *P. oregana* populations were higher (average 0.86, standard deviation 0.03).

Differentiation Within *Perideridia erythrorhiza*. Isozyme analysis supported the existence of important geographic subdivision within *Perideridia erythrorhiza*. Differentiation among populations made a significant contribution to intraspecific variation in *Perideridia erythrorhiza*, contributing about half the variation (see Fst; Table 5). Dividing populations into two groups, east and west of the Cascades, did not explain *Perideridia erythrorhiza* variation as well as dividing them into three regions. When east/west regions are compared, the contribution of regional differentiation to the species (Fpt = 0.197) is less than the contribution of populations to the intraregional variation (Fsp = 0.385). When the species is divided into three or four geographic regions, regional differences make a larger contribution to intraspecific differentiation (Fpt = 0.338 and 0.337, respectively; Table 5). Dividing populations into four groups (by Counties) explains no more about the variation in *P. erythrorhiza* than when dividing populations into the three regional areas.

DISCUSSION

Genetic Variation. In the three species of *Perideridia* examined in this study, isozyme variation was greater than compared to the average perennial herbaceous plant or the animal-pollinated plant with a mixed mating system (Hamrick and Godt 1990). They were more genetically variable than the average endemic plants, and even more variable than widespread species. Within *P. erythrorhiza*, each of the three regions was more variable than typical for plants with similar lifestyles. It might seem that the variation in *P. erythrorhiza*

resulted from scoring a tetraploid plant as diploid, but even the small samples of diploid *P. oregana* were much more variable than the average widespread perennial (Hamrick and Godt 1990).

Although it is highly variable, *Perideridia erythrorhiza* appears to be somewhat inbred (*He* >> *Ho*, *F* > 1). The species is known to be capable of both outcrossing and selfing (Meinke 1998), so some degree of inbreeding is expected. It is unclear whether the inbreeding found in this species is a normal result of its mixed mating system or the result of the very small size of some populations, though the largest Klamath Falls area population (Mud Flat) also shows evidence of inbreeding.

Seed Transfer. Can seed be transferred among Klamath Falls area populations to augment declining populations and offset negative effects of inbreeding in the smaller populations? Genetic identities of the Klamath Falls area populations vary from 0.907 to 0.996. Moving seed among the most similar populations (Howard Prairie and Mud Flat, or Pelican Barn and Wampler, each pair with genetic identity > 0.99) will cause no great change. However, of greater concern is using seed from the large Mud Flat population to augment the small Pelican Barn population. Genetic identity of these populations is 0.907, higher than the genetic similarity of these populations with populations in other regions but the lowest observed among the Klamath Falls area populations tested.

If the very small size of Klamath County populations such as Wampler make their survival unlikely, then augmenting them by introducing seeds or seedlings from elsewhere may be worthwhile. Using other nearby populations is best, but using plants in the general region (e.g. Mud Flat seed for Wampler) might not be harmful, even though it would change Wampler allele frequencies. Wampler alleles would not be lost (unless they were less fit than corresponding Mud Flat alleles). They would continue to exist, mostly in heterozygotes in the resulting mixed population.

Although transferring seed within a region can be justified, seed should not be transferred between the three relatively differentiated geographic areas sampled. The Roseburg, Cave Junction, and Klamath Falls area gene pools are distinct should be kept separate.

Identity of the Leather Population. We identify the Leather 1 population as *Perideridia erythrorhiza*, but questions remain. The Leather 1 and Leather 2 populations cluster together (Figure 2). However, they share a larger genetic group with the third Cave Junction area *P. erythrorhiza* population, Illinois River, and also with the two populations of *P. oregana*. The Leather 1 and 2 samples were collected in an area where *P. erythrorhiza* and *P. oregana* grow in close proximity. Leather 1 and 2 allele frequencies were still somewhat divergent from other *P. erythrorhiza* populations, with PGI allele frequencies closer to those of *P. oregana* and ME allele frequencies closest to those of *P. gairdneri*. Their low inbreeding coefficients (F = 0.004 and 0.061; Table 2) are anomalous, and indicate higher levels of outcrossing in these populations. If the Leather 1 and 2 collection are simply mixed collection of *P. erythrorhiza* and *P. oregana,* we would expect that allele frequencies in all loci would be intermittent between *P. erythrorhiza* and *P. oregana,* but they are not. It is possible that the Leather 1 and 2 populations include F1 hybrids or backcrosses, or have allele frequencies shifted by introgression from *P. oregana.* Alternatively, they may be simply divergent *P. erythrorhiza* populations.

If the status of Leather populations of *P. erythrorhiza* is addressed again, perhaps a different genetic marker should be used. Alternatives might include DNA markers like

nucleotide sequences, organelle haplotypes, or AFLPs. In the aneuploid *Perideridia* complex, ploidy could be determined by flow cytometry.

Taxonomy. Should *Perideridia erythrorhiza* be split taxonomically? Isozyme analysis alone cannot answer that question, but isozyme analysis provides evidence on that issue. Speciation may occur with little or no isozyme differentiation, so that populations of different, closely related species are no more different than those within either species. On the other hand, great differentiation in the enzymes studied in this kind of analysis can occur within a species, especially if some of the populations are isolated geographically (Crawford 1989). Some generalizations can be made. Populations within one species usually have genetic identities (Nei 1978) greater than 0.90, though they may be lower. Genetic identities among populations of different, closely related (congeneric) species tend to be lower, averaging 0.67, but can be very low (e.g. 0.25) or extremely high (>0.99). Populations of different geographic races or named subspecies or varieties usually have genetic identities greater than 0.90, but their identities may be much lower (Crawford 1989). Genetic identities of *Perideridia erythrorhiza* populations from different regions are low, averaging 0.75 to 0.86 (Table 5), comparable to the genetic identities between *P. erythrorhiza* populations and *P. oregana* (average 0.83).

The suggestion has been made on morphological and phenological grounds (Meinke 1998) to split *Perideridia erythrorhiza* into two groups, east and west of the Cascades. This would split the Douglas County + Josephine County populations from the Klamath Falls area populations (Jackson + Klamath Counties). This two-way split does not explain the isozyme variation observed as fully as does a three-way split. In a two-way split, the contribution of region to variation is relatively low (Fpt ~ 0.20). In a three-way split (Douglas County vs. Josephine County vs. Klamath Falls area Counties), the contribution of regions is higher (Fpt = 0.38). The genetic identities of the three groups with each other are all moderately low (0.75 to 0.86). Meinke's (1998) study found differences among all three of these regions (Table 6), and a PCA of morphological traits found four clusters, corresponding to the Douglas County, Josephine County, Klamath County, and Jackson County populations.

Does this mean that any taxonomic split within *P. erythrorhiza* must divide the species into three or four units? Not necessarily. The decision to recognize clusters of *P. erythrorhiza* as species or subspecies depends on a preponderance of biological evidence, including isozymes, morphology, and phenology, organized in line with the human need to create unambiguous mutually exclusive categories (Hey 2001). If taxonomists working with this group feel that the morphological and other differences that distinguish populations from the Klamath Falls area warrant taxonomic recognition, the low genetic identities of these populations compared to the others supports that decision. A two-way split would leave the western species with two divergent clusters of populations (Douglas and Josephine Counties) but that is not unprecedented.

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Table 1. Description of *Perideridia* populations studied. 'Year' = year of seed collection. E/W refers to populations located east or west of the Cascades. Elevation in feet. N = population size in thousands: estimates from Meinke (1998), except for the Wampler population, estimated by P. Berrang, and Pelican Barn, estimated by Sarah Malaby.

Project	Year	Population	TRS	County	Area	E/W	Elev.	N
P. erythrorhiza								
Pj.78	1999	Howard Prairie	T38S R3E S 36	Jackson	Klamath Falls	E	4,200	3–4
Pj.78	1999	Mud Flat	T40S R5E S 14/24	Klamath	Klamath Falls	E	4,170	>250
Pj.78	1999	Pelican Barn	T36S R6E S 9	Klamath	Klamath Falls	E	4,150	50
Pj.78	1999	Illinois River	T38S R8W S 20/29	Josephine	Cave Junction	W	1,300	25–50
Pj.78	1999	Leather 1	T38S R8W S 30	Josephine	Cave Junction	W	1,450	75–100
Pj.78	1999	Umpqua River	T26S R5W S 11	Douglas	Roseburg	W	830	2–4
Pj.78	1999	Roseburg	T27S R5W S 7	Douglas	Roseburg	W	900	1–5
Pj.136	2002	Wampler	T36S R6E S 23	Klamath	Klamath Falls	E	4,160	0.75
Pj.136	2002	Leather 2	T38S R8W S 30	Josephine	Cave Junction	W	1,450	75–100
P. oregana								
Pj.136	2002	Leather 3	T38S R8W S 30	Josephine	Cave Junction	W	1,450	N/A
Pj.136	2002	Odessa	T36S R6E S 24	Klamath	Klamath Falls	E	4,150	N/A
P. gairdneri								
Pj.136	2002	7-mile Guard Station	T33S R6E S 13	Klamath	Klamath Falls	E	4,200	N/A
Pj.136	2002	Fort Klamath	T33S R7.5E S15	Klamath	Klamath Falls	E	4,190	N/A

Table 2. Summary of genetic variability in *Perideridia* populations. S = number of individuals sampled; N = mean number of individuals scored per locus; P = % of all loci that are polymorphic; A = average number of alleles at all loci; 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 = fixation index, = $(H_e-H_o)/H_e$; s.d. = standard deviation.

Population	Pops	S	N	#Loci	P	A	(s.d.)	Ae	(s.d.)	Ho	(s.d.)	He	(s.d.)	F
P. erythrorhiza	**9**	**396**	**324.7**	**12**	**91.7**	**3.4**	**(1.2)**	**1.6**	**(0.6)**	**0.123**	**(0.092)**	**0.279**	**(0.228)**	**0.561**
KLAMATH FALLS AREA	**4**	**188**	**163.0**	**12**	**83.3**	**2.3**	**(0.8)**	**1.3**	**(0.4)**	**0.094**	**(0.107)**	**0.160**	**(0.192)**	**0.411**
Jackson County	**1**	**57**	**52.3**	**12**	**58.3**	**1.8**	**(0.8)**	**1.1**	**(0.3)**	**0.060**	**(0.105)**	**0.089**	**(0.155)**	**0.322**
Howard Prairie	1	57	52.3	12	58.3	1.8	(0.8)	1.1	(0.3)	0.060	(0.105)	0.089	(0.155)	0.322
Klamath County	**3**	**131**	**221.0**	**12**	**66.7**	**2.1**	**(0.9)**	**1.4**	**(0.5)**	**0.111**	**(0.123)**	**0.183**	**(0.217)**	**0.393**
Mud Flat	1	61	55.2	12	50.0	1.7	(0.8)	1.2	(0.4)	0.103	(0.126)	0.144	(0.192)	0.281
Pelican Barn	1	37	31.7	12	50.0	1.5	(0.5)	1.3	(0.4)	0.126	(0.224)	0.144	(0.210)	0.125
Wampler	1	33	23.8	12	33.3	1.4	(0.7)	1.2	(0.4)	0.106	(0.181)	0.139	(0.211)	0.239
CAVE JUNCTION AREA														
Josephine County	**3**	**114**	**81.2**	**12**	**83.3**	**3.1**	**(1.3)**	**1.5**	**(0.6)**	**0.217**	**(0.181)**	**0.276**	**(0.233)**	**0.213**
Illinois River	1	42	30.9	12	66.7	2.4	(1.4)	1.3	(0.4)	0.133	(0.161)	0.170	(0.201)	0.216
Leather 1	1	41	32.4	12	50.0	2.0	(1.2)	1.6	(0.8)	0.232	(0.278)	0.233	(0.281)	0.004
Leather 2	1	31	19.5	11	81.8	2.2	(0.9)	1.6	(0.4)	0.359	(0.318)	0.383	(0.291)	0.061
ROSEBURG AREA														
Douglas County	**2**	**94**	**80.4**	**12**	**58.3**	**2.0**	**(1.2)**	**1.3**	**(0.5)**	**0.077**	**(0.092)**	**0.173**	**(0.222)**	**0.555**
Roseburg	1	50	45.8	12	50.0	1.8	(1.0)	1.3	(0.4)	0.090	(0.124)	0.146	(0.206)	0.382
Umpqua River	1	44	34.6	12	50.0	1.6	(0.7)	1.2	(0.3)	0.047	(0.089)	0.108	(0.167)	0.561
P. oregana	**2**	**56**	**36.1**	**12**	**83.3**	**2.9**	**(1.2)**	**1.9**	**(0.9)**	**0.256**	**(0.206)**	**0.375**	**(0.267)**	**0.318**
Odessa	1	29	12.3	11	72.7	2.4	(1.2)	1.7	(0.8)	0.274	(0.231)	0.342	(0.273)	0.200
Leather 3	1	27	24.8	12	83.3	2.8	(1.2)	1.9	(0.9)	0.261	(0.211)	0.362	(0.267)	0.281
P. gairdneri	**2**	**12**	**7.6**	**10**	**60.0**	**1.8**	**(0.8)**	**1.2**	**(0.2)**	**0.130**	**(0.151)**	**0.147**	**(0.143)**	**0.116**
Ft. Klamath	1	6	5.2	10	50.0	1.6	(0.7)	1.2	(0.3)	0.140	(0.198)	0.174	(0.203)	0.197
7-mile Guard Sta.	1	6	3.4	7	28.6	1.4	(0.8)	1.2	(0.3)	0.129	(0.222)	0.115	(0.197)	-0.116

Table 3. Allele frequencies of loci that distinguish the three *Perideridia* species examined in this study. See text of Methods for enzyme abbreviations. **Boldface** frequencies are those most common in the species.

Locus	Allele	*P. erythrorhiza*	*P. oregana*	*P. gairdneri*
ADH	1	0.1739	0.1538	
ADH	2	**0.6902**	**0.3846**	
ADH	3	0.0326		no data
ADH	4	0.1033	**0.4615**	
ADH	5			
ME	1	**0.6909**	0.1771	
ME	3	0.0247	0.0833	
ME	4	0.0299	**0.4062**	**0.9167**
ME	5	0.0078		
ME	6	0.2468	**0.3333**	0.0833
6PGD	1	**0.3305**	0.0909	
6PGD	2	0.2514	**0.7273**	no data
6PGD	3	**0.4181**	0.1818	
PGI	1	**0.8532**	**0.5224**	0.0455
PGI	2	0.0899	0.2463	
PGI	3	0.0468	0.1791	**0.9091**
PGI	4	0.0101	0.0522	0.0455
PGM1	1	0.2392	**0.4143**	**0.8000**
PGM1	2	**0.6225**	**0.2143**	0.2000
PGM1	3			
PGM1	4	0.0821	0.1571	
PGM1	5	0.0533	0.1857	
PGM1	6	0.0029	0.0286	
PGM2	1	**0.9719**	**0.7973**	
PGM2	4	0.0033	0.0270	**1.0000**
PGM2	5	0.0248	0.1757	

Table 4. Nei's unbiased genetic identities (Nei 1978), based on 12 isozyme loci, among species of *Perideridia* species and geographic areas of *P. erythrorhiza*. GI = genetic identity. s.d. = standard deviation.

Unit	compare	Unit	Average GI	s.d.
P. erythrorhiza	to	*P. erythrorhiza*	0.828	(0.085)
P. oregana	to	*P. oregana*	0.910	
P. gairdneri	to	*P. gairdneri*	0.779	
P. erythrorhiza	to	*P. oregana*	0.832	(0.050)
P. erythrorhiza	to	*P. gairdneri*	0.483	(0.148)
P. oregana	to	*P. gairdneri*	0.616	(0.162)
Cave Junction Area	to	Cave Junction Area	0.877	(0.039)
Roseburg Area	to	Roseburg Area	0.910	
Klamath Falls Area	to	Klamath Falls Area	0.941	(0.044)
Cave Junction Area	to	Roseburg Area	0.773	(0.042)
Cave Junction Area	to	Klamath Falls Area	0.764	(0.080)
Roseburg Area	to	Klamath Falls Area	0.846	(0.048)
Klamath County	to	Klamath County	0.935	
Josephine County	to	Klamath County	0.831	
Josephine County	to	Jackson County	0.770	
Douglas County	to	Klamath County	0.894	
Douglas County	to	Jackson County	0.838	
Klamath County	to	Jackson County	0.981	
Cave Junction Area	to	*P. oregana*	0.901	
Roseburg Area	to	*P. oregana*	0.870	
Klamath Falls Area	to	*P. oregana*	0.811	
Klamath County	to	*P. oregana*	0.842	
Jackson County	to	*P. oregana*	0.780	

Table 5. Wright's F statistics for a three level sampling hierarchy of populations within regions within *Perideridia erythrorhiza*, based on 12 isozyme loci. Two regions: east (Klamath Falls Area) and west (Cave Junction Area + Roseburg Area). Three regions: Cave Junction Area, Roseburg Area, and Klamath Falls Area. Four regions: Josephine, Douglas, Klamath, and Jackson Counties.

F	Level	within	level	2 regions	3 regions	4 regions
Fis	Individuals	within	populations	0.233	0.233	0.233
Fip	Individuals	within	regions	0.528	0.432	0.345
Fit	Individuals	within	species	0.621	0.624	0.611
Fsp	Populations	within	regions	0.385	0.260	0.185
Fst	Populations	within	species	0.506	0.510	0.493
Fpt	Regions	within	species	0.197	0.338	0.337

Table 6. Morphological, ecological, and phenological traits of *Perideridia erythrorhiza* in three regions, from Meinke (1998).

Trait	**Klamath Falls Area**	**Roseburg Area**	**Cave Junction Area**
Elevation	4182 ft.	677 ft.	1375 ft.
Flowering date	July/August	July/August	August/September
Fruit set date	early	early	late
Seed set	lower	highest	high
# of umbels/plant	lower	higher	higher
leaf/shoot ratio	higher (=/> 0.18)	lower (=/< 0.5)	lower (=/< 0.5)
root weight	higher (=/> 4.5g)	lower (=/ 4.4g)	lower (=/<3.9g)
shoot/root ratio	smaller (=/> 0.83)	higher (=/.0.92)	higher (=/> 0.95)
tubers	lack a neck	goose-neck	
longest tubers	7.8 cm	14.6 cm	
tuber inner epidermal surfaces	off-white to pale chestnut brown	brick-red to cherry-red	brick red to cherry red
tuber sprout dates	earlier	later	
growth in 'fridge	longer	shorter	
PCA infl. traits	less floriferous	more floriferous	more floriferous
PCA root morphology	more massive, shorter, few	less massive, longer, more numerous	less massive, longer, more numerous
fruit shape	longer, larger*	smaller, ovoid	longer, larger

*Jackson County fruits smaller, ovoid

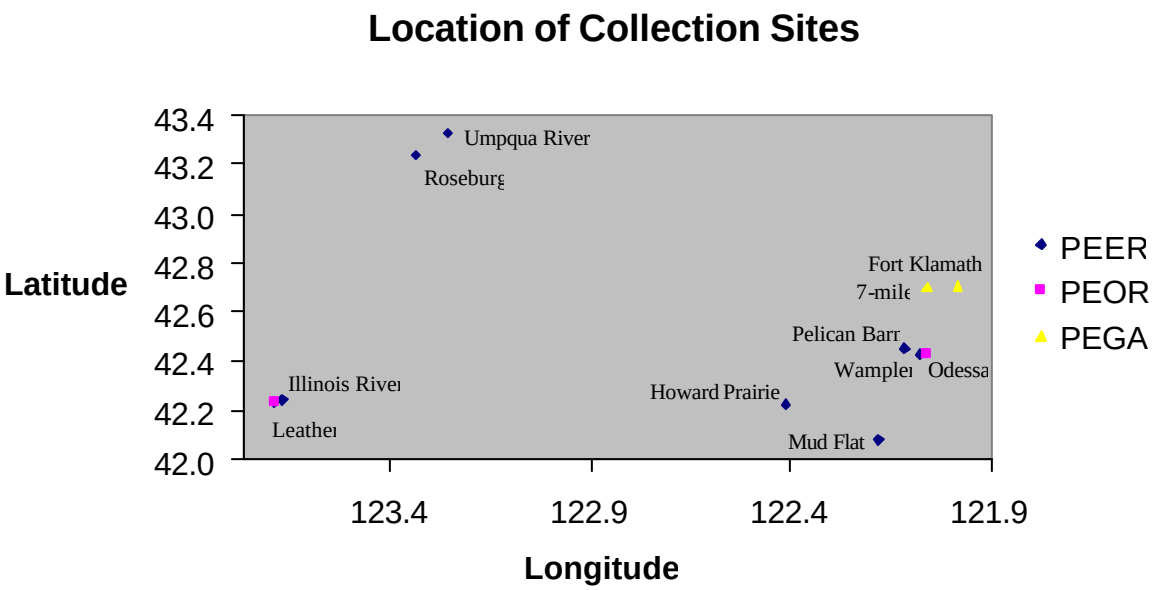
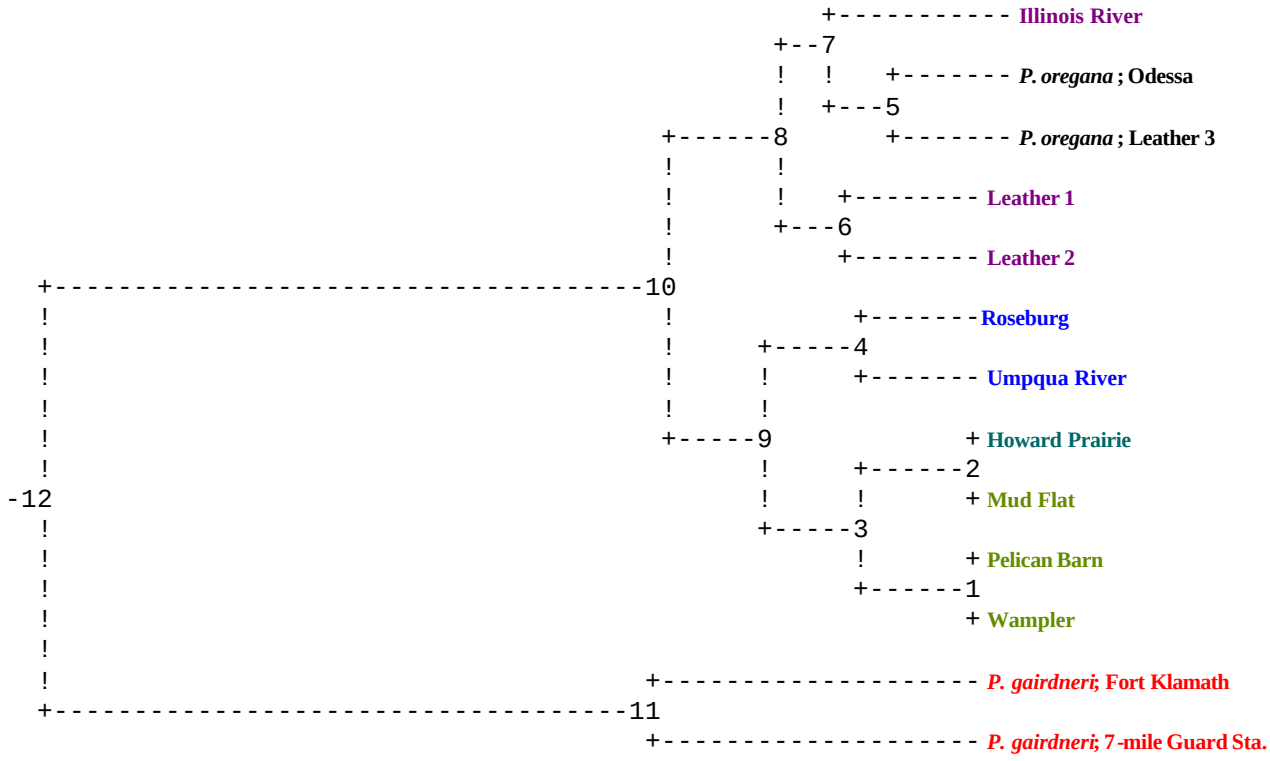


Figure 1. Location by latitude and longitude of *Perideridia* collection sites.

Figure 2. Similarities among *Perideridia* populations, based on Nei's genetic identities, calculated using 12 isozyme loci (AAT ADH GDH ME 6PGD2 PGI2 PGM1 PGM2 TPI1 TPIf TPIs UGPP2).

P. erythrorhiza; Cave Junction Area
    Illinois River
    Leather 1
    Leather 2
P. erythrorhiza; Roseburg Area
    Roseburg
    Umpqua River
P. erythrorhiza; Klamath Falls area; Jackson County
    Howard Prairie
P. erythrorhiza; Klamath Falls area; Klamath County
    Mud Flat
    Pelican Barn
    Wampler
P. gairdneri
    7-mile Guard Sta.
    Fort Klamath
P. oregana
    P. oregana; Leather 3
    P. oregana; Odessa



Implications of Isozyme Variation for the Taxonomy of the Rare California Plant *Silene campanulata ssp. campanulata*

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ABSTRACT

Silene campanulata is an uncommon, morphologically variable species generally considered to include two subspecies, *S. campanulata* ssp. *campanulata* and *S. campanulata* ssp. *glandulosa*. *Silene campanulata* ssp. *campanulata*, thought to be endemic to ultramafic (serpentine) substrates, is listed as an endangered plant in California. Populations morphologically intermediate between the two subspecies have called into question the taxonomic validity of the subspecies. In genetics as in morphology, *S. campanulata* is variable, consisting of individuals with a bimodal distribution of genotypes. One extreme consists of plants that contain genetic similarity to known individuals of what is considered *S. campanulata* ssp. *glandulosa*, while the other extreme are those plants genetically similar to *S. campanulata* ssp. *campanulata*. A significant proportion of individuals belong to neither extreme and are instead genetic intermediates (much like the morphological intermediates that are observed). Most Trinity County populations are a mix of the genetic extremes and their intermediates. Both glandulosa-type and campanulata-type individuals are found on ultramafic soils, whereas metamorphic substrates seem to favor those individuals that show genetic similarity to *S. campanulata* ssp. *glandulosa*. Isozyme variation does not clearly support the division of *S. campanulata* into subspecies, at least in Trinity County, California. The variation observed may be reflective of (1) geographic variants of a single species, or (2) a rapidly evolving lineage where morphological characters and basic metabolic enzymes used in isozyme analysis do not adequately track their relationships. Although both morphology and genetics are indicating the presence of two extremes within the species, the existence of a significant proportion of intermediate individuals and populations makes the subspecific designation less valid for management purposes.

INTRODUCTION

The uncommon species *Silene campanulata* S. Watson (1875) consists of two subspecies restricted to northwest California and southwest Oregon (Wilken 1993). *Silene campanulata* ssp. *glandulosa* C. Hitchcock & Maguire (1947) is uncommon but by no means rare, growing on non-serpentine substrates throughout the species range. *Silene campanulata* ssp. *campanulata* was at one time considered limited to serpentine habitats on Red Mountain (Mendocino County) were its survival has been threatened by mining activity, and in Colusa County. This subspecies is a listed Endangered species in California (California Department of Fish and Game 1999) and was at one time a candidate for federal listing (Anonymous 2000).

Recent discoveries have challenged the Endangered status of *Silene campanulata* ssp. *campanulata* in two ways. First, the species may be more widespread than originally thought. Plants from Trinity County, California, collected by Julie Nelson in 1996 and submitted to J. K. Morton for verification have extended its known range. Subsequently, more than 15 populations identified as *S. campanulata* ssp. *campanulata* have been recorded in the Shasta Trinity National Forest, covering over 30 acres geographically and containing over 1400 individuals. Second, morphologically intermediate individuals and populations call into question the validity of the taxa. Distinctions between *S. campanulata* ssp. *campanulata* and *S. campanulata* ssp. *glandulosa* are slight (Table 1). Typically, *S. campanulata* ssp. *campanulata* are short, narrow-leaved, glabrous plants of serpentine substrates, and *S. campanulata* ssp. *glandulosa* are taller, broad-leaved, glandular pubescent

plants of non-serpentine substrates. Many populations seem to have characteristics of both subspecies, with great overlap in morphological characteristics. The greatest problems observed on the Shasta-Trinity National Forest are (1) individual plants with intermediate size characteristics and 10 mm leaf width, (2) glandular individuals that grow on serpentine substrates or that otherwise fit the description of *S. campanulata* ssp. *campanulata*, and (3) individuals with the characteristics of *S. campanulata* ssp. *campanulata* found growing on what are clearly granitic (non-serpentine) substrates.

This isozyme analysis of *Silene campanulata* populations was initiated to address two questions. First, does isozyme analysis provide evidence to support or refute the hypothesis that *S. campanulata* ssp. *campanulata* and *S. campanulata* ssp. *glandulosa* are valid taxa? Second, assuming that they are valid taxa, how should ambiguous populations from the Shasta-Trinity National Forest be identified?

METHODS

Sampling. Samples consisting of three to five leaves per plant were collected in the Shasta-Trinity National Forest in Trinity County, California (Table 2). Certain of these population represented subpopulations collected within ¼ mile of each other, within metapopulations. Specifically, populations 1, 2, and 3 were subpopulations of metapopulation 5140010, populations 5 and 6 were subpopulations of metapopulation 5140009, and populations 7 and 8 belong to metapopulation 5140008 (Table 2). Comparative material from two populations of known *S. campanulata* ssp. *campanulata* and two of known *S. campanulata* ssp. *glandulosa* were collected in Mendocino, Del Norte, and Humboldt Counties (Table 2). Leaves from each plant were bagged separately, and samples were shipped on ice to NFGEL.

Tissue Preparation. Three to five leaves per individual were ground in a mortar using liquid nitrogen. Approximately 0.4 ml of Tris buffer, pH 7.5 (Gottlieb 1981) was added to the ground power and mixed. As the resulting tissue mass thawed, a 120 µl/sample of slurry was transferred to a microtiter plate well, and a total of three replicate plates were made. Plates were stored at -70°C. On the morning of electrophoresis, the samples were thawed and soaked onto 3mm wide wicks made of Whatman 3MM chromatography paper.

Electrophoresis. Methods of sample preparation and electrophoresis follow the general methodology of Conkle et al. (1982), with some modifications (USDA Forest Service 2003). 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 aconitase (ACO), leucine aminopeptidase (LAP), malic enzymes (ME), phosphoglucose isomerase (PGI), and phosphoglucomutase (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 aspartate aminotransferase (AAT), catalase (CAT), glutamate dehydrogenase (GDH), 6-phosphogluconate dehydrogenase (6-PGD), triosephosphate isomerase (TPI), and uridine diphosphoglucose pyrophosphorylase (UGPP). A morpholine citrate electrode and gel buffer (pH 7) (USDA Forest Service 2003) was used to resolve diaphorase (DIA), fluorescent esterase (FEST), isocitrate dehydrogenase (IDH), malate dehydrogenase (MDH), phosphogluconate dehydrogenase (6PGD), and shikimate dehydrogenase (SKD). Enzyme stain recipes follow USDA Forest Service (2003). Two loci were resolved for TPI, PGM, and UGPP, and three were resolved for MDH, for a total of 20 loci. Eighteen of the 20 loci (excluding FEST and GDH) consistently resolved in all samples.

For quality control, 10% of the individuals were run and scored twice. Gels were photographed, and the photographs consulted to resolve quality control issues. 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). We are unaware of a chromosome count for *Silene campanulata* ssp. *campanulata,* but *S. campanulata* ssp. *glandulosa* has 2n = 48 (Wilken 1993) and is presumably tetraploid. Isozyme band patterns indicated that *S. campanulata* ssp. *campanulata* is an autopolyploid.

Data Analysis. Basic measures of genetic variability and Nei's (1973) genetic identity were calculated from autotetraploid genotypes using PyPolyPloid version 0.91 (Groth 2003). Hypotheses tests and first ordinations were done by canonical analysis (also known as canonical variate analysis and canonical discriminant analysis) using SAS' PROC CANDISC. Classification and probabilities of membership was done by discriminant analysis (SAS PROC DISCRIM). Classification probabilities were computed by the Bayesian method. Graphical summaries of the data were done in SAS' JMP.

RESULTS

Genetic Diversity. *Silene campanulata* is a genetically variable species, with 79% polymorphic loci and 3.1 alleles per locus (Table 3). Individual populations were also variable, except for the small 28N48A Road population, which had 21% polymorphic loci (Table 3). Red Mountain ssp. *campanulata* and the Humboldt and Del Norte County ssp. *glandulosa* were also variable. Samples of *S. californica* and *S. lemmonii* used in this study were less variable than all *S. campanulata* samples except for 28N48A Road. The lower variation is likely because of the small sample sizes in these two taxa (n = 10 and 8, respectively).

Isozyme variation indicates that there are two genetic extremes among individuals of *S. campanulata* (Figure 1). One extreme contains known ssp. *glandulosa* individuals (as determined by morphological characterization); the other extreme encompasses those individuals that are clearly morphological ssp. *campanulata.* Approximately 45% of the individuals studied have the genetic make-up of the 'glandulosa' group, roughly 30% of the individuals have the genetics of the 'campanulata' group, and the remaining 25% are genetic intermediates sharing similarity to both genetic extremes.

Twelve of the 16 populations of *S. campanulata* studied contain a mix of individuals identifies as the glandulosa-type, campanulata-type, or genetic intermediate (Figure 2). Three populations (Diamond Creek (D), 28N48A Road (9), and Post Mountain (17)) are pure glandulosa-type populations (all individuals have the genetic make-up of the glandulosa group). Only one population, Red Mountain-Mendocino (M2), contained all individuals belonging to the campanulata group. The majority of populations contained individuals that were not clearly either glandulosa or campanulata, but instead had genetic variation in common with each group (indicated with yellow in Figure 2). Therefore, the populations that are strongly comprised of glandulosa-type individuals are: Diamond Creek (D), 28N48A Road (9), Post Mountain (17), Red Mountain-Humboldt (H), New River Trail (16), Rusch Creek (15), and 29N48 Road (3). Those populations that are strongly comprised of campanulata-type individuals are Red Mountain-Mendocino (M1 and M2). Seven populations are mixes of both extremes and their genetic intermediates: Mud Spring (1),

Smoky Creek (2), Red Mountain-Trinity (5), Bramlet Road (6), Wild Mad Road (7), Prospect Creek (8), and Graves Creek Rd (11).

Association With Soil Substrate. In general, the populations growing on ultramafic substrates appear slightly more variable than those from metamorphic substrates (Table 3). Ten alleles at seven loci (AAT-s allele C, FEST allele E, GDH allele B, LAP allele C, LAP allele E, MDH-m allele C, MDH-s allele B, MDH-s allele D, TPI-f allele A, and TPI-f allele D) were observed only in populations from ultramafic substrates (Table 5). No alleles were confined to metamorphic substrates. The alleles observed only in populations from ultramafic substrates were uncommon to rare (frequencies averaging 0.056/population, s.d. 0.059). These alleles occurred in 1 to 8 (average 3.2) of the 11 populations from ultramafic substrates. Each population on ultramafic substrate had a least one of these alleles. It is tempting to relate the higher observed variation on ultramafic substrates to adaptations to those substrates, but it may be an artifact of the sampling design. Not only were 2.75 times as many populations sampled on ultramafic as metamorphic substrates, but sample size per population was 1.5 times larger on ultramafic as on metamorphic substrates.

Both the glandulosa-type and campanulata-type genetic individuals were found to grow in roughly equal numbers on ultramafic soils (Figure 3A). A range of genetic intermediates were also detected on the ultramafic substrate. This contrasts sharply with metamorphic soils. Metamorphic substrates tend to strongly favor the presence of glandulosa-type individuals (Figure 3B), with a lower proportion of campanulata-type plants and genetic intermediates. Neither substrate is unique to a given genetic extreme.

Species Comparisons. *Silene lemmonii* was distinguished from the *S. campanulata / S. californica* pair by fixed differences at five loci (GDH, MDH-m, PGM-f, TPI-s, and UGPP-s) and extreme differences in allele frequencies at six additional loci. Therefore, genetic identities between *S. lemmonii* and the other populations are very low (<73% genetic similarity; Table 4), as would be expected when comparing different species.

S. campanulata and S. californica share a high degree of genetic similarity. These two species share 97.5% genetic similarity (Table 4). Except in three loci, the same allele was most common in all populations of the *Silene campanulata / S. californica* pair. Exceptions included single populations of *S. campanulata* for PGM-f and PGM-s. For PGI-f, which was highly variable and therefore had low frequencies for each allele, the most common allele in *S. californica* was different than in *S. campanulata* (Table 5).

Within *Silene campanulata,* genetic identities based on tetraploid genotypes were high, averaging 0.978 (Table 4). Genetic identities between the one *S. californica* population and *S. campanulata* populations were nearly as high, averaging 0.975. Closer examination of the *S. campanulata / S. californica* pair reveals no pattern related to substrate, subspecific identification (or even species identification), or metapopulation.

DISCUSSION

Taxonomic divergence is generally related to isozyme differentiation. Intraspecific populations, and some subspecies, usually have genetic identities (based on isozymes) above 0.90. Members of different, closely related species usually have much lower genetic identities, averaging 0.68 though varying from 0.25 to 0.99. Distinct species are usually (but not always) distinguished by fixed differences at some isozyme loci (Crawford 1989).

Differentiation among *Silene campanulata* and *S. lemmonii* populations is consistent with these trends. All *S. campanulata* populations are similar (genetic identity > 0.94), and

their similarity with *S. lemmonii* is much lower (averaging 0.70). Fixed differences at five loci distinguished *S. lemmonii* from *S. campanulata.* Surprisingly, the *S. californica* population sampled is nearly as similar to the *S. campanulata* populations as they are to each other (genetic identities averaging above 0.97). This suggests that *S. campanulata* and *S. californica* may be part of a rapidly evolving lineage in which morphology may be diverging faster than the basic metabolic enzymes studied in isozyme analysis.

Like the morphological evidence, the isozyme evidence on the validity of the *Silene campanulata* subspecies is equivocal. The unambiguously identified samples of *S. campanulata* ssp. *campanulata* from Mendocino County are relatively different from the unambiguously identified *S. campanulata* ssp. *glandulosa* from Humboldt and Del Norte Counties (genetic identities averaging 0.96; Figure 2). Isozyme analysis shows that the *S. campanulata* individuals are predominantly separated into one of two genetic groups (see Figure 1). One of the groups contains the *S. campanulata* ssp. *glandulosa* from Humboldt and Del Norte Counties, and we refer to it as the glandulosa group. The other group, the campanulata group, contains the *S. campanulata* ssp. *campanulata* from Mendocino County. A significant proportion of the individuals analyzed are genetic intermediates, sharing genes with both groups. Most (but not all) of the ambiguous Trinity County samples that are the focus of this study are mixes of individuals belonging to the glandulosa group, campanulata group, and the genetic intermediates (see Figure 2).

In isozymes, as in morphology, the Trinity County plants are intermediate. Genetics, like morphology, suggests that variation in *Silene campanulata* does not meet our human need for neat, mutually exclusive taxon categories (Hey 2001). For the subspecies designation to be informative, individuals need to be reliably classified into one of the two taxa. This is not usually possible in the Trinity County *S. campanulata.* The species instead shows a range of both morphological and genetic variants between two extremes. This suggests that (1) the subspecies designation is not valid in this species and we are instead looking at a single species reflecting geographic variants, or (2) that we are seeing the beginning of the subspeciation process (geographic segregates that exhibit a trend toward speciation, perhaps in part being driven by a metamorphic habitat favoring glandulosa-type individuals).

Despite what names we choose to associate with these *Silene campanulata* populations, we need to maintain their existing capacity to respond to habitat change by preserving their known morphological and genetic diversity. Such a plan would preserve some populations with narrow, eglandular leaves as well as some of the more common populations with broad, glandular leaves. It would maintain populations on ultramafic sites (and thus their possibly unique rare alleles) as well as populations on metamorphic substrates (which tend to favor glandulosa-type individuals).

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Table 1. Characteristics of taxonomic importance in subspecies of *Silene campanulata* (Wilken 1993).

Subspecies	Size	Stem	Leaf	Habitat	Elevation
campanulata	5-20 cm	puberulent	1.5-4 cm 2-10 mm wide, linear to lanceolate	Serpentine (ultramafic), chaparral, coniferous forest	500-1000 m.
glandulosa	15-40 cm	glabrous to puberulent; glandular or not	1-3(5) cm, 10-30 mm wide, lanceolate to +/- round	Non-serpentine (metamorphic), open or shaded areas, coniferous forest	300-1900 m.

Table 2. Populations sampled for this isozyme study of *Silene campanulata* in northwest California. Taxa (as identified morphologically during field collection) are *S. campanulata* ssp. *campanulata* (S.c.s.camp), *S. campanulata* ssp. *glandulosa* (S.c.s.gland), *S. californica*, and *S. lemmonii*. Code = population designation. Metapop = metapopulation identifier. N = number of individuals sampled. Soil Type = M, metamorphic; U, ultramafic. Samples in Trinity County were collected by Susan Erwin, USDA Forest Service. Those in other counties were collected by David Imper, US Fish and Wildlife Service.

Taxon	Code	Metapop	Collection Date	N	Soil Type	County	Location	TRS	Elev. (m)	Pop. Size
S. c. s. camp.?	1	05140010	30-May-02	30	U	Trinity	Mud Spring	T29N R12W S13	1600	10 acre
S. c. s. camp.?	2	05140010	30-May-02	30	M	Trinity	Smoky Creek	T29N R12W S23	1300	1 acre
S. c. s. camp.?	3	05140010	30-May-02	30	U	Trinity	29N48 Road	T29N R12W S23	1460	1 acre
S. c. s. camp.?	5	05140009	30-May-02	25	U	Trinity	Red Mt. (Trinity Co.)	T28N R11W S05	1600	2 acre
S. c. s. camp.?	6	05140009	30-May-02	29	U	Trinity	Bramlet Road	T28N R11W S08	1450	50 pl.
S. c. s. camp.?	7	05140008	30-May-02	25	U	Trinity	30 Road (Wild Mad Road)	T28N R11W S04	1460	0.5 acre
S. c. s. camp.?	8	05140008	30-May-02	12	U	Trinity	Prospect Creek	T28N R11W S04	1460	0.25 acre
S. c. s. camp.?	9	05140007	30-May-02	13	M	Trinity	28N48A Road	T28N R11W S09	1360	10 pl.
S. c. s. gland.	11	05140004	30-May-02	23	U	Trinity	Graves Creek Road	T39N R07W S35	939	10 pl.
S. c. s. camp.?	15	05140999	30-May-02	30	U	Trinity	2N39C Road & Rusch Cr.	T02N R08E S08	1040	20 pl.
S. c. s. gland.	16	05140500	7-May-03	7	M	Trinity	E. Fk. New River Trail	T07N R08E S18	660	10 pl.
S. c. s. gland.	17	05140501	7-Jul-03	15	M	Trinity	Post Mountain	T30N R12W S18	1200	15 pl.
S. c. s. camp.	M1	----------	26-Jun-02	15	U	Mendocino	Red Mt. (Mendocino Co.)	T24N R16W S20	1220	>500 pl.
S. c. s. camp.	M2	----------	26-Jun-02	28	U	Mendocino	Red Mt. (Menodcino Co.)	T24N R16W S19	1230	>500 pl.
S. c. s. gland.	H	----------	28-Jun-03	26	U	Humboldt	Red Mt. (Humboldt Co.)	T01S R05E S14	1420	>1000 pl.
S. c. s. gland.	D	----------	10-June-03	24	U	Del Norte	Diamond Creek	T19N R02E	420	>500 pl.
S. californica	cal	05140504	13-Jul-03	10	M	Trinity	Brown's Mountain Road	T33N R02W S20	600	30 – 100 pl.
S. lemmonii	lem	05140503	13-Jul-03	8	U	Trinity	Graves Cr. Road	T39N R07W S35	989	8 pl.

Table 3. Genetic diversity statistics for *Silene campanulata* and relatives. Code = population designation. N = number of plants sampled; N* = average number of plants providing data for each locus. %P = percent polymorphic loci. A = average number of alleles per locus. Ho = observed heterozygosity.

Code	Population	N	N*	%P	A	Ho
1	Mud Spring	30	27.5	63	2.1	0.228
2	Smoky Creek	30	27.3	58	2.1	0.235
3	29N48 Road	30	28.3	68	2.5	0.311
5	Red Mt. (Trinity)	25	22.1	63	2.2	0.279
6	Bramlet Road	29	23.5	68	2.2	0.251
7	Wild Mad Road (30 Road)	25	21.9	58	2.3	0.271
8	Prospect Creek	12	10.4	42	1.9	0.269
9	28N48A Road	13	11.9	21	1.5	0.150
11	Graves Creek Rd.	23	19.0	47	2.0	0.205
15	Rusch Creek	30	26.6	63	2.1	0.287
16	E. Fork New River Trail	7	6.7	53	1.8	0.299
17	Post Mountain	15	14.0	48	1.7	0.135
M1	Red Mt. (Mendocino)	15	14.3	53	1.7	0.221
M2	Red Mt. (Mendocino)	28	25.2	63	1.8	0.253
D	Diamond Creek	24	19.0	58	1.9	0.149
H	Red Mt. (Humboldt)	26	22.3	58	2.2	0.239
Silene campanulata (entire study)		364	321.0	79	3.1	0.244
Silene campanulata (population mean)		22.8	20.0	55	2.0	0.236
ultramafic substrate		269	237.8	79	3.0	0.269
metamorphic substrate		65	60.3	79	2.6	0.216
cal	*Silene californica*	10	8.6	39	1.5	0.155
lem	*Silene lemmonii*	8	7.3	32	1.4	0.144

Table 4. Summary of genetic identities (Nei 1973) among populations of *Silene campanulata* and relatives, based on the 18 tetraploid loci that resolved in all populations.

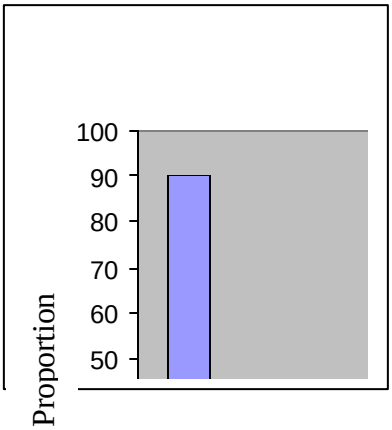
Taxon	to	Taxon	Mean	s.d.
S. campanulata	to	*S. campanulata*	0.978	(0.012)
S. campanulata	to	*S. californica*	0.975	(0.013)
S. campanulata	to	*S. lemmonii*	0.701	(0.029)
S. californica	to	*S. lemmonii*	0.724	

Table 5. *Silene campanulata* isozyme allele frequencies in relation to substrate. **Boldface** = the most common allele. *Italics* = allele confined to one substrate. * = statistically significant difference in allele frequencies between metamorphic and ultramafic substrates (p = 0.001).

Soil Type:		Metamorphic				Ultramafic											
Pop. Code:		2	9	16	17	1	3	5	6	7	8	11	15	M2	M1	D	H
Enzyme/Allele																	
AAT-S	A	**0.984**	**1.000**	**0.625**	**0.929**	**0.759**	**0.856**	**0.880**	**0.875**	**0.385**	**1.000**	**1.000**	**1.000**	**0.656**	**0.750**	**0.714**	**0.820**
AAT-S	B			0.375	0.071	0.036	0.096	0.120		0.442				0.344	0.250		0.100
AAT-S	*C*					*0.196*	*0.048*			*0.115*						*0.286*	*0.080*
AAT-S	D	0.016				0.009			0.125	0.058							
DIA	A	**0.586**	**1.000**	**0.679**	**1.000**	**0.705**	**0.741**	**0.603**	**0.750**	**0.670**	**0.591**	**0.602**	**0.714**	**0.500**	**0.550**	**0.935**	**0.826**
DIA	B	0.414		0.321		0.295	0.259	0.397	0.250	0.330	0.409	0.398	0.286	0.500	0.450	0.065	0.174
DIA	F																
FEST	A	0.035				0.013		0.011		0.045	0.023	0.063					
FEST	B	0.181		0.179		0.067	0.167	0.159		0.046	0.091		0.028				
FEST	C	**0.707**	**1.000**	**0.714**	**1.000**	**0.862**	**0.824**	**0.796**	**0.968**	**0.909**	**0.818**	**0.875**	**0.805**	**0.875**	**0.967**		
FEST	D	0.078		0.107		0.057	0.009	0.034	0.032		0.045	0.062	0.167	0.125	0.033		
FEST	*E*					*0.001*					*0.023*						
GDH	A	**1.000**	**1.000**	**1.000**	**1.000**	**0.978**		**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**
GDH	*B*					*0.022*											
GDH	C																
GDH	D																
GDH	E																
IDH	A*	**0.767**	**0.909**	**0.893**	**0.928**	**0.710**	**0.724**	**0.709**	**0.656**	**0.570**	**0.536**	**0.750**	**0.892**	**0.500**	**0.714**	**0.990**	**0.590**
IDH	B	0.181	0.023	0.107	0.036	0.290	0.206	0.208	0.302	0.390	0.429	0.250	0.054	0.500	0.286		0.390
IDH	C		0.068		0.036		0.069	0.083	0.021	0.020	0.035		0.054			0.010	0.020
IDH	F	0.052							0.021	0.020							
LAP	A	**1.000**	**1.000**	**0.750**	**0.938**	**0.931**	**0.897**	**0.990**	**1.000**	**1.000**	**1.000**	**1.000**	**0.892**	**0.875**	**1.000**	**0.826**	**0.979**
LAP	B			0.071	0.063	0.052	0.034	0.010					0.058	0.125		0.011	
LAP	*C*															*0.043*	
LAP	*E*					*0.017*											
LAP	F			0.179			0.069						0.050			0.120	0.021
MDH-f1	A	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**
MDH-F2	B	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**
MDH-m	A				0.063		0.050		0.018				0.017	0.143			
MDH-m	B	**1.000**	**1.000**	**1.000**	**0.937**	**1.000**	**0.950**	**1.000**	**0.982**	**1.000**	**1.000**	**1.000**	**0.983**	**0.857**	**0.900**	**0.979**	**1.000**
MDH-m	*C*														*0.100*	*0.021*	
MDH-m	F																
MDHS	A	0.026	0.192	0.143		0.052	0.158	0.312	0.411	0.110	0.068	0.057	0.225	0.009			0.010
MDHS	*B*						*0.008*		*0.134*			*0.080*	*0.058*				
MDHS	C	**0.974**	**0.808**	**0.857**	**1.000**	**0.948**	**0.817**	**0.688**	**0.455**	**0.830**	**0.909**	**0.852**	**0.667**	**0.991**	**1.000**	**1.000**	**0.990**
MDHS	*D*						*0.017*			*0.060*	*0.023*	*0.011*	*0.050*				
ME	A	**0.982**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**0.911**	**1.000**	**1.000**	**1.000**
ME	B	0.018												0.089			
PGD-f	A	**0.967**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**0.578**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**0.848**	**0.980**
PGD-f	B	0.033						0.230	0.422							0.152	0.020
PGD-s	A	**0.850**	**1.000**	**0.917**	**1.000**	**0.969**	**0.883**	**0.900**	**0.713**	**0.875**	**0.600**	**1.000**	**0.933**	**1.000**	**0.933**	**1.000**	**1.000**
PGD-s	C	0.150		0.083		0.031	0.117	0.100	0.287	0.125	0.400		0.067		0.067		

Soil Type:		Metamorphic				Ultramafic											
Pop. Code:		2	9	16	17	1	3	5	6	7	8	11	15	M2	M1	D	H
PGI-S	A	**0.455**	**0.673**	**0.536**	**0.550**	**0.472**	**0.500**	**0.563**	**0.695**	**0.522**	**0.500**	**0.667**	**0.559**	**0.661**	**0.534**	**0.557**	**0.500**
PGI-S	B	0.134	0.077	0.250	0.133	0.204	0.157	0.042	0.111	0.130	0.056	0.225	0.308	0.330	0.233	0.295	0.198
PGI-S	C	0.250	0.250	0.143	0.250	0.213	0.250	0.250	0.194	0.294	0.389	0.048	0.133	0.009	0.083	0.125	0.167
PGI-S	D	0.098		0.071			0.037	0.062			0.055						0.073
PGI-S	E				0.067	0.111	0.019	0.083		0.043		0.048			0.150		0.062
PGI-S	F	0.063					0.037			0.011		0.012				0.023	
PGM-F	A				0.115	0.009	0.241		0.219	0.214		0.071	0.074	0.010		0.441	**0.730**
PGM-F	B	**1.000**	**1.000**	**1.000**	**0.885**	**0.991**	**0.759**	**1.000**	**0.781**	**0.786**	**1.000**	**0.929**	**0.926**	**0.990**	**1.000**	**0.559**	0.270
PGM-F	C																
PGM-S	A	0.028	0.192				0.036	0.114	0.014	0.098		0.114	0.067	0.196		0.067	0.020
PGM-S	B	0.361	**0.385**	**0.607**	**0.817**	**0.583**	**0.625**	**0.557**	**0.806**	**0.598**	0.292	**0.659**	**0.683**	**0.739**	**0.821**	**0.917**	**0.550**
PGM-S	C	**0.602**	0.173	0.214	0.150	0.358	0.143	0.136	0.042	0.152	**0.667**	0.136	0.167	0.011	0.054	0.017	0.210
PGM-S	D	0.009	0.173	0.143	0.033	0.042	0.187	0.136	0.111	0.152	0.042	0.091	0.058	0.054	0.107		0.200
PGM-S	E		0.077	0.036		0.017	0.009	0.057	0.028				0.025		0.018		0.020
TPI-f	*A**					*0.025*	*0.050*	*0.013*	*0.065*	*0.070*		*0.076*		*0.018*			*0.020*
TPI-f	B	**1.000**	**1.000**	**1.000**	**0.968**	**0.925**	**0.850**	**0.938**	**0.902**	**0.880**	**0.958**	**0.880**	**0.975**	**0.920**	**0.933**	**0.948**	**0.940**
TPI-f	C				0.031	0.050	0.100	0.050	0.033	0.010	0.021			0.062	0.067		0.010
TPI-f	*D*									*0.040*	*0.021*	*0.044*	*0.025*			*0.052*	*0.030*
TPI-s	A	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**
TPI-s	B																
UGPP-f	A	0.025			0.063	0.116	0.058	0.060	0.241	0.020		0.022				0.033	0.212
UGPP-f	B	**0.892**	**1.000**	**0.821**	**0.938**	**0.884**	**0.853**	**0.900**	**0.724**	**0.970**	**1.000**	**0.946**	**0.858**	**1.000**	**0.942**	**0.934**	**0.712**
UGPP-f	C	0.058		0.179			0.083	0.040		0.010		0.033	0.142		0.058	0.033	0.076
UGPP-f	D	0.025							0.035								
UGPP-f	F																
UGPP-S	A	**0.792**	**0.500**	**0.823**	**0.891**	**0.707**	**0.708**	**0.770**	**0.569**	**0.850**	**0.771**	**0.837**	**0.533**	**0.663**	**0.538**	**0.591**	**0.586**
UGPP-S	B	0.033		0.107	0.031	0.112	0.075	0.060	0.198	0.020	0.229	0.109	0.100	0.087	0.077		0.135
UGPP-S	C	0.158	0.500		0.078	0.181	0.208	0.170	0.233	0.130		0.054	0.367	0.250	0.385	0.409	0.279
UGPP-S	D	0.017					0.008										
UGPP-S	E																

Figure 1. Genetic classification of *Silene campanulata* individuals.



glandulosa

individuals with intermediate genotypes

Figure 2. Plot by longitude/latitude of 16 populations of *Silene* analyzed for genetic diversity. Population names are indicated using the population code (see Table 1 for key). Each population 'pie-chart' indicates the proportion of individuals that are made up of each putative subspecies. Blue = 'glandulosa'; Red = 'campanulata'; Yellow = genetic intermediates between glandulosa and campanulata.

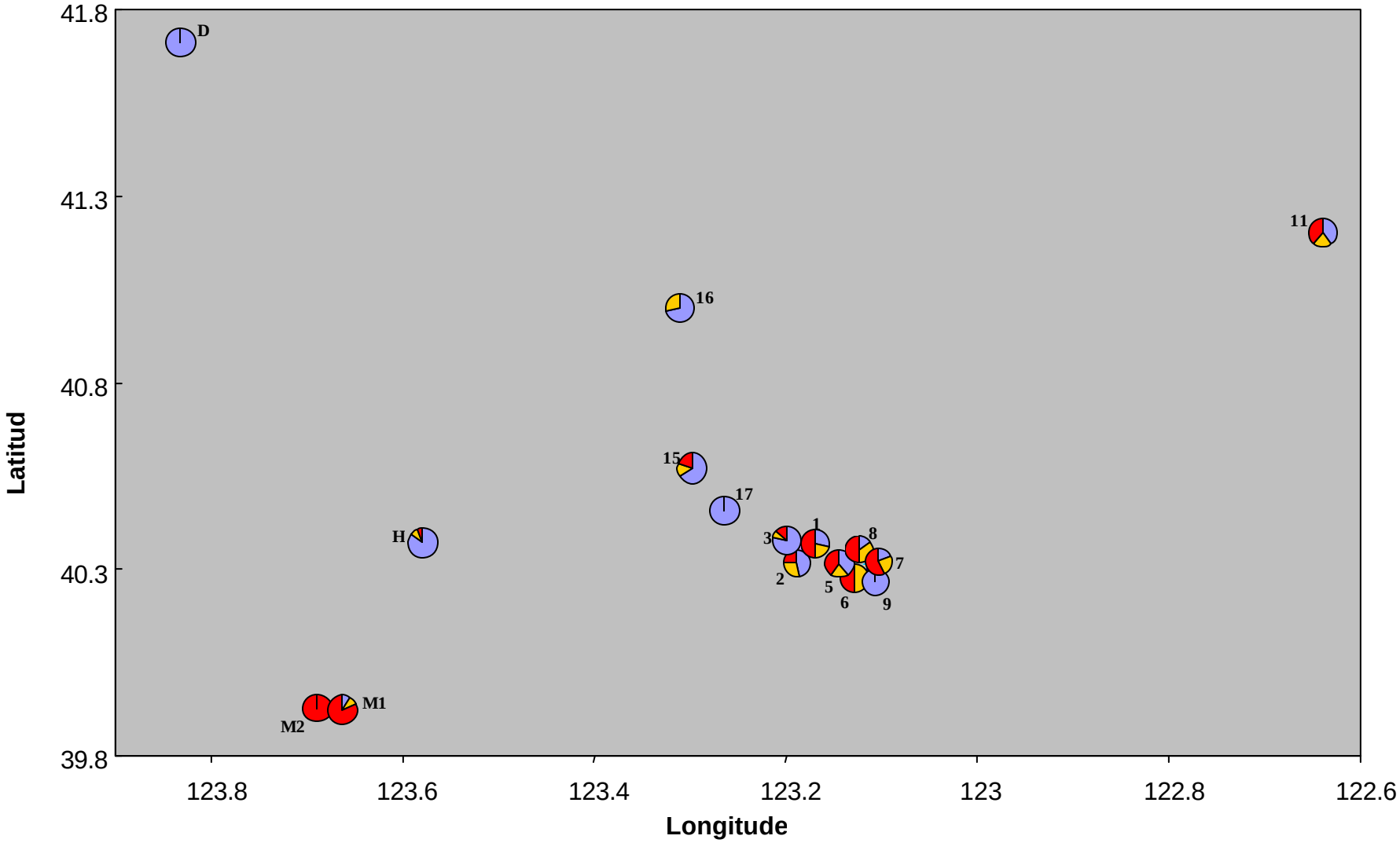
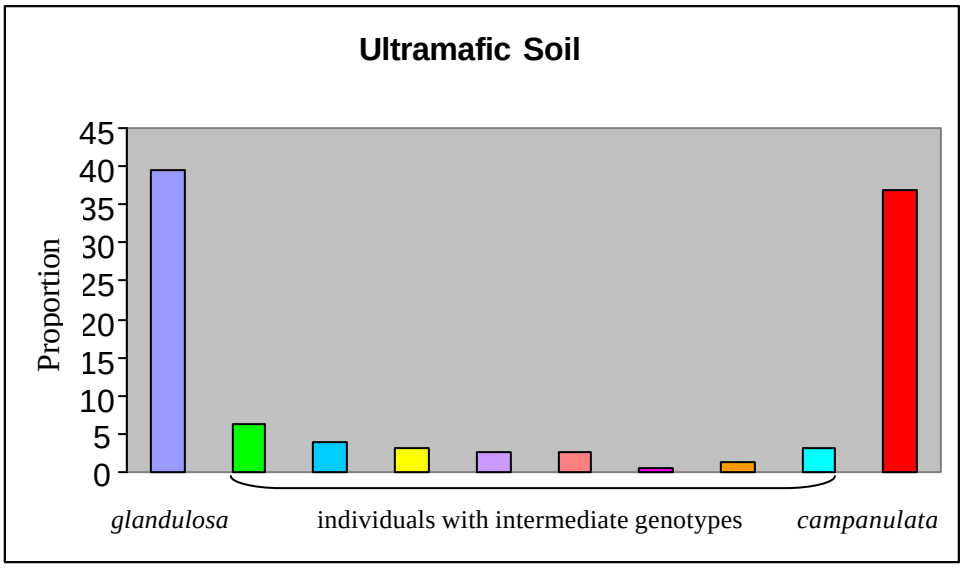
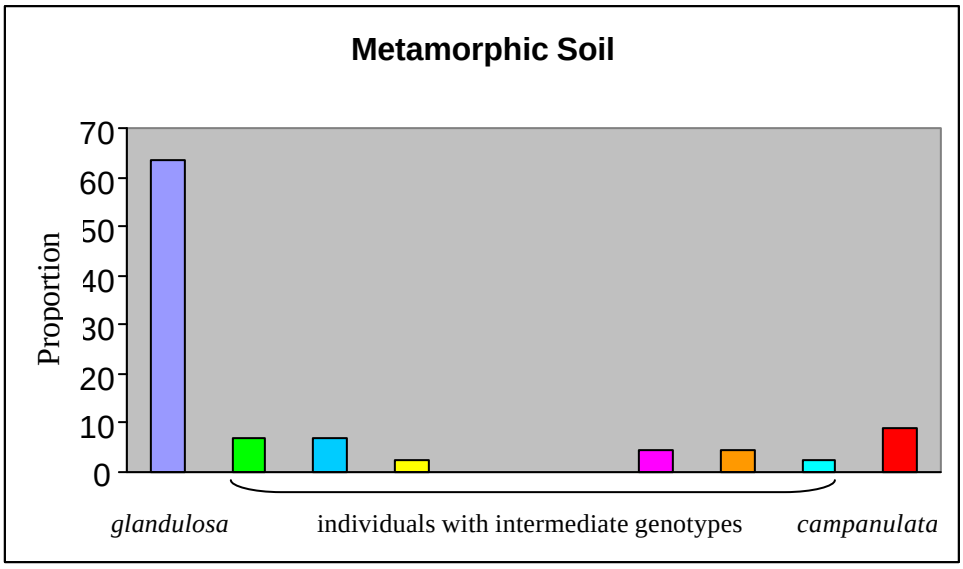


Figure 3. Genetic classification of *Silene campanulata* individuals growing on **(A)** ultramafic substrates, or **(B)** metamorphic substrates.

A.



B.



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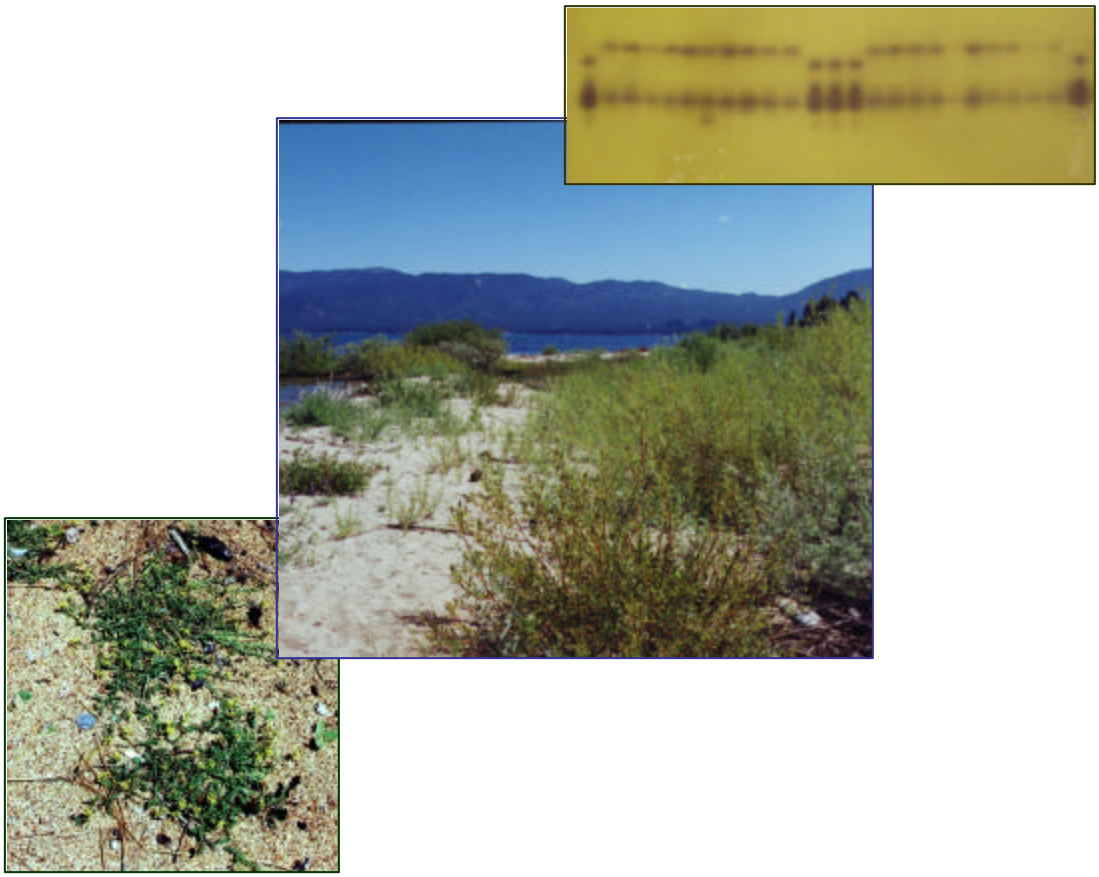
Final Report

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

(Service Agreement 14320-2-H401)



Report prepared by Jennifer DeWoody and Valerie D. Hipkins
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ABSTRACT

Rorippa subumbellata, a small perennial Brassicaceae endemic to the shores of Lake Tahoe, was assessed for genetic variation using isozyme and DNA techniques. Samples from a total of twenty-five sites were collected over two years (2002-2003) and assayed for variation at 23 isozyme loci. Of the 553 total individuals genotyped, 540 (97.6%) had the same isozyme genotype. This genotype was the same as the common genotype found among 95.0% of the individuals sampled in the previous isozyme study (Service Agreement 14-48-0001-95813) conducted in 1999. The thirteen individuals found to contain variation in the 2002-03 collections were distributed among four populations: Eagle Creek (1 plant), Sugar Pine (9 plants), Tahoe Keys (2 plants), and Tallac Creek (1 plant). Neither random amplified polymorphic DNA (RAPD) nor sequences of the chloroplast genome resolved variation in individuals displaying isozyme variation. Of the ten populations sampled over more than one year, two showed evidence of some change in genetic structure between years based on isozyme analysis, including the apparent loss of one rare allele from the Upper Truckee East population. No variation was detected in the samples from the out-planted population at Emerald Bay Avalanche, but as the natural population at this site displayed only the common genotype, these plantings do not change the genetic structure of the population. Due to the lack of variation in most populations, the movement of seed among populations during restoration efforts will likely not affect the overall genetic structure of the species, although efforts to conserve the limited variation observed in the species are warranted.

INTRODUCTION

Rorippa subumbellata (Roll.), Tahoe yellow cress, is a small, perennial plant endemic to the sandy beaches of Lake Tahoe in California (El Dorado and Placer counties) and Nevada (Carson City Rural Area, Washoe and Douglas counties). Occurring only where beaches are wide enough to offer a back beach area protected from wave action (Ferreira 1987), populations are subject to annual variation in size and distribution (*i.e.* metapopulation dynamics), although population censuses have observed a net decline in the species over the past ten years (Pavlik *et al.* 2002). Based on its narrow habitat and declining population sizes, the California and Nevada state governments, local municipalities, and non-profit organizations have identified *R. subumbellata* as an endangered species, and the species is currently a candidate for federal protection under the U.S. Endangered Species Act of 1973, as amended (Pavlik *et al*. 2002).

The current conservation strategy (CS) for *R. subumbellata*, implemented by a coalition of federal, state, and local agencies and private organizations, is described in detail by Pavlik *et al.* (2002). Previous research established that metapopulation dynamics, or the local extinction and colonization of populations, are common in this species. Restoring these processes is considered important to the survival of this species. However, a complete understanding of the demographic processes contributing to these metapopulation dynamics is still lacking.

Metapopulation, migratory, and even reproductive processes can be interpreted from genetic data given sufficient variation in the species (Leberg 1996). In addition, conserving the genetic variation within and among populations is an important component of maintaining the long-term survival and evolutionary potential of a species (Sherwin and Moritz 2000). In the hope of applying genetic information to *R. subumbellata* conservation efforts, the National Forest Genetics Laboratory (NFGEL) was contracted in 1999 to assess 11 sites of *R. subumbellata* for genetic variation at 23 isozyme loci (Saich and Hipkins, 2000). This

previous study found very low levels of variation (eight of the eleven populations displayed no variation), and as a result could identify no patterns of genetic differentiation in the species.

As an extension of the previous genetic work performed by NFGEL, the current study was designed to screen 25 sites for variation at the same 23 isozyme loci. In order to determine if genetic variation is present but undetected by isozymes, additional analyses were completed using two DNA-based markers on a subset of samples. The isozyme analysis revealed low amounts of variation within and among populations, similar to those reported by Saich and Hipkins (2000). Neither of the DNA-based analyses detected variation in the samples screened. These results are consistent with those found in other narrowly-distributed endemic herbs (Hamrick and Godt 1990), and imply that maintaining patterns of genetic differentiation among populations of *Rorippa subumbellata* may be less critical than capturing genetic variation in seed collection and *ex situ* propagation activities.

METHODS

Study Area: Study sites were located along the southern half of Lake Tahoe, on the western, southern, and eastern shores (Figure 1). Samples were collected from 25 sites in 2002-2003 in conjunction with annual census performed by the U.S. Fish and Wildlife Service in cooperation with several federal, state, and local organizations. Several populations were located within protective enclosures, while other populations contained out-planted individuals established as part of restoration efforts (Table 1). In the latter case, collections from naturally occurring populations were collected and labeled separately from restored material (Native and Planted, respectively).

Sample Collection: Up to 30 samples per site of *R. subumbellata* were collected September 2002 and September 2003 for a total of 553 sampled individuals (Table 1). In addition, twenty samples from each of two populations of *R. curvisiliqua* were collected in September 2002. When fewer than 30 *R. subumbellata* plants were present, all plants were sampled. When more than 30 plants were present, samples were collected at sufficient intervals to insure individuals were sampled from throughout the range of the population. On one occasion, up to 60 samples were collected due to misunderstanding as to where the borders of the site were defined. One or two leaves were taken from each plant and placed in zip-lock bags. Bags were kept cool in ice chests during transport to NFGEL in Placerville, CA, and kept refrigerated until prepared for analysis.

Isozyme Analysis: Samples were prepared according to NFGEL Standard Operating Procedures (USDA Forest Service 2003) by submerging a 1cm long section of leaf (40 mm^2) in 100 uL of Tris buffer pH 7.5 (Gottlieb 1981). Samples were stored at –80°C until electophoresis.

Starch gel electrophoresis took place following NFGEL Standard Operating Procedures (USDA Forest Service 2003). A total of 23 loci were resolved in three buffer systems: a lithium borate electrode buffer-tris citrate gel buffer combination (LB), a sodium borate electrode buffer-tris citrate gel buffer system (SB), and a morpholine citrate electrode and gel buffer system (MC6). Eleven loci were resolved in system LB: aconitase (ACO1), leucine aminopeptidase (LAP1), malic enzyme (ME(7)1), phosphoglucose isomerase (PGI1 and PGI2), phosphoglucomutase (PGM1 and PGM2), and fluorescent esterase (FEST1, FEST2, FEST3, and FEST4). Six loci were resolved in system SB: aspartate aminotransferase (AAT1), catalase (CAT1), glycerate-2-dehydrogenase (GLYDH1), triosephosphate isomerase (TPI1 and TPI2), and uridine diphosphoglucose pyrophosphorylase

(UGPP1). Six loci were resolved in system MC6: diaphorase (DIA1), isocitrate dehydrogenase (IDH1), malate dehydrogenase (MDH1), phosphogluconate dehydrogenase (6PGD1 and 6PGD2), and shikimic acid dehydrogenase (SKD1). Two people independently scored each gel, and a third person resolved any disagreements in scores. As part of the NFGEL quality assurance (QA) program, duplicate preparations of 25 individuals (3%), and complete re-runs of 5 sets of 30 individuals (19%) were analyzed in order to confirm observed variation.

DNA Analysis: DNA was extracted from a total of 22 samples using a Qiagen DNEasy Mini Kit following the manufacturer's instructions. Six of the samples were chosen because they displayed the three genotypes observed at the locus UGPP1 during isoyzme analysis: TE-1, UTW-1, and ZS-3 were homozygous for the common allele; SP-1 and SP-9 were heterozygous at the locus, and TC-4 was homozygous for the rare allele. The remaining samples (LH-1, LH-10, LH-5, LH-6, R-21, R-22, RAT-1, RAT-18, SP-2, SP-3, TCW-30, TCW-31, UTE-1, UTE-20, UTW-10, and UTW-20) were selected at random in order to screen DNA markers for variation in the species.

The six samples chosen for their genotype at UGPP (herein the set of six samples) were assessed for variation using three DNA marker systems: random amplified polymorphic DNA (RAPDs), chloroplast DNA sequences in the *trn*L-*trn*F intergenic region (cpDNA), and three microsatellite loci (SSRs). The remaining samples were assessed for variation only using the cpDNA sequences. All amplification reactions took place on a MJ Research® PTC-100 thermalcycler.

The set of six samples were screened for variation using 10 RAPD primers obtained from Operon primer set B: primers 3 thru 12 (available through Qiagen DNA Oligos). Amplification reactions were carried out in a total volume of 25.0 uL, with 3.0 ng sample DNA, 1X reaction buffer (provided with enzyme), 0.2 mM each dNTP, 1.5 mM $MgCl_2$, 20.0 pmol primer, and 1.0 U Taq DNA Polymerase (Qiagen). Amplification reactions involved 1-min. 30-sec. melting at 94 °C, 40 cycles of 1-min. 94 °C, 1-min. 40 °C, and 2-min. 72 °C, followed by a final extension of 10-min. at 72 °C. Products were separated on 1.4% agarose gels and visualized using ethidium bromide.

Bleeker and Hurka (2001) identified intra- and interspecific variation in the *trn*L-*trn*F intergenic region of the chloroplast genome in their study of three European *Rorippa* species. This variation was observed within and among populations at the intraspecific level. All 22 samples of *R. subumbellata* were screened for sequence variation at this locus using primer sequences from Taberlet *et al.* (1991). The intergenic region was first amplified using a standard polymerase chain reaction with a total volume of 25.0 uL, with 8.0 ng sample DNA, 1X reaction buffer (provided with enzyme), 200 uM each dNTP, 2.5 mM $MgCl_2$, 1 uM each primer, and 1.0 U Taq DNA Polymerase (Qiagen). Amplification reactions involved 2-min melting at 95°C, 35 cycles of 30-sec. 95 °C, 30-sec. 55 °C, and 2-min. 72 °C, followed by a final extension of 5-min. at 72 °C. PCR products were purified using Qiagen QiaQuick PCR Purification kits following the manufacturer's protocol. The concentration of the PCR product recovered was quantified using either fluorometery or agarose gel electrophoresis. The sequencing reaction took place in a total volume of 10.0 uL, with 100 ng DNA, 9.5 uM primer, and 4.0 uL of BigDye® Terminator v3.1 Cycle Sequencing Mix (Applied Biosystems, Inc.). The sequencing reaction involved 25 cycles of 10-sec. 96 °C, 5-sec. 50 °C, and 4-min. at 60 °C, with a temperature change rate of 1 °C/second between each step. Sequences were detected on an ABI-3100 capillary system.

Finally, the set of six samples was assessed for variation at three microsatellite loci (or simple sequence repeats, SSRs) developed by Suwabe *et al.* (2002) for *Brassica rapa*:

BRMS-020, BRMS-025, BRMS-044. Although most microsatellite primers cannot readily transfer across genera, these three primers have been shown to transfer to *Arabidopsis*, which is classified in a different tribe than *Brassica* (Heywood *et al*. 1993). As a result, these markers had a greater probability of transferring to *Rorippa* than other microsatellite markers currently available. The amplification reaction recipe and conditions followed those described by Suwabe *et al*. (2002). Primers were fluorescently labeled, and products were detected on the ABI-3100 capillary system.

Data Analysis: A variety of species- and population-level parameters were estimated from the isozyme data. For each population, the percent polymorphic loci (*P*), mean alleles per locus (*A*), mean alleles per polymorphic locus (A_p), observed heterozygosity (H_o), and heterozygosity expected under Hardy-Weinberg equilibrium (H_e) were calculated. These six parameters were also estimated at the species level for the 2002 and 2003 collections.

In order to identify the genetic structure of the entire species, data from the 2002-2003 collections and the 1999 study were combined to create population phenograms, using the two *R. curvisiliqua* populations as outgroups. Nei's (1978) unbiased estimate of genetic distance was estimated for the isozyme data for all possible pairs of populations. The resulting distance matrix was then used to create two population phenograms using cluster analysis (UPGMA) and Neighbor-Joining (NJ) methods. All phenograms were executed using the software PHYLIP (Felsenstein 1993).

Finally, annual changes in genetic structure are not unexpected in species displaying metapopulation dynamics, and such variability in *R. subumbellata* may provide insight to the dynamics of this species. In order to test for annual differences in genetic structure in *R. subumbellata* populations, data from the 2002-2003 collections were combined with the data from the 1999 study, providing multi-year data for ten sites (Table 1). Temporal differences in genetic structure were identified from the isozyme data based on the genetic identity and genetic distance (Nei's (1978) unbiased estimate) among years for each site.

RESULTS

Isozyme Analysis: Isozyme analysis was marginal or failed for samples collected at 13 of the sites in 2002, and as a result, samples were recollected from those sites in 2003. Data for each year a site was sampled was analyzed independently. The resulting data set includes six sites each sampled twice (resulting in 12 "populations") and nineteen sites sampled once (19 populations), for a total of 31 populations in this analysis. Although isozyme analysis of the two samples collected at the D.L. Bliss site in 2002 failed, the site was not revisited in 2003, and has not been included in this report.

Twenty-seven of the 31 populations were monomorphic at the 23 isozyme loci analyzed (Appendix 1). All variation observed in the remaining four populations occurred at low frequency. Two populations, Sugar Pine 2002 and Tallac Creek 2002, contained variation at a locus previously found to be variable, UGPP1. The other two populations displayed novel variation: Eagle Creek 2003 at the locus FEST1, and Tahoe Keys 2003 at PGM2 (Table 2, Appendix 2). For the 2002-2003 collections, this variation results in a species-level estimate of percent polymorphic loci of 13.04%. The expected heterozygosity for all populations was 0.0000 except Sugar Pine 2002: 0.0159 (S.E. = 0.0154), Tallac Creek 2002: 0.0103 (S.E. = 0.0100); Eagle Creek 2003: 0.0062 (S.E. = 0.0061); and Tahoe Keys 2003: 0.0049 (S.E. = 0.0048). *R. curvisiliqua* contains greater levels of variation than does *R. subumbellata* (He=0.032 vs He=0.002, respectively; Table 3).

The population phenograms produced using UPGMA and NJ methods display a similar topology, each rooted by the two populations of *R. curvisiliqua* and illustrating the similarity in most populations. The UPGMA phenogram distinguishes only the Taylor Creek 1999 and Tahoe Meadows 1999 populations from the other *R. subumbellata* sites (available upon request). The Neighbor-Joining method depicts not only these sites as unique, but also reflects the variation observed in Sugar Pine 2002, Tallac Creek 2002, Upper Truckee East 1999, and Tahoe Keys 2003 (Figure 2).

Genetic distances detect temporal variation in genetic structure between the 1999 and 2003 collections of Tahoe Meadows, as variable between 1999 and 2003 (genetic distance = 0.005), although the single variable locus (DIA1) was not resolved in the 2003 collection. A change in allele frequency was detected in the samples collected at Upper Truckee East, with the rare allele at UGPP1 occurring at low frequency in 1999, but missing from the 2002 collections. All remaining pairs of collections produced an index of genetic identity of 1.000.

***DNA Analyses*:** Of the ten RAPD primers screened in the set of six samples, none produced consistent variation among individuals. Attempts to replicate potential variation failed to produce repeatable banding patterns. Despite their great potential due to the anonymous nature of primer binding, banding patterns produced by RAPD analyses are not always consistent or appropriate for genetic diversity studies (Jones *et al.* 1998), and the results of the *Rorippa* screening indicate that RAPDs are not appropriate for this species. As a result, none of the banding patterns were analyzed further. All 22 samples screened for variation at the *trn*L-*trn*F spacer of the chloroplast genome produced identical sequences (sequences available upon request.) Finally, none of the microsatellite primers produced peaks in the six samples screened in this test, indicating that these primers may not be easily transferred across species within this genus.

DISCUSSION

Genetic structure of *Rorippa subumbellata*

Low levels of variation were detected in only a handful of *R. subumbellata* populations, which is consistent with the findings of Saich and Hipkins (2000). Of the 31 populations (25 sites) sampled in this study, four contained variation at a single locus. Two populations, Sugar Pine 2002 and Tallac Creek 2002, contained the same alternate allele at the locus UGPP1 that Saich and Hipkins (2000) reported in previous collections. The other two populations displayed novel variation: Eagle Creek 2003 contained a single alternate allele at the locus FEST1, and Tahoe Keys 2003 contained an alternate allele at the locus PGM2 (Table 2, Appendix 1, Appendix 2). No variation was observed at two loci previously reported as variable: PGI1 and DIA1. The remaining 27 populations were monomorphic for the common allele at all loci. Other studies of extreme endemics (plants restricted to narrow habitats) have reported consistently low levels of variation, including *Pedicularis furbishiae* (Waller *et al.* 1987) and *Iris lacustris* (Simonich and Morgan 1994). However, *R. subumbellata* displays levels of variation that are consistently lower than the mean of 40% polymorphic loci reported in rare and endemic herbs by Hamrick and Gott (1990).

Given that all populations sampled contain the common genotype (*i.e.* individuals homozygous for the common allele at all loci) in high frequencies, efforts to supplement or reestablish populations with *ex situ* plants that contain only the common genotype should maintain the current genetic structure observed among populations. However, care should be taken to preserve those populations containing genetic variation (see Gene Conservation below).

Saich and Hipkins (2000) discussed the possible causes of the low levels of variation in *R. subumbellata,* including genetic bottlenecks, clonal reproduction, and a mating system displaying high rates of selfing. The genetic findings of this study, together with the life history of the species described by Pavlik *et al.* (2002), reveals additional factors that may contribute to the observed genetic structure. The recurring extinction and colonization of populations in a metapopulation may lead to genetic bottlenecks and random genetic drift, and eventually to a decrease in heterozygosity in the species (Thrall *et al*. 2000). If metapopulation dynamics were historically important to the survival of this species, frequent turnover of populations may have maintained the low levels of variation currently observed.

Although metapopulation dynamics may play an important role in the structure of this species, these studies indicate that gene flow among established populations is rare. The presence of rare alleles in only one or a couple of populations is consistent over years (Pavlik *et al.* 2002, Saich and Hipkins 2000), and no evidence has been found that these rare alleles have moved (presumably via seed flow) into neighboring populations. In addition, the population differentiation reported by Saich and Hipkins (2000) is the consequence of rare alleles being restricted to no more than 2 populations, another indication that gene flow is rare in this species.

Temporal variation

Temporal differences in genetic variation were detected in two of the ten sites (20%) sampled over more than one year (1999-2003). These differences were indicated by genetic distances greater than 0.000, and by a difference in allele frequencies between collection years. Differences at one site, Tahoe Meadows, are due not to a potential change in allele frequency in the population over a four year period, but rather due to the fact that the single locus displaying variation in 1999, DIA1, was unresolved in the 2003 collections. Alternatively, the absence of the rare allele UGPP1-2 in the Upper Truckee East samples collected in 2002, indicates that the allele has likely been lost through random genetic drift since the 1999 collections (see Gene Conservation, below), although the allele may still be present at low frequencies due to the large size of the population. These patterns of temporal differentiation are consistent with the genetic consequences expected from the local extinction and colonization dynamics that define a metapopulation.

Gene conservation

Although the high frequency of the common alleles in *R. subumbellata* indicates that outplanting efforts using the common genotype should not change the genetic structure of the metapopulation, the conservation of the species as a whole will not be complete if the rare alleles are lost through genetic drift. One goal of conservation biology is to conserve patterns and levels of genetic diversity in species. Theoretically, maintaining levels of genetic variation is important in order to maximize the adaptive potential of the species of concern (Lande and Barrowclough 1987).

The genetic variation observed in *R. subumbellata* occurs in low levels and in a limited number of populations (Table 1, 2, 3). As a result, care should be taken to conserve these rare alleles and preserve their functional role in each population. Two rare alleles present in the 1999 samples, DIA1-2 and PGI1-2, were not observed in the present study. In one case, the locus DIA1 was not resolved in the 2003 collection from the Tahoe Meadows population (where it was previously observed), so the allele may still be present but not detected. In another case, *R. subumbellata* was not recollected from the Taylor Creek Enclosure, where the PGI1-2, as well as the UGPP1-2 allele, were observed in 1999. These

alleles were not observed in any of the neighboring sites, however (Taylor Creek East, Taylor Creek Middle, and Taylor Creek West; Appendix 1). In the last case, the allele UGPP1-2 was not observed in the 2002 collections at Upper Truckee East. In this case, the rare allele has likely been lost through genetic drift, since we would have expected to observe the allele based on the frequency of the allele in the 1999 collections.

If a rare allele is present at low frequencies in a large population of *R. subumbellata*, how many copies would we expect to observe in a sample of 30 individuals? This can be estimated as the product of the allele frequency of the rare allele (conservatively, the lowest population-level frequency observed for the allele) and the number of alleles sampled from the population (since *R. subumbellata* behaves as diploid, sampling 30 individuals resolves 60 alleles from the population). From this information, we expect to observe the following number of rare alleles for each variable locus (the allele frequency used in each estimate is given in parentheses): 22.5 occurrences of DIA1-2 (0.375), 4.02 of FEST1-2 (0.067), 6.0 of PGI1-2 (0.100), 3.12 of PGM2-3 (0.052), and 3.66 of UGPP1-2 (0.061). Allele frequencies reported by Saich and Hipkins (2000) were used to estimate the values for DIA1, PGI1, and UGPP1. These estimates indicate that, based on a sample of 30 individuals, the rare allele should have been observed if present in a population. Given these probabilities, we conclude that those populations containing more individuals than those sampled (4H, Blackwood North, Blackwood South, Emerald Avalanche, Emerald Point, Lighthouse, Rubicon, Sugar Pine, Tahoe Keys, Taylor Creek West, Upper Truckee East, and Upper Truckee West) likely do not contain rare alleles not reported herein. The remaining populations were sampled exhaustively, so allele frequencies at each site are conclusive.

Future directions

Isozyme variation can be considered a proxy for the total genetic variation that may be contained in a species. Based on the observed isozyme variation and the lack of variation at DNA markers in individuals known to display protein variation, this battery of isozyme loci currently provides the best tool to monitor the genetic structure of populations of *R. subumbellata.* In contrast, other DNA marker systems may prove more variable than isozymes, but are often either expensive to develop and screen (*e.g.*, microsatellites) or require a larger amount of tissue than may be available from a single *R. subumbellata* rosette without significantly damaging its chances for survival (*e.g.* AFLPs).

Rather than expanded searches for variable markers in *R. subumbellata*, the addition of populations from the northern half of Lake Tahoe may provide additional information about the genetic structure of this species. Pavlik *et al.* (2002) identified several sites along the northern shores of the lake that were once known to contain *R. subumbellata* populations (Figure 1), although recent surveys have determined populations at some of the sites to be extinct.

Finally, one goal of conservation biology is to conserve genes under the theory that species (or populations) displaying higher levels of variation have a greater chance of adapting to changing environments (Lande and Barrowclough 1987). To this end, care should be taken to conserve the genetic variation known to exist in *R. subumbellata.* With the goal of preserving the limited genetic variation observed in this species, efforts to tag individual plants containing rare alleles and track their survival over years, systematically collect seed from these individuals, or even vegetatively propagate these plants would be reasonable additions to the conservation strategy. Sites containing known genetic variants should receive special attention for future seed collections (Table 2, Appendix 2).

SUMMARY AND CONCLUSIONS

Analysis of 693 individuals from 28 sites revealed low levels of variation in *Rorippa subumbellata* over three collection years (1999, 2002, and 2003). DNA analyses resolved no variation in individuals known to contain different isozyme genotypes. Genetic differences among populations and between years are due to the presence of rare alleles in five loci: DIA1, FEST1, PGI1, PGM2, and UGPP1 (Table 2). Populations found to contain variation are: Eagle Creek (2003), Sugar Pine (2002), Tahoe Keys (2003), Tahoe Meadows (1999), Tallac Creek (2002), Taylor Creek (1999), and Upper Truckee East (1999). Temporal changes in genetic variation observed at Upper Truckee East is likely due to the loss of a rare allele at one locus (UGPP1) between 1999 and 2002, although the allele may be present in low frequencies due to the large size of this population. The high frequency of the common alleles in every population sampled indicates that restoration activities using plants that are homozygous for the common genotype will not significantly change the genetic structure of the metapopulation. However, care should be taken to conserve the limited genetic variation observed in order to preserve the evolutionary potential of the species.

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Table 1. Name, abbreviation, and collection date of *Rorippa* study sites. Previous Name is the label used by Saich and Hipkins (2000), and is provided for reference. The number of individuals analyzed, if different from the number collected, is indicated in parentheses. Names or Dates in **bold** indicate collections that displayed isozyme variation. 1999 collections are part of the previous isozyme study conducted under Service Agreement 14-48-0001-95813; 2002 and 2003 collections are part of the current study, Service Agreement 14320-2-H401.

Population	Abbrev.	Previous Name	Date Collected	# Collected
Rorippa subumbellata				
4H	4H		9-4-2002	21 (0)
			9-9-2003	30
Baldwin	B	Baldwin West – N of lot	8-15-1999	4
			9-3-2002	3(2)
			9-2-2003	3
Blackwood North	BN		9-2-2003	24[a]
Blackwood South	BS	Blackwood South	9-1-1999	27
			9-4-2002	28 (5)
			9-2-2003	30
Cascade West	CW		9-3-2002	4 (0)
			9-2-2003	8
Eagle Creek	EC		9-4-2002	4 (0)
			9-3-2003	15
Edgewood	E		8-15-1999	18
Emerald Bay Avalanche, Native	EAN		9-4-2002	21 (1)
			9-3-2003	60
Emerald Bay Avalanche, Planted	EAP		9-3-2003	15
Emerald Point	EP		9-4-2002	11 (7)
			9-3-2003	30
Kahle/Nevada	K		9-1-1999	7
Lighthouse	L	Lighthouse	9-1-1999	11
		Lighthouse Beach	9-1-1999	7
			9-4-2002	31 (10)
			9-2-2003	35
Meeks Bay	MB		9-4-2002	12 (5)
			9-2-2003	7
Pope Beach	P		9-4-2002	7 (0)
			9-2-2003	9 (4)
Regan/Al Tahoe	RAT		9-3-2002	18
Rubicon	R		9-4-2002	30
Sugar Pine	SP		**9-4-2002**	30
Tahoe Keys	TK		9-4-2002	31 (0)
			9-2-2003	30
Tahoe Meadows	TM	Tahoe Meadows	**9-1-1999**	8
			9-4-2002	20 (0)
			9-9-2003	12
Tallac Creek	TC		**9-3-2002**	11
Tallac Enclosure	TE	Baldwin West (enclosure)	8-15-1999	13
			9-3-2002	10

[a]More than 24 plants present.

Table 1. Continued.

Population	Abbrev.	Previous Name	Date Collected	# Collected
Taylor Creek Enclosure	TAY		**8-15-1999**	10
Taylor Creek East	TCE		9-3-2002	12
Taylor Creek Mouth	TCM		9-3-2002	10
Taylor Creek West	TCW		9-3-2002	31
Upper Truckee East	UTE	Upper Truckee East	**8-15-1999**	33
			9-3-2002	30
Upper Truckee West	UTW	Upper Truckee West	8-15-1999	2
			9-3-2002	30
Zephyr Spit	ZS		9-4-2002	8
Rorippa curvisiliqua				
Tallac Creek	ROCUT		9-3-2002	20
Taylor Creek Enclosure	ROCUE		9-3-2002	20

Table 2. Genotype scores at the five variable isozyme loci for the 20 *R. subumbellata* individuals showing genetic variation in both studies (20 out of 693 total plants sampled; 2.9%). Allele numbers (1, 2, or 3) are defined in Appendix 1. All individuals are homozygous for the common allele ('11') at the other 18 loci (not listed).

Site	**Year**	**# of Plants**	**Locus**				
			UGPP1	PGM2	FEST1	DIA1	PGI1
Sugar Pine	2002	9	12	11	11	11	11
Tallac Creek	2002	1	22	11	11	11	11
Upper Truckee East	1999	2	22	11	11	11	11
Taylor Creek Enclosure	1999	2	22	11	11	11	12
Tahoe Meadows	1999	3	11	11	11	22	11
Eagle Creek	2003	1	11	11	22	11	11
Tahoe Keys	2003	1	11	13	11	11	11
		1	11	33	11	11	11

Table 3. Summary of genetic variability in *Rorippa* species. *N* = mean number of individuals per locus per population; *P* = % polymorphic loci; *A* = mean number alleles per locus; A_p = mean alleles per polymorphic locus; H_o = oberserved frequency of heterozygotes; H_e = frequency of heterozygotes expected under Hardy-Weinberg equilibrium. Standard errors given in parentheses.

	N	*P*	*A*	*A*p	*H*o	*H*e
Species level						
R. subumbellata						
All populations (1999, 2002, 2003)	693	13.04	1.1304 (0.3444[a])	2.0000	0.0008 (0.0034[a])	0.0015 (0.0045[a])
R. curvisiliqua						
All populations (2002)	28	30.43	1.3478(0.1168)	2.1429	0.0457 (0.0414)	0.0324 (0.0221)
Population level						
(*R.subumbellata)*						
2002 mean	250	4.35	1.0588 (0.2425[a])	2.0000	0.0021 (0.0088[a])	0.0025 (0.0105[a])
2003 mean	303	8.70	1.0952 (0.3008[a])	2.0000	0.0002 (0.0008[a])	0.0009 (0.0029[a])
4H 2003	28.714	0.00	1.0000 (0.0000)		0.0000 (0.0000)	0.0000 (0.0000)
B 2002	1.929	0.00	1.0000 (0.0000)		0.0000 (0.0000)	0.0000 (0.0000)
B 2003	3.000	0.00	1.0000 (0.0000)		0.0000 (0.0000)	0.0000 (0.0000)
BN 2003	21.750	0.00	1.0000 (0.0000)		0.0000 (0.0000)	0.0000 (0.0000)
BS 2002	5.000	0.00	1.0000 (0.0000)		0.0000 (0.0000)	0.0000 (0.0000)
BS 2003	28.000	0.00	1.0000 (0.0000)		0.0000 (0.0000)	0.0000 (0.0000)
CW 2003	7.882	0.00	1.0000 (0.0000)		0.0000 (0.0000)	0.0000 (0.0000)
EC 2003	14.150	5.00	1.0500 (0.0487)	2.0000	0.0000 (0.0000)	0.0062 (0.0061)
EAN 2002	1.000	0.00	1.0000 (0.0000)		0.0000 (0.0000)	0.0000 (0.0000)
EAN 2003	52.588	0.00	1.0000 (0.0000)		0.0000 (0.0000)	0.0000 (0.0000)
EAP 2003	14.500	0.00	1.0000 (0.0000)		0.0000 (0.0000)	0.0000 (0.0000)
EP 2002	6.539	0.00	1.0000 (0.0000)		0.0000 (0.0000)	0.0000 (0.0000)
EP 2003	17.947	0.00	1.0000 (0.0000)		0.0000 (0.0000)	0.0000 (0.0000)
L 2002	10.000	0.00	1.0000 (0.0000)		0.0000 (0.0000)	0.0000 (0.0000)
L 2003	33.810	0.00	1.0000 (0.0000)		0.0000 (0.0000)	0.0000 (0.0000)
MB 2002	4.692	0.00	1.0000 (0.0000)		0.0000 (0.0000)	0.0000 (0.0000)
MB 2003	6.824	0.00	1.0000 (0.0000)		0.0000 (0.0000)	0.0000 (0.0000)
P 2003	3.895	0.00	1.0000 (0.0000)		0.0000 (0.0000)	0.0000 (0.0000)
RAT 2002	16.500	0.00	1.0000 (0.0000)		0.0000 (0.0000)	0.0000 (0.0000)
R 2002	27.188	0.00	1.0000 (0.0000)		0.0000 (0.0000)	0.0000 (0.0000)
SP 2002	26.875	6.25	1.0625 (0.0605)	2.0000	0.0188 (0.0182)	0.0159 (0.0154)
TK 2003	25.700	5.00	1.0500 (0.0487)	2.0000	0.0017 (0.0017)	0.0049 (0.0048)
TM 2003	10.350	0.00	1.0000 (0.0000)		0.0000 (0.0000)	0.0000 (0.0000)
TC 2002	10.063	2.000	1.0625 (0.0605)	2.0000	0.0000 (0.0000)	0.0103 (0.0100)
TE 2002	10.000	0.00	1.0000 (0.0000)		0.0000 (0.0000)	0.0000 (0.0000)
TCE 2002	10.133	0.00	1.0000 (0.0000)		0.0000 (0.0000)	0.0000 (0.0000)
TCM 2002	9.438	0.00	1.0000 (0.0000)		0.0000 (0.0000)	0.0000 (0.0000)
TCW 2002	23.625	0.00	1.0000 (0.0000)		0.0000 (0.0000)	0.0000 (0.0000)
UTE 2002	28.235	0.00	1.0000 (0.0000)		0.0000 (0.0000)	0.0000 (0.0000)
UTW 2002	28.353	0.00	1.0000 (0.0000)		0.0000 (0.0000)	0.0000 (0.0000)
ZS 2002	8.000	0.00	1.0000 (0.0000)		0.0000 (0.0000)	0.0000 (0.0000)

[a]Standard deviations

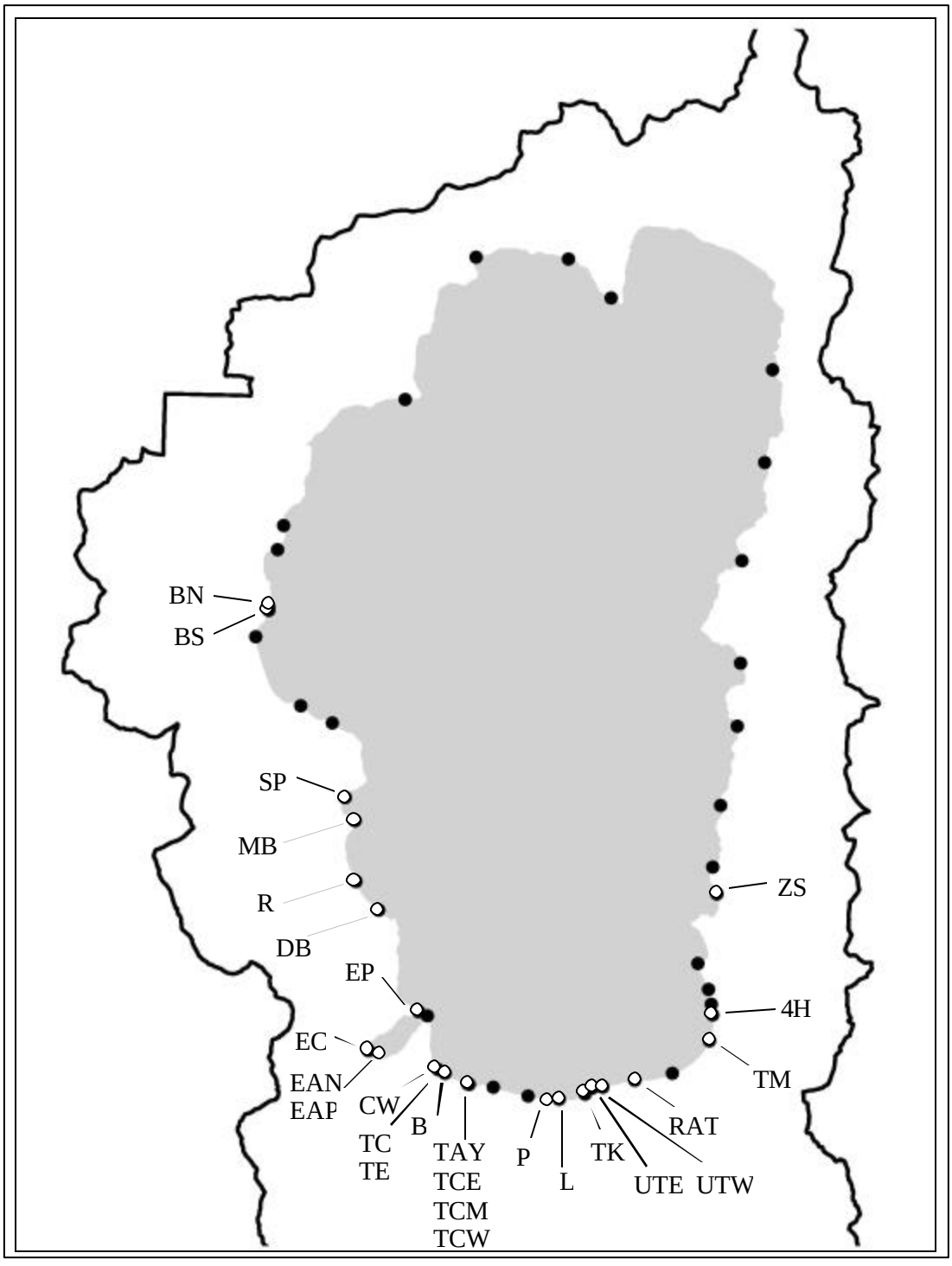


Figure 1. Locations of *R. subumbellata* populations sampled in this study. Open circles represent those populations sampled, with abbreviations identifying each site. Solid gray circles represent historic locations of populations. From Pavlik *et al.* 2002.

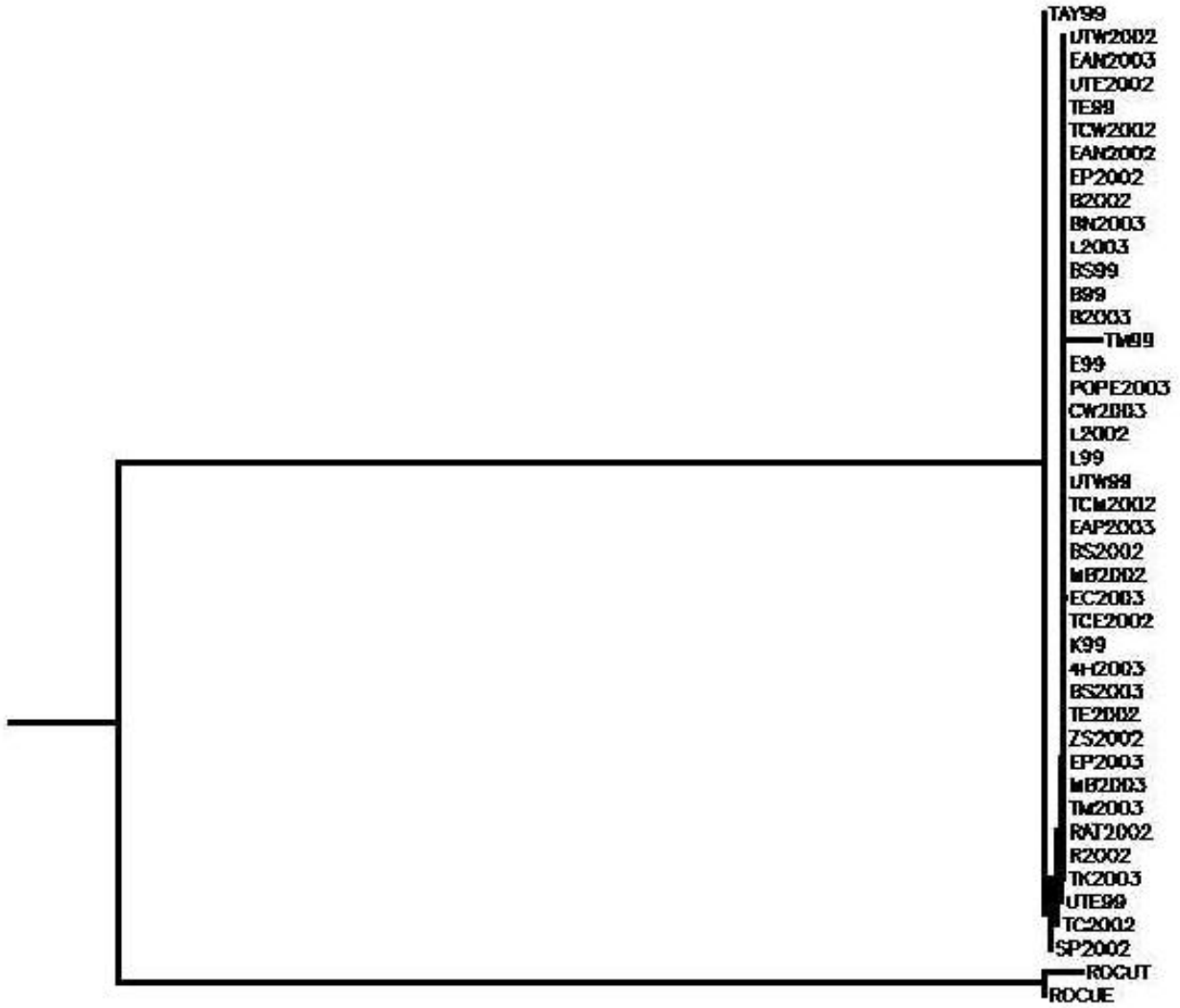


Figure 2. Population phenogram for 41 populations of *Rorippa subumbellata* and 2 populations of *R. curvisiliqua*. Phenogram is built from Nei's (1978) unbiased genetic distance using Neighbor Joining methods. Population abbreviations found in Table 1.

Appendix 1. Allele frequencies at 23 isozyme loci for *Rorippa subumbellata* and *R. curvisiliqua*. Alleles were numbered in the order they were observed, not in order of migration speed or frequency. Migration is the distance (mm) the allele migrated from the origin. * indicates missing data.

Locus	AAT1	ACO1			CAT1	DIA1		FEST1	
Allele	1	1	2	3	1	1	2	1	2
Migration	48/45/42	41	45	43	18	26	23	51	54
Rorippa subumbellata									
4H 2003	1.000	1.000	0.000	0.000	1.000	1.000	0.000	1.000	0.000
Baldwin 2002	1.000	1.000	0.000	0.000	*	*	*	*	*
Baldwin 2003	1.000	*	*	*	1.000	*	*	1.000	0.000
Blackwood North 2003	1.000	1.000	0.000	0.000	1.000	*	*	1.000	0.000
Blackwood South 2002	1.000	1.000	0.000	0.000	*	*	*	*	*
Blackwood South 2003	1.000	1.000	0.000	0.000	1.000	*	*	1.000	0.000
Cascade West 2003	1.000	*	*	*	1.000	*	*	1.000	0.000
Eagle Creek 2003	1.000	*	*	*	1.000	1.000	0.000	0.933	0.067
Emerald Bay Avalanche, Native 2002	1.000	1.000	0.000	0.000	*	*	*	*	*
Emerald Bay Avalanche, Native 2003	1.000	*	*	*	1.000	*	*	1.000	0.000
Emerald Bay Avalanche, Planted 2003	1.000	*	*	*	1.000	1.000	0.000	1.000	0.000
Emerald Point 2002	1.000	1.000	0.000	0.000	*	*	*	*	*
Emerald Point 2003	1.000	1.000	0.000	0.000	*	*	*	1.000	0.000
Lighthouse 2002	1.000	1.000	0.000	0.000	*	*	*	*	*
Lighthouse 2003	1.000	1.000	0.000	0.000	1.000	1.000	0.000	1.000	0.000
Meeks Bay 2002	1.000	1.000	0.000	0.000	*	*	*	*	*
Meeks Bay 2003	1.000	*	*	*	1.000	*	*	1.000	0.000
Pope Beach 2003	1.000	1.000	0.000	0.000	1.000	*	*	1.000	0.000
Regan/Al Tahoe 2002	1.000	1.000	0.000	0.000	1.000	*	*	*	*
Rubicon 2002	1.000	1.000	0.000	0.000	1.000	*	*	*	*
Sugar Pine 2002	1.000	1.000	0.000	0.000	1.000	*	*	*	*
Tahoe Keys 2003	1.000	1.000	0.000	0.000	1.000	*	*	1.000	0.000
Tahoe Meadows 2003	1.000	1.000	0.000	0.000	1.000	*	*	1.000	0.000
Tallac Creek 2002	1.000	1.000	0.000	0.000	1.000	*	*	*	*
Tallac Enclosure 2002	1.000	1.000	0.000	0.000	1.000	*	*	*	*
Taylor Creek East 2002	1.000	1.000	0.000	0.000	1.000	*	*	*	*
Taylor Creek Mouth 2002	1.000	1.000	0.000	0.000	1.000	*	*	*	*
Taylor Creek West 2002	1.000	1.000	0.000	0.000	1.000	*	*	*	*
Upper Truckee East 2002	1.000	1.000	0.000	0.000	1.000	*	*	*	*
Upper Truckee West 2002	1.000	1.000	0.000	0.000	1.000	*	*	*	*
Zephyr Spit 2002	1.000	1.000	0.000	0.000	*	*	*	*	*
R. curvisiliqua									
Tallac Enclosure 2002	1.000	0.500	0.500	0.000	1.000	*	*	*	*
Taylor Creek Enclosure 2002	1.000	0.475	0.475	0.050	1.000	*	*	*	*

Appenix 1 (cont'd)

Locus	FEST2	FEST3	FEST4	GLYDH1	IDH1		LAP1		MDH1
Allele	1	1	1	1	1	2	1	2	1
Migration	46	39	34	7	17/27	17/21	42	45	24
Rorippa subumbellata									
4H 2003	1.000	1.000	1.000	1.000	*	*	1.000	0.000	1.000
Baldwin 2002	*	*	*	1.000	1.000	0.000	1.000	0.000	1.000
Baldwin 2003	*	1.000	*	1.000	*	*	1.000	0.000	1.000
Blackwood North 2003	1.000	1.000	1.000	1.000	*	*	1.000	0.000	1.000
Blackwood South 2002	*	*	*	1.000	*	*	1.000	0.000	1.000
Blackwood South 2003	*	*	*	1.000	*	*	1.000	0.000	1.000
Cascade West 2003	*	1.000	*	1.000	*	*	1.000	0.000	1.000
Eagle Creek 2003	1.000	1.000	1.000	1.000	*	*	1.000	0.000	1.000
Emerald Bay Avalanche, Native 2002	*	*	*	1.000	*	*	1.000	0.000	1.000
Emerald Bay Avalanche, Native 2003	*	1.000	*	1.000	*	*	1.000	0.000	1.000
Emerald Bay Avalanche, Planted 2003	1.000	1.000	1.000	1.000	*	*	1.000	0.000	1.000
Emerald Point 2002	*	*	*	1.000	*	*	1.000	0.000	1.000
Emerald Point 2003	1.000	1.000	1.000	1.000	*	*	1.000	0.000	1.000
Lighthouse 2002	*	*	*	1.000	1.000	0.000	1.000	0.000	1.000
Lighthouse 2003	1.000	1.000	1.000	1.000	*	*	1.000	0.000	1.000
Meeks Bay 2002	*	*	*	1.000	*	*	1.000	0.000	1.000
Meeks Bay 2003	*	1.000	*	1.000	*	*	1.000	0.000	1.000
Pope Beach 2003	1.000	1.000	*	1.000	*	*	1.000	0.000	1.000
Regan/Al Tahoe 2002	*	*	*	1.000	1.000	0.000	1.000	0.000	1.000
Rubicon 2002	*	*	*	1.000	1.000	0.000	1.000	0.000	1.000
Sugar Pine 2002	*	*	*	1.000	1.000	0.000	1.000	0.000	1.000
Tahoe Keys 2003	1.000	1.000	1.000	1.000	*	*	1.000	0.000	1.000
Tahoe Meadows 2003	1.000	1.000	1.000	1.000	*	*	1.000	0.000	1.000
Tallac Creek 2002	*	*	*	1.000	1.000	0.000	1.000	0.000	1.000
Tallac Enclosure 2002	*	*	*	1.000	*	*	1.000	0.000	1.000
Taylor Creek East 2002	*	*	*	1.000	*	*	1.000	0.000	1.000
Taylor Creek Mouth 2002	*	*	*	1.000	*	*	1.000	0.000	1.000
Taylor Creek West 2002	*	*	*	1.000	*	*	1.000	0.000	1.000
Upper Truckee East 2002	*	*	*	1.000	*	*	1.000	0.000	1.000
Upper Truckee West 2002	*	*	*	1.000	*	*	1.000	0.000	1.000
Zephyr Spit 2002	*	*	*	1.000	*	*	1.000	0.000	1.000
R. curvisiliqua									
Tallac Enclosure 2002	*	*	*	1.000	0.000	1.000	0.000	1.000	1.000
Taylor Creek Enclosure 2002	*	*	*	1.000	0.000	1.000	0.050	0.950	1.000

Appenidx 1 (cont'd)

Locus	ME(7)1	6PGD1		6PGD2	PGI1		PGI2		
Allele	1	1	2	1	1	2	1	2	3
Migration	25	28/25	32/27	13	36	40	27	30	28
Rorippa subumbellata									
4H 2003	1.000	1.000	0.000	1.000	1.000	0.000	1.000	0.000	0.000
Baldwin 2002	1.000	1.000	0.000	*	1.000	0.000	1.000	0.000	0.000
Baldwin 2003	1.000	1.000	0.000	1.000	1.000	0.000	1.000	0.000	0.000
Blackwood North 2003	1.000	1.000	0.000	1.000	1.000	0.000	1.000	0.000	0.000
Blackwood South 2002	1.000	1.000	0.000	1.000	1.000	0.000	1.000	0.000	0.000
Blackwood South 2003	1.000	1.000	0.000	1.000	1.000	0.000	1.000	0.000	0.000
Cascade West 2003	1.000	1.000	0.000	1.000	1.000	0.000	1.000	0.000	0.000
Eagle Creek 2003	1.000	1.000	0.000	1.000	1.000	0.000	1.000	0.000	0.000
Emerald Bay Avalanche, Native 2002	1.000	1.000	0.000	1.000	1.000	0.000	1.000	0.000	0.000
Emerald Bay Avalanche, Native 2003	1.000	1.000	0.000	1.000	1.000	0.000	1.000	0.000	0.000
Emerald Bay Avalanche, Planted 2003	1.000	1.000	0.000	1.000	1.000	0.000	1.000	0.000	0.000
Emerald Point 2002	1.000	1.000	0.000	1.000	1.000	0.000	1.000	0.000	0.000
Emerald Point 2003	1.000	1.000	0.000	1.000	1.000	0.000	1.000	0.000	0.000
Lighthouse 2002	1.000	1.000	0.000	1.000	1.000	0.000	1.000	0.000	0.000
Lighthouse 2003	1.000	1.000	0.000	1.000	1.000	0.000	1.000	0.000	0.000
Meeks Bay 2002	1.000	1.000	0.000	1.000	1.000	0.000	1.000	0.000	0.000
Meeks Bay 2003	1.000	1.000	0.000	1.000	1.000	0.000	1.000	0.000	0.000
Pope Beach 2003	1.000	1.000	0.000	1.000	1.000	0.000	1.000	0.000	0.000
Regan/Al Tahoe 2002	1.000	1.000	0.000	1.000	1.000	0.000	1.000	0.000	0.000
Rubicon 2002	1.000	1.000	0.000	1.000	1.000	0.000	1.000	0.000	0.000
Sugar Pine 2002	1.000	1.000	0.000	1.000	1.000	0.000	1.000	0.000	0.000
Tahoe Keys 2003	1.000	1.000	0.000	1.000	1.000	0.000	1.000	0.000	0.000
Tahoe Meadows 2003	1.000	1.000	0.000	1.000	1.000	0.000	1.000	0.000	0.000
Tallac Creek 2002	1.000	1.000	0.000	1.000	1.000	0.000	1.000	0.000	0.000
Tallac Enclosure 2002	1.000	1.000	0.000	1.000	1.000	0.000	1.000	0.000	0.000
Taylor Creek East 2002	1.000	1.000	0.000	1.000	1.000	0.000	1.000	0.000	0.000
Taylor Creek Mouth 2002	1.000	1.000	0.000	1.000	1.000	0.000	1.000	0.000	0.000
Taylor Creek West 2002	1.000	1.000	0.000	1.000	1.000	0.000	1.000	0.000	0.000
Upper Truckee East 2002	1.000	1.000	0.000	1.000	1.000	0.000	1.000	0.000	0.000
Upper Truckee West 2002	1.000	1.000	0.000	1.000	1.000	0.000	1.000	0.000	0.000
Zephyr Spit 2002	1.000	1.000	0.000	1.000	1.000	0.000	1.000	0.000	0.000
R. curvisiliqua									
Tallac Enclosure 2002	1.000	0.000	1.000	*	1.000	0.000	0.000	1.000	0.000
Taylor Creek Enclosure 2002	1.000	0.000	1.000	*	1.000	0.000	0.000	0.975	0.025

Appendix 1 (cont'd)

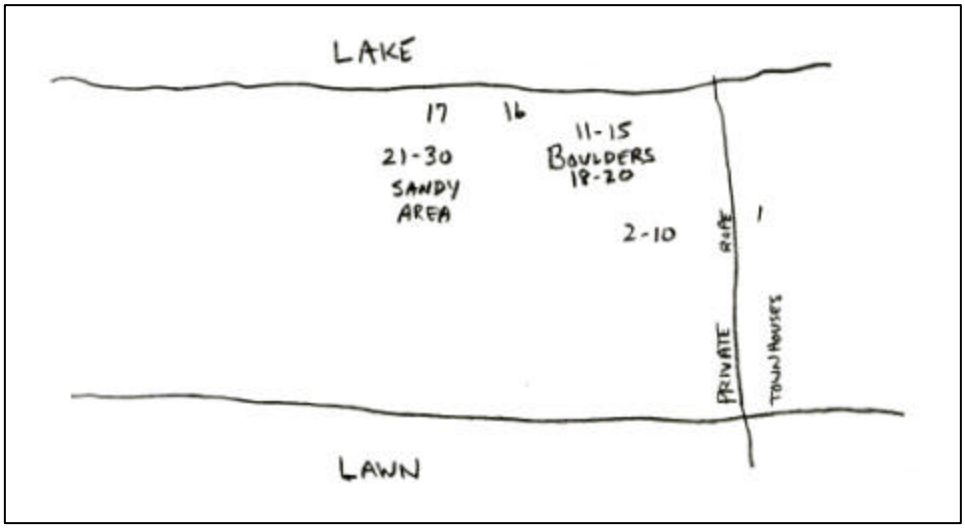
Locus	PGM1	PGM2			SKD1		TPI1		TPI2
Allele	1	1	2	3	1	2	1	2	1
Migration	43	31	33	24.5	32	37	55	51	43
Rorippa subumbellata									
4H 2003	1.000	1.000	0.000	0.000	1.000	0.000	1.000	0.000	*
Baldwin 2002	1.000	1.000	0.000	0.000	1.000	0.000	*	*	*
Baldwin 2003	1.000	1.000	0.000	0.000	1.000	0.000	1.000	0.000	*
Blackwood North 2003	1.000	1.000	0.000	0.000	1.000	0.000	1.000	0.000	*
Blackwood South 2002	1.000	1.000	0.000	0.000	1.000	0.000	*	*	*
Blackwood South 2003	1.000	1.000	0.000	0.000	1.000	0.000	1.000	0.000	*
Cascade West 2003	1.000	1.000	0.000	0.000	1.000	0.000	1.000	0.000	*
Eagle Creek 2003	1.000	1.000	0.000	0.000	1.000	0.000	1.000	0.000	*
Emerald Bay Avalanche, Native 2002	1.000	1.000	0.000	0.000	1.000	0.000	*	*	*
Emerald Bay Avalanche, Native 2003	1.000	1.000	0.000	0.000	1.000	0.000	1.000	0.000	*
Emerald Bay Avalanche, Planted 2003	1.000	1.000	0.000	0.000	1.000	0.000	1.000	0.000	*
Emerald Point 2002	1.000	1.000	0.000	0.000	1.000	0.000	*	*	*
Emerald Point 2003	1.000	1.000	0.000	0.000	1.000	0.000	1.000	0.000	*
Lighthouse 2002	1.000	1.000	0.000	0.000	1.000	0.000	1.000	0.000	*
Lighthouse 2003	1.000	1.000	0.000	0.000	1.000	0.000	1.000	0.000	*
Meeks Bay 2002	1.000	1.000	0.000	0.000	1.000	0.000	*	*	*
Meeks Bay 2003	1.000	1.000	0.000	0.000	1.000	0.000	1.000	0.000	*
Pope Beach 2003	1.000	1.000	0.000	0.000	1.000	0.000	1.000	0.000	*
Regan/Al Tahoe 2002	1.000	1.000	0.000	0.000	1.000	0.000	1.000	0.000	*
Rubicon 2002	1.000	1.000	0.000	0.000	1.000	0.000	1.000	0.000	*
Sugar Pine 2002	1.000	1.000	0.000	0.000	1.000	0.000	1.000	0.000	*
Tahoe Keys 2003	1.000	0.948	0.000	0.052	1.000	0.000	1.000	0.000	*
Tahoe Meadows 2003	1.000	1.000	0.000	0.000	1.000	0.000	1.000	0.000	*
Tallac Creek 2002	1.000	1.000	0.000	0.000	1.000	0.000	1.000	0.000	*
Tallac Enclosure 2002	1.000	1.000	0.000	0.000	1.000	0.000	1.000	0.000	1.000
Taylor Creek East 2002	1.000	1.000	0.000	0.000	1.000	0.000	1.000	0.000	*
Taylor Creek Mouth 2002	1.000	1.000	0.000	0.000	1.000	0.000	1.000	0.000	1.000
Taylor Creek West 2002	*	1.000	0.000	0.000	1.000	0.000	1.000	0.000	1.000
Upper Truckee East 2002	1.000	1.000	0.000	0.000	1.000	0.000	1.000	0.000	1.000
Upper Truckee West 2002	1.000	1.000	0.000	0.000	1.000	0.000	1.000	0.000	1.000
Zephyr Spit 2002	1.000	1.000	0.000	0.000	1.000	0.000	1.000	0.000	*
R. curvisiliqua									
Tallac Enclosure 2002	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	*
Taylor Creek Enclosure 2002	1.000	0.025	0.975	0.000	0.950	0.050	0.975	0.025	*

Appendix 1 (cont'd)

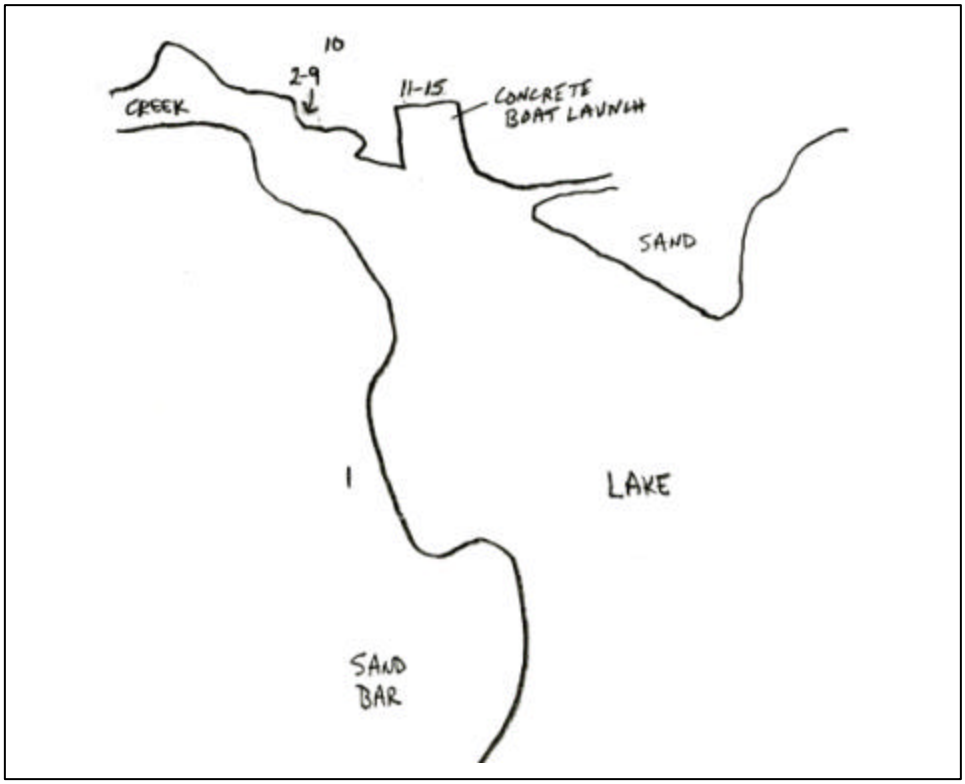
Locus	UGPP1		
Allele	1	2	3
Migration	47	40	52
Rorippa subumbellata			
4H 2003	1.000	0.000	0.000
Baldwin 2002	1.000	0.000	0.000
Baldwin 2003	1.000	0.000	0.000
Blackwood North 2003	1.000	0.000	0.000
Blackwood South 2002	1.000	0.000	0.000
Blackwood South 2003	1.000	0.000	0.000
Cascade West 2003	1.000	0.000	0.000
Eagle Creek 2003	1.000	0.000	0.000
Emerald Bay Avalanche, Native 2002	1.000	0.000	0.000
Emerald Bay Avalanche, Native 2003	1.000	0.000	0.000
Emerald Bay Avalanche, Planted 2003	1.000	0.000	0.000
Emerald Point 2002	1.000	0.000	0.000
Emerald Point 2003	1.000	0.000	0.000
Lighthouse 2002	1.000	0.000	0.000
Lighthouse 2003	1.000	0.000	0.000
Meeks Bay 2002	1.000	0.000	0.000
Meeks Bay 2003	1.000	0.000	0.000
Pope Beach 2003	1.000	0.000	0.000
Regan/Al Tahoe 2002	1.000	0.000	0.000
Rubicon 2002	1.000	0.000	0.000
Sugar Pine 2002	0.850	0.150	0.000
Tahoe Keys 2003	1.000	0.000	0.000
Tahoe Meadows 2003	1.000	0.000	0.000
Tallac Creek 2002	0.909	0.091	0.000
Tallac Enclosure 2002	1.000	0.000	0.000
Taylor Creek East 2002	1.000	0.000	0.000
Taylor Creek Mouth 2002	1.000	0.000	0.000
Taylor Creek West 2002	1.000	0.000	0.000
Upper Truckee East 2002	1.000	0.000	0.000
Upper Truckee West 2002	1.000	0.000	0.000
Zephyr Spit 2002	1.000	0.000	0.000
R. curvisiliqua			
Tallac Enclosure 2002	0.000	0.000	1.000
Taylor Creek Enclosure 2002	0.050	0.000	0.950

Appendix 2. Location maps of 2002-2003 collection sites showing genetic variation. Maps are not to scale. Individual plant collections are indicated with number.

Site = Tahoe Keys (2003 collection). Genetically variable plants = #11 and #18. (Site mapped by J. DeWoody).

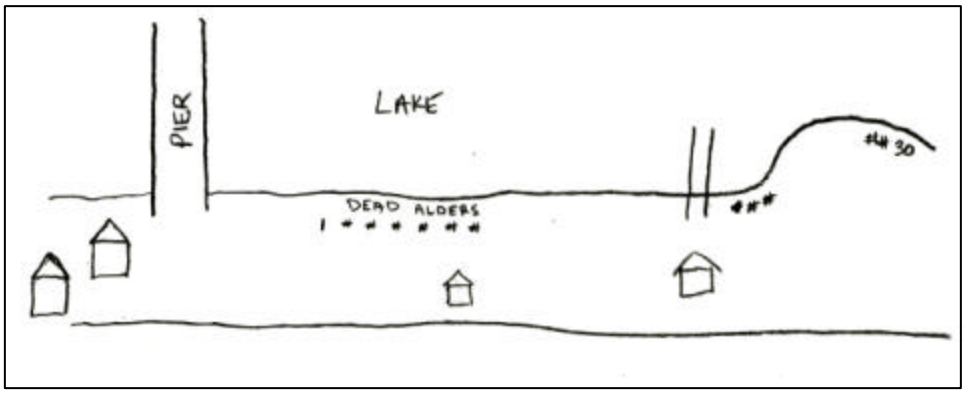


Site = Eagle Creek (2003 collection). Genetically variable plant = #3. (Site mapped by J. DeWoody).

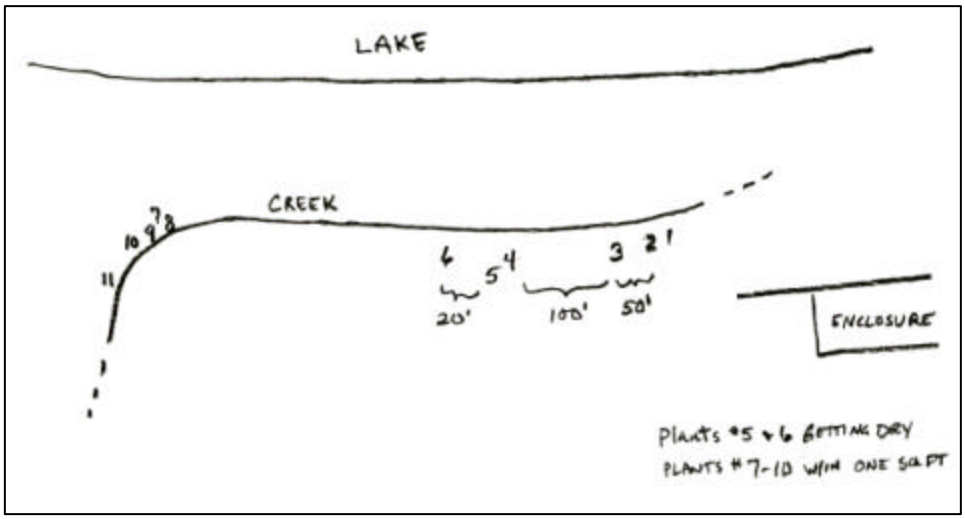


Appendix 2, continued.

Site = Sugar Pine (2002 collection). Genetically variable plants = #1 thru #9. (Site mapped by J. Fraiser). Individual plants were collected in order from #1 (northern most sample) to #30 (southern most sample).



Site = Tallac Creek (2002 collection). Genetically variable plant = #4. (Site mapped by V. Hipkins).



Appendix 3. Summary of Expenditures.

Item	Cost (dollars)
Rorippa subumbellata	
Plant material collection (24 sites, 546 plants)	
NFGEL staff (1 GS-9, 2 GS-5), 10 hrs	$924
Supplies and mileage	$120
Plant material laboratory preparation	
546 individuals/site	$1,818
Genetic Analysis	
546 individuals	$11,591
Analysis and Reporting	$310
Overhead (18%)	$2,658
Subtotal	$17,421
Rorippa curvisiliqua	
Plant material collection (2 sites, 20 plants per site)	
NFGEL staff labor	$128
Supplies and mileage	0
Plant material laboratory preparation	
40 individuals	$133
Genetic Analysis	
40 individuals	$849
Overhead (18%)	$200
Subtotal	$1,310
Total	$18,731

United States Department of Agriculture | **Forest Service** | **National Forest Genetic Electrophoresis Laboratory (NFGEL)** | **2480 Carson Road Placerville, CA 95667 (530) 622-1609 (530) 622-2633 (fax)**

CHARACTERIZING PLOIDY LEVEL VARIATION USING FLOW CYTOMETRY

NFGEL Project #167

February 5, 2004

Ploidy level was assessed in 24 individuals from three species: 5 individuals of *Lotus crassifolius,* 5 individuals of *Lupinus latifolius,* and 14 individuals of *Bromus carinatus*. The one-step PARTEC method was used for sample preparation. No incubation was used on the *Lupinus* and *Bromus* samples. *Lotus* sample used a total of 650ul staining buffer, a 5 minute incubation time, and the green celltrics filters. *Lupinus* and *Lotus* samples resolved well. *Bromus* samples were more problematic, and resolution was poor in some samples.

Lupinus

Sample 7-22 looks like a tetraploid (peak position at 180); samples 7-2, 7-3, 7-13, and 7-23 look to be diploid (peak = 90).

Lotus

No variation was observed that would indicate diploidy vs tetraploidy. All samples actually look diploid with some variation (haploid dosage?): sample #184 has a peak at position 50; the other four samples have a peak at roughly position 70.

Bromus

Observed variation may be the result of haploid dosage or aneuploidy (not simple ploidy differences). There appears to be four groups:
Peak=100 (samples 165501-5 and 161801-5); Peak=120 (146801-6, 147001-6, 141801-6, 160401-6, 141801-5, and 41101-6); Peak=140 (164801-6, 441001-5, and 147901-5); Peak=160 (166101-5, 168401-6, and 140201-6).

Date Submitted: 1/15/04 Date Report Prepared: 2/5/04
Client: USDA Forest Service, PNW, Richard Cronn

STAFF ACTIVITIES

Meetings, Shortcourses, and Workshops

Presentations

2003. E. Durant McArthur, Valerie Hipkins, and Richard Cronn. The role of population genetics in revegetation: Philosophy and empirical data. Society of Range Management Meeting. Casper, WY. February 3 – 5.

2003. V. Hipkins. Linking science to management. Presentation to the USDA Forest Service, Pacific Southwest Research Station Leadership Team. Albany, CA. May 5.

2003. V. Hipkins. Genetic issues on the National Forests. USDA Forest Service, Institute of Forest Genetics Annual Retreat. Placerville, CA. June 4 – 5.

2003. V. Hipkins. Genetic study of aspen on the Eagle Lake Ranger District, Lassen National Forest. Meeting with the USDA Forest Service and Rocky Mountain Elk Foundation. Susanville, CA. August 24 – 25.

2003. V Hipkins. Genetics in silviculture: a National perspective. USDA Forest Service, National Silvicultural Meeting. Granby, CO. September 7 – 9.

Posters

2003. DeWoody, J., V. D. Hipkins, and J. Fraser. Integrating genetic information into conservation strategies for Tahoe yellow cress, *Rorippa subumbellata* (Brassicaceae). Society for Conservation Biology, 17th Annual Meeting. Duluth, MN. June 28 – July 2.

2003. Valerie D. Hipkins, David Burton, Vicky J. Erickson, Barbara L. Wilson, and Jay Kitzmiller. Genetic diversity and structure of Quaking Aspen (*Populus tremuloides*) in the Western U.S.: Implications for conservation and management. USDA Forest Service, National Silviculture Meeting. University Granby, CO. September 7 – 9.

2003. J.H. Braatne, S.J. Brunsfeld, V. Hipkins, P. Berrang, and D. Ecker. Naturalization of Plains Cottonwood (*Populus deltoides* subsp. *monilifera*) along river drainages west of the Rocky Mountains.

2003. David L. Doede, Caitlin Cray, Joan Trindle, Dale Darris, and Valerie Hipkins. Geographic patterns of genetic variation and isozyme variation in broadleaf lupine (*Lupinus latifolius*) on the Mt Hood National Forest. Applied Ecology Meeting, Corvallis, OR. February 3.

Attended

2003. V. Hipkins, R. Saich, and J. DeWoody. Met with project collaborators to analyze Douglas-fir SSR data for Project 148 (Estimating Supplemental Mass Pollination Success in Douglas-fir). Dr. Glenn Howe and Gancho Slavov, Department of Forest Science, Oregon State University, Corvallis, OR. June 11 – 13.

2003. V. Hipkins, D. Burton, and J. Kitzmiller. Planning meeting for the Lassan NF aspen study. Susanville, CA. April 23.

Publications

2003. Hipkins, V.D., B.L. Wilson, R.J. Harrod, and C. Aubry. Isozyme variation in showy stickseed, a Washington Endemic Plant, and relatives. Northwest Science, 77:170-177.

Internal Activities

Member of the National Forest Service Safety Committee (R Meyer)
Union President – Pacific Southwest Research Station (R Meyer)

Hosted

NFGEL continues to host a variety of visitors. Tours of the facility and operation were provided to Forest Service employees, members of the public and private industry, university faculty and classes, foreign scientists, and employees from other state and federal government agencies.

Collaborations and Cooperations

NFGEL formed collaborations with FS Research Stations, Bureau of Land Management, California Department of Transportation, US Fish and Wildlife Service, University of California at Davis, private

companies, and non-profit groups. We hosted a Region 6 FS employee for one week to provide training in molecular methods. We also collaborate internally within the Agency to lend expertise in the area of genetics.

STAFFING

During FY03 (10/1/02 to 10/1/03), NFGEL was staffed with three permanent full-time, 1 TERM, and six temporary employees.

Name	Position	Term	E-mail Address
Valerie Hipkins	Director	PFT	vhipkins@fs.fed.us
Jennifer DeWoody	Lab Manager/Biologist	TERM (arrived 1/03)	jdewoody@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	rcsaich@fs.fed.us
Barbara Wilson	Scientist	Temp (4/03 – 9/03)	--
Ricardo Hernandez	Lab Biotechnician	Temp	ricardohernandez@fs.fed.us
Ashley Linsdstrom	Lab Biotechnician	Temp (arrived 6/03)	alindstrom@fs.fed.us
Bernardo Ortiz	Lab Biotechnician	Temp (arrived 6/03)	bortiz@fs.fed.us
David Villasenor	Lab Biotechnician	Temp (3/03 – 6/03)	--

BUDGET

Activity	FY02	FY03
Receipts (in thousands)		
Allocation	343.0	378.0
Carryover	0.0	52.0
Soft Money	66.2	233.5
-Fire Transfer		-30.0
Total	409.2	633.5
Expenditures (in thousands)		
Salary (permanant)	*198.5	**201
(temperary)	29.3	80.9
Overhead to Headquarters	39.6	40.0
Overhead to Site	41.5	38.2
Chemicals/Supplies	29.1	77.0
Equipment	8.1	97.6
Travel/Training	5.5	7.8
Awards	1.3	2.0
Books/subscriptions	0.3	0.4
Computers (not including FOR)	0.1	18.9
Repair	0.8	4.7
Photos/Slides/Publications	0.6	1.6
Postage	0.2	0.7
Office Supplies	0.0	0.7
Furniture	0.4	2.1
Total	355.3	573.6

* does not include $18.0 in salary due to alternate salary sources
** does not include $17.9 in salary due to alternate salary sources

FY 03 Soft Money

Source	Amount ($)	Percentage
FS-NFP (WO)	174,958	74.9%
FS-R9	7,000	3.0%
FSR-RMRS	18,000	7.7%
Private Companies	9,023	3.9%
BLM	24,500	10.5%
Total	233,481	100.0%

Project Workload, FY03

ISOZYMES (starch gel electrophoresis)

By Project

Reagion or Agency	Project#	Species	# gels	# run days	# weeks
R-1	103	*Pinus ponderosa*	116.0	14.5	7.50
R-6/BLM	125	*Pseudotsuga menziesii*	26.0	6.0	3.00
Mexico	130	*Pseudotsuga menziesii*	12.0	3.0	1.50
RMRS	132	*Atriplex canescens*	12.0	2.0	1.00
R-6	136	*Perideridia erythrorhiza*	20.0	4.0	2.00
R-5/USFWS	137	*Silene campanulata*	70.0	10.0	5.00
R-6/BLM	138	*Pinus lambertiana*	103.0	12.0	6.00
R-5/USFWS	143	*Rorippa subumbellata*	116.0	12.0	6.00
USFWS	144	*Erysimum*	3.0	1.0	0.50
Pope Ind.	145	*Pseudotsuga menziesii*	6.0	2.0	1.00
R-6	146	*Chamaecyparis lawsoniana*	7.0	2.5	2.25
Weyerhaeuser	148	*Pseudotsuga menziesii*	3.0	1.0	0.50
R-6	149	*Populus/Salix*	6.0	1.0	0.50
RMRS	151	*Balsamorhiza sagittata*	3.0	0.5	0.25
RMRS	153	*Purshia tridentata*	3.0	0.5	0.25
R-6	157	*Pinus albicaulis*	1.5	0.5	0.25
NPS	158	*Oenothera wolfii*	1.5	0.5	0.25
RMRS	159	*Oryzopsis hymenoides*	3.0	1.0	0.50
R-10	160	*Arnica lessingii*	3.0	1.0	0.50
R-6	161	*Chamaecyparis lawsoniana*	6.0	2.0	1.00
NFGEL		testing	35.0	6.5	3.25
TOTAL			**556.0**	**83.5**	**43.0**

By Forest Service Region or Agency

Region or Agency	#gels	#days	#weeks
Forest Service			
National Forest System			
R-1	116.0	14.5	7.50
R-5/USFWS	186.0	22.0	11.00
R-6	40.5	10.0	6.00
R-6/BLM	129.0	18.0	9.00
R-10	3.0	1.0	0.50
NFGEL	35.0	6.5	3.25
Research			
RMRS	21.0	4.0	2.00
National Park Service	1.5	0.5	0.25
Private Companies	9.0	3.0	1.50
USFWS	3.0	1.0	0.50
International - Mexico	12.0	3.0	1.50

R = Region
RMRS = Rocky Mountain Research Station
USFWS = United Stated Fish and Wildlife Service
BLM = Bureau of Land Management
NPS=National Park Service

DNA

By Project

Region or Agency	Project #	Species	# DNA Extractions	Extraction Method	# PCR Reactions	# ABI runs (# capillaries)	# days	# weeks	# employee hours
FS-NFS-R1	103	*Pinus ponderosa*	126	FastPrep					41.6
			252	DNEasy 96-well					31.5
FS-FSR-RMRS	105	*Viguiera multiflora*	22	DNEasy Mini					5.5
FS-FSR-RMRS	111	*Astragalus utahensis*	16	DNEasy Mini					4
			4	DNEasy 96-well					0.5
FS-FSR-RMRS	120	*Vicia americana*	90	DNEasy Mini					22.5
FS-FSR-RMRS	133	*Atriplex canescens*	76	DNEasy Mini					19
		Atriplex tridentata	20	DNEasy Mini					5
FS-FSR-RMRS	135	*Stipa comata*	20	DNEasy 96-well					2.5
FS-FSR-RMRS	140	*Artemesia tridentata*	36	DNEasy Mini					9
			4	DNEasy 96-well					0.5
FS-NFS-R5 /USFWS	143	*Rorippa subumbellata*	28	DNEasy Mini	255	5			100
FS-NFS-R6	146	*Chamaecyparis lawsoniana*	19	DNeasy Mini	152 RAPD	0	10 lab / 12 analysis	4.4	
FS-NFS-R5	147	*Panicum virgatum*	36	DNEasy 96-well					4.5
		Schizachyrium scoparium	166	DNEasy 96-well					20.8
Private	148	*Psuedotsuga menziesii*	576	DNeasy 96	920 SSR	57.5 (920)	37	7.4	
FS-FSR-RMRS	151	*Balsamorhiza sagittata*	55	DNEasy Mini					13.8
			24	DNEasy 96-well					3
FS-FSR-RMRS	152	*Crepis occidentalis*	16	DNEasy Mini					4
			4	DNEasy 96-well					0.5

DNA By Project Continued

Region or Agency	Project #	Species	# DNA Extractions	Extraction Method	# PCR Reactions	# ABI runs (# capillaries)	# days	# weeks	# employee hours
FS-FSR-RMRS	153	*Purshia tridentata*	48	DNEasy Mini					12
			4	DNEasy 96-well					0.5
Private	154	*Psuedotsuga menziesii*	3	DNeasy Mini	9 SSR	1 (9)	2	0.4	
FS-NFS-R9	155	*Pinus strobes*	420	DNEasy 96-well					52.5
NPS	156	*Pinus albicaulis*	600	DNEasy 96-well					75
FS-NFS-R6	157	*Pinus albicaulis*	440	DNEasy 96-well					55
FS-FSR-RMRS	159	*Oryzopsis hymenoides*	14	DNEasy Mini					3.5
FS-NFS-R6	161	*Chamaecyparis lawsoniana*	50	DNeasy 96	250 SSR	15.625 (250)	5 lab/ 2 analysis	1.4	
Private	162	*Populus trichocarpa, Populus nigra, Populus deltoides*	107	DNeasy 96(96)/ DNeasy Mini(11)	2400 SSR	150 (2400)	27 lab/ 27 analysis	10.8	
FS-NFS-R6	164	*Pinus* spp.	44	DNEasy Mini	75	0			70

By Region or Agency

Region or Agency	# DNA Extractions	# PCR Reactions	# ABI runs (# capillaries)	# employee hours
FS-NFS-R1	378			73.1
FS-NFS-R5	202			25.3
FS-NFS-R5 /USFWS	28	255	5	100
FS-NFS-R6	553	477	15.625 (250)	367
FS-NFS-R9	420			52.5
FS-FSR-RMRS	453			105.8
Private	686	3329	208.5 (3329)	372
NPS	600			75
TOTAL	3320	4061	230 (3659)	1170.7

FS=Forest Service
FSR=Forest Service Research
RMRS=Rocky Mountain Research Station
NFS=National Forest System
R#=Region Number
Private=Private Company
BLM=Bureau of Land Management
USFWS=US Fish and Wildlife Service
NPS=National Park Service