NATIONAL FOREST GENETICS LABORATORY (NFGEL)

ANNUAL REPORT, FY13

USDA Forest Service, Washington Office, Forest Management

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NFGEL FY13 ANNUAL REPORT

NFGEL Overview

This report covers laboratory activities and accomplishments during Fiscal Year 2013 (October 1, 2012 through September 30, 2013).

MISSION AND PURPOSE

The National Forest Genetics Laboratory (NFGEL) provides genetic testing and information for integrated solutions to on-the-ground problems faced by natural resource managers and policy makers. Solutions are provided for public agencies, non-government organizations, and private industries across the United States, often spanning geographical and organizational boundaries. NFGEL addresses conservation, restoration, and management of all plant species using molecular genetic techniques.

The purpose of NFGEL is to analyze molecular genetic markers (protein and DNA) in plant material submitted by Forest Service employees and those from other cooperating entities. NFGEL provides baseline genetic information, determines the effect of management on the genetic resource, supports genetic improvement program, and contributes information in the support of conservation and restoration programs, especially those involving native and TES (threatened, endangered, and sensitive) species. NFGEL serves the needs of the national forests and provides natural resource managers with the means for evaluating the genetic consequences of vegetation establishment actions.

STAFFING AND OPERATIONAL HIGHLIGHTS

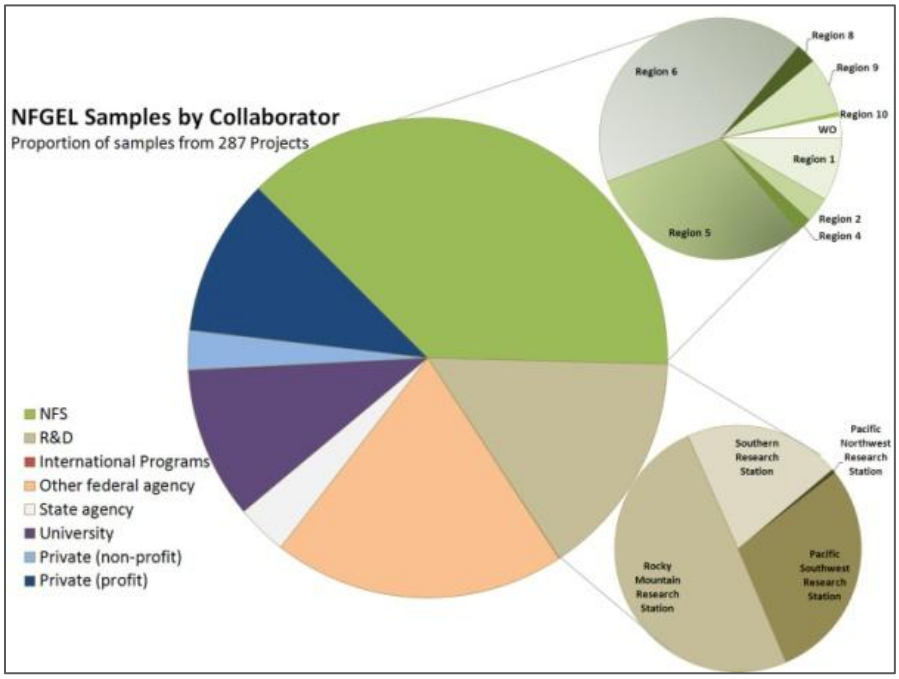
I am delighted that we were able to fill our permanent Geneticist vacancy this year with the hire of Dr. Jennifer DeWoody. Jennifer has extensive experience studying the genetics of natural plant populations. Jennifer holds a Doctorate in Biological Sciences from the University of Southampton (UK) where she examined genetic and morphological variation in European black poplar. Her Master' degree is in Ecology and Evolutionary Biology where she studied the conservation genetics of native plant species, and she holds a Bachelor's degree in Conservation Biology. In a postdoctoral position at Colorado State University, she applied some of the most current, cutting-edge molecular techniques (next generation sequencing, RNA sequencing) to plant genetic analyses, and transferred this newest technology to plant conservation projects. Welcome Jennifer!

This year brought a significant change to our Steering Committee composition. NFGEL has been historically guided by a Steering Committee made up of Regional Geneticists and a WO Forest Management lead that assisted with determining and evaluating the Lab's program of work. In order to expand the representation on the Committee to include other staff groups that have a national role in the areas of vegetation management and genetics, nominations were solicited for an expanded committee membership. The new NFGEL Steering Committee is now comprised of individuals from Regional and National groups that have an interest in the genetic assessment of our natural resources. I greatly look forward to working with the Committee to assure that NFGEL products meet Agency needs.

WORKLOAD HIGHLIGHTS

Fifteen projects providing genetic data on 11 different plant species and one butterfly were completed during Fiscal Year 2013 for a variety of partners. Project summaries are provided within this report.

In an effort to provide a comprehensive picture of the work conducted by NFGEL over our 25 year history, an MS-Access database was built to summarize the genetic studies accomplished in over 280 completed projects. The database contains the metadata for each Project, including project objectives, collaborators, species, submitted samples, and the type of genetic markers used in the study. Plans to present this information in the form of a GTR and/or peer-reviewed publication(s) will follow in FY14.



ALIGNMENT TO NATIONAL STRATEGIC PLAN

NFGEL's work is consistent with the strategic direction outlined in the USDA Strategic Plan (2011 – 2015) and the Forest Service Strategic Plan (2007 – 2012). Our work aligns to the following Agency Strategic Plan measures:

- Goal 1 (Restore, Sustain and Enhance the Nation's Forests and Grasslands)
- Goal 2 (Provide, Sustain, and Enhance Benefits to the American People).
- Goal 4 (Sustain and Enhance Outdoor Recreation Opportunities)
- Goal 6 (Engage Urban America with Forest Service Programs)
- Goal 7 (Provide Science-Based Applications and Tools for Sustainable Natural Resources Management)

Valerie D. Hipkins
NFGEL Director
October 1, 2013

NFGEL Projects

NFGEL projects were processed to meet a variety of management objectives. Project results were used to guide restoration and conservation projects, assist in silviculture and tree improvement activities, and identify illegally harvested timber. This year, NFGEL processed its first non-plant project: a genetic study to determine the taxonomic identity of a butterfly (Project #274). Fifteen project summaries are included in this Annual Report.

NFGEL PROJECTS COMPLETED IN FY13

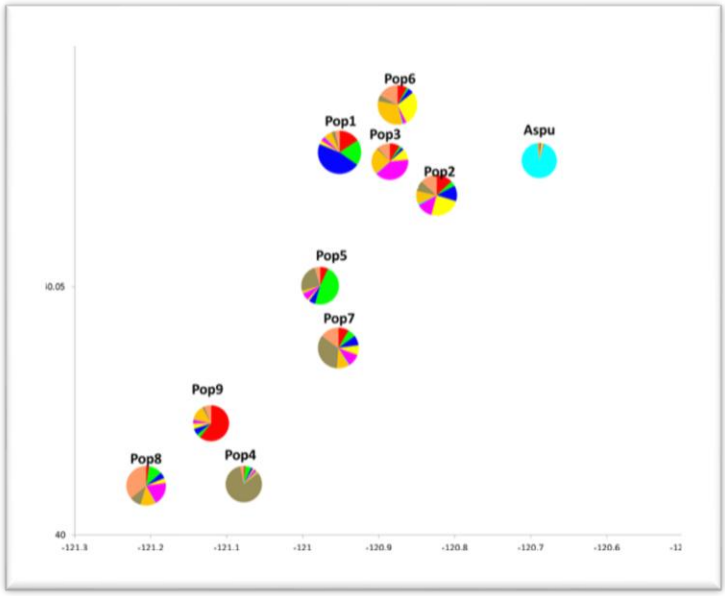
NFGEL Project #	Cooperator	Project Title
256	US Forest Service, Region 5	Genetic variation of Webber's milkvetch (*Astragalus webberi*)
278	University of California-Davis	Parentage verification in Douglas-fir
276	US Forest Service, Region 6	DNA extraction for purposes of ramet and parental identification in western white pine
264	Sierra Pacific Industries	Determining the validity of parent IDs in a Ponderosa Pine seed orchard program
265	US Forest Service, Region 6	DNA extraction from pollen: confirming parent identity or contamination in controlled crosses
269	US Forest Service, Region 6	Genetic diversity and population structure of Baker Cypress (*Hesperocyparis bakerii*)
271	US Forest Service, Region 6	Confirmation of family identities and diversity for Port-Orford-cedar inbreeding depression study
280	US Forest Service, Region 5	Genetic relatedness among resistant Port-Orford-cedar trees
272	US Forest Service, Region 6	Identification of western white pine clones at Beaver Creek Seed Orchard
283	US Forest Service, Region 5	Clonal identification of Quaking Aspen in the Bald Mountain Project Area.
284	US Forest Service, Region 6	Genetic structure of Pacific madrone (*Arbutus menziesii*)
287	US Forest Service, Region 10	DNA content variation in Alaska *Vaccinium* spp
233	US Forest Service, Region 1	Idaho fescue (Festuca idahoensis) ploidy level in the Northern Region and three agricultural releases
275	US Forest Service, Region 6 and International Programs	Big Leaf Maple timber theft
274	US Forest Service, Region 6	Taxonomic identity of putative Taylor's Checkerspot (butterfly) populations

GENETIC VARIATION OF WEBBER'S MILKVETCH (ASTRAGALUS WEBBERI A. Gray *ex* W.H. Brewer & S. Watson).

US Forest Service – Region 5. NFGEL Project # 256

New York Botanic Garden

- *Astragalus webberi* A. Gray ex W.H. Brewer & S. Watson is a rare endemic restricted to a small area of Northern California in and around the Plumas National Forest. Management plans include habitat enhancement and population augmentation projects in an effort to increase population sizes and stability. An understanding of the genetic structure of *A. webberi* will aid in management efforts.
- To investigate the genetic structure of this rare species, samples were collected from all nine extant occurrences of *A. webberi*, and from one population of *A. pulsiferae*, a common congener. Samples were examined at 18 isozyme loci to quantify measures of genetic variation and differentiation, and to qualitatively examine the genetic structure across the species range. In addition, five microsatellite loci were screened in order to determine the feasibility of a DNA-based assay.
- Low levels of allelic variation were observed. Moderate levels of observed heterozygosity were lower than the expected values, resulting in positive fixation indices. Significant differences were observed between the two species and among populations within *A. webberi*. No evidence of isolation by distance was found, meaning neighboring populations were no more likely to be genetically similar than those geographically separated.



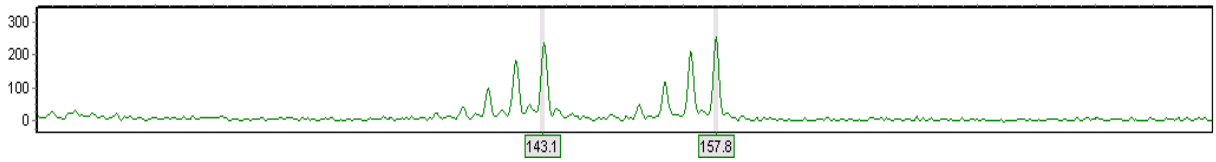
Individual assignment tests identified most populations of *A. webberi* as admixed, or composed of more than one genetic group. Some neighboring populations were assigned to the same genetic cluster, whereas other populations were highly heterogeneous. Principal coordinate analyses revealed most populations to be overlapping in genetic similarity, though two pairs of populations appeared genetically differentiated. These similarities and difference will help inform seed transfer guidelines.

PARENTAGE VERIFICATION IN DOUGLAS-FIR

University of California - Davis. NFGEL Project # 278

Eleven Douglas-fir trees were genotyped at six SSR loci using a combination of needle and megagametophytic tissue for the purpose of clonal identification. The genotype of the ramets in question matched the genotype of the ortet provided; the seed analyzed are from the designated clone; and the genotype of the ortet did not match the genotype of an alternate clone provided for analysis.

The six SSR markers were developed by the Pacific Northwest Tree Improvement Research Cooperative (PNWTIRC): 2C3, 3B2, 2G12, 3G9, 1C3, and 4A7 (the marker names are all preceded by 'OSUPCT_ssrPmOSU_'); *SMP Success, PNWTIRC protocol v3*). PCR/ABI conditions followed NFGEL Standard Operating Procedures and DNA fragments were analyzed on an ABI 3130x instrument. All data were scored by the GeneMarker software program.

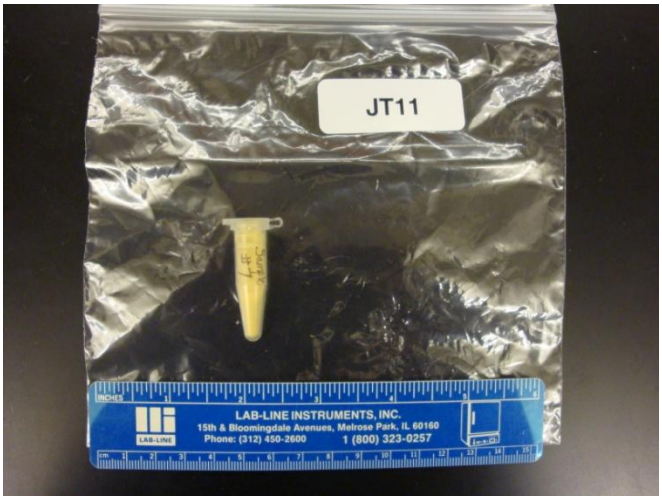


DNA EXTRACTION FOR PURPOSES OF RAMET AND PARENTAL IDENTIFICATION IN WESTERN WHITE PINE

US Forest Service – Region 6. NFGEL Project # 276

Extract DNA from western white pine (wwp) pollen and foliar samples and ship up to 10ug DNA per sample to partner in Canada for further marker development and analysis.

DNA was isolated using Qiagen DNeasy-96 Plant kits with the liquid nitrogen procedure and proteinaseK modification from a total of 18 samples: 12 pollen and 6 vegetative samples. Each sample was extracted twice to ensure that we had a final DNA yield per sample of at least 10ug. One foliar sample consisted of dead branches which were prepared with the understanding yield and quality of DNA may be poor. DNA was quantified using picogreen on a Gemini spectrophotometer.



An average DNA yield per sample of 9.1ug was obtained. Up to 10ug of DNA per submitted sample was ethanol precipitated and shipped dry to Canada.

DETERMINING THE VALIDITY OF PARENT IDS IN A PONDEROSA PINE SEED ORCHARD PROGRAM

Sierra Pacific Industries. NFGEL Project # 264

Project Background and Objectives

CalPhoto

A ponderosa pine breeding program was obtaining unexpected general and specific combining abilities within some crosses. The expectation based on previous open-pollinated testing of Diallel parents was that certain parents should be outstanding (high GCA) and others should be poor (low GCA). Two crosses that share a female parent, however, showed the reverse trend from that expected. This suggests that male parents are reversed (mis-indentified).

A project objective was to verify the parents used in two specific controlled crosses. The three parents involved in the two crosses were genotyped (two ramets per parent clone) using vegetative material, and the two seedlots were genotyped using 20 seed per lot. Meg/embryo pairs were genotyped individually to determine the maternal and paternal contribution to each seed, and therefore to check the parents of each controlled cross.

Additionally, a selfed seedlot was performing much better than expected. There is suspicion that this seedlot was not really the product of a self. *A second project objective was to check the identity of the male and female parents of the seedlot by genotyping between 10 and 20 meg/embryo pairs, and comparing that data to the genotype of ramets obtained through analyzing vegetative tissue.*

Project Results Summary

Objective 1: The parents are correctly identified in both controlled cross seedlots. However, further analysis showed that the paternal identity of the planted progeny was switched at both planting sites. Since both sites show the same switch, it is likely the labeling error occurred before material was received at the sites for planting.

Objective 2: The seedlot is not the product of a self, however the parent tree can be the maternal parent of the seedlot.

DNA EXTRACTION FROM POLLEN: CONFIRMING PARENT IDENTITY OR CONTAMINATION IN CONTROLLED CROSSES

US Forest Service – Region 6. NFGEL Project # 265

Project Background and Objectives

Being able to use DNA extracted directly from pollen collections would help in confirming parent identity or contamination in controlled crosses. Knowing the parental identities is key to the success of a breeding program. Efficiency in the breeding program can be improved because one source of contamination can be examined. The objective of this project was to achieve successful DNA isolation from *Chamaecyparis lawsoniana* (Port Orford-cedar, POC) and *Pinus monticola* (western white pine, WWP) pollen.

Methods

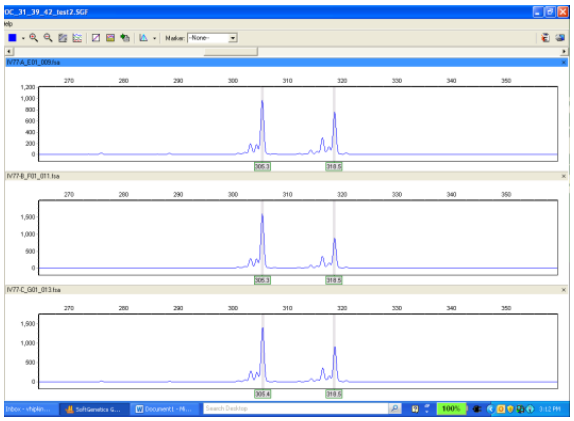
DNA was extracted using the Qiagen DNEasy-96 plant kit with the liquid nitrogen and proteinaseK modifications. Because the DNEasy filter-based kits are sensitive to an excess of sample material, three amounts of pollen were extracted per species based on both volume and weight (WWP: between 7 and 25mg; POC: between 10 and 40mg). DNA concentrations were quantified using a Gemini XPS Microplate Spectrofluorometer with PicoGreen.

Western White Pine. SSR methodology from: Liu, J.-J., Sniezko, R. A., and Ekramoddoullah, A. K. M. 2011. Association of a novel *Pinus monticola* chitinase gene (*PmCh4B*) with quantitative resistance to *Cronartium ribicola*. Phytopathology 101:904-911. All reactions were performed as single, per locus, PCR amplifications. PCR reactions were multiplexed in groups of three and run on an ABI-3130xl using ROX-500.

Port Orford-cedar. SSR methodology from: Tara N. Jennings, Brian J. Knaus, Scott Kolpak, and Richard Cronn. 2011. Microsatellite primers for the Pacific Northwest endemic conifer C*hamaecyparis lawsoniana* (Cupressaceae). American Journal of Botany: e1–e3. All reactions were performed as single, per locus, PCR amplifications. PCR reactions were multiplexed in groups of three to four and run on an ABI-3130xl using ROX-500.

Results Summary

DNA was successfully extracted from all three preparations of both pollen samples. DNA yields from the three WWP extractions ranged from 10.5ug to 14.5ug. DNA yields from the three POC extractions ranged from 10.6ug to 28.5ug. Extractions were carried out carefully and cleanly to eliminate potential contamination. The presence of DNA was not detected in our negative control samples.



All DNA extractions amplified cleanly using the species specific primers. There was no amplification in negative control samples.

GENETIC DIVERSITY AND POPULATION STRUCTURE OF BAKE R CYPRESS (*HESPEROCYPARIS BAKERII*)

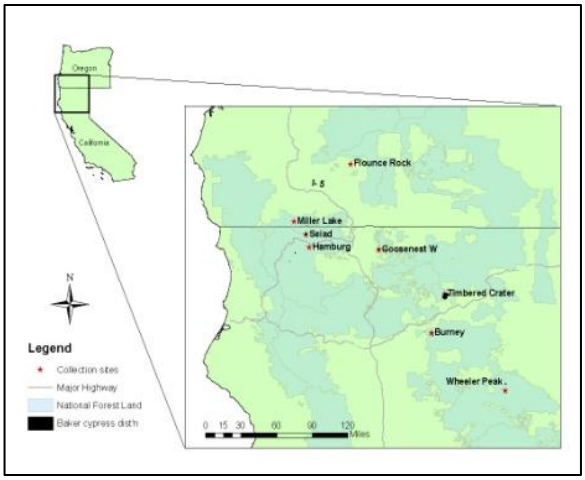
US Forest Service – Region 6. NFGEL Project # 269
(Andy Bower and Tom Blush)

Hesperocyparis bakerii (Baker cypress or Modoc cypress) is found on appoximately 12 isolated stands in northern California and southwestern Oregon. It has serotinous cones and is dependent on fire for reproduction. Many of the existing stands are suffering because of fire suppression which has allowed other conifer species to encroach into these stands. This species was also rated highly vulnerable to the potential effects of climate change in a vulnerability assessment for southwestern Oregon. Seed is to be collected from all existing stands for gene conservation, but to-date no information is available on the level of genetic diversity of population structure of this species.

Fresh foliage samples were collected from 30 widely spaced individuals at 8 of 11 stands across the range of the species. Fourteen isozyme loci were utilized to assess genetic diversity and population structure.

CONCLUSIONS

- Flounce Rock, the smallest (<1 acre) and northernmost population of cypress had lower % polymorphic loci (35% vs. 58% across all other populations), lowest H_o, and was genetically differentiated from all other populations. This population may have gone through (or is currently experiencing) a bottleneck which has led to fixation of several alleles and a reduction in genetic diversity.
- Despite the lower genetic diversity, there was no evidence of a heterozygote deficiency at the Flounce Rock site.
- Because Baker cypress seed lacks a wing and is only released after fires, it is possible that familial "islands" of siblings establish after fires, leading to some inbreeding and the observed heterozygote deficiency in many populations.
- Except for Flounce Rock, little population differentiation was observed among sites, with pairwise variation among sites of only 1.8 - 9.1%.



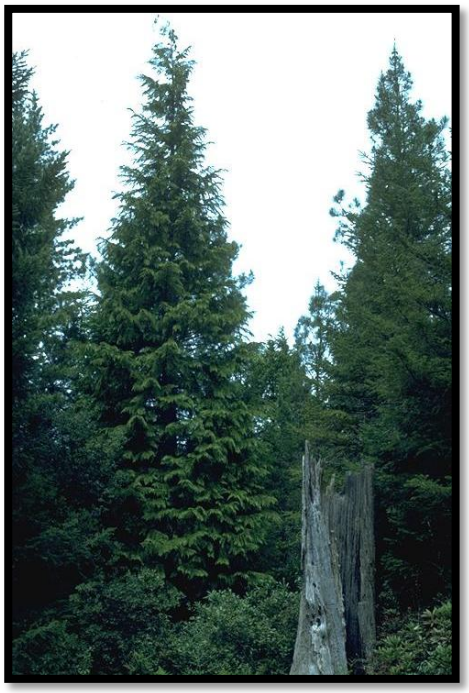
CONFIRMATION OF FAMILY IDENTITIES AND DIVERSITY FOR PORT-ORFORD-CEDAR INBREEDING DEPRESSION STUDY

US Forest Service – Region 6. NFGEL Project # 271

Differences in height between pairs of selfed and open pollinated families was measured in an inbreeding depression study in Port-Orford-cedar. In most pairs, the mean height was greater in the open pollinated families than in the selfed families. However, there were six pairs in which the selfed families had a greater mean height.

Project objectives include:

1. Confirm that the individuals in the S_1 families from 19 parents tested are indeed progeny of that particular selfed mating.
2. Confirm that the seedlings from the open pollinated families are progeny of that particular seed parent.
3. Determine the relative diversity of the 19 open pollinated families to see if the level of pollen parent diversity is correlated with vigor.



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Selfing of Port-Orford-cedar trees is desirable to develop populations of disease resistant trees and to produce resistant seed for reforestation and restoration. However, a major drawback to selfing in other species has been inbreeding depression. The evaluation of the 19 self-pollinated families will provide a first look of the potential level of contamination in control crosses as well as confirm that there are 100% S_1 progenies. The examination of diversity in the 19 open-pollinated families will provide some basic information on level of genetic diversity generated in containerized orchard of POC, as well as confirming they provide the proper contrast in the S_1 vs OP test. NFGEL genotyped 804 *Chamaecyparis lawsoniana* (Port Orford-cedar, POC) trees at eight SSR loci to answer project objectives.

Summary

(1) There are genotype mismatches among ramets in five of the 19 parent clones.
(2) Similar levels of genetic diversity was observed among open pollinated families.

There is some contamination in the S_1 and OP progenies of some families. Nine seed parents have no detectable contamination issues within their S_1 or OP families. Four of the seed parents had some minor levels of outcrossing in the S_1 families, or seed contamination in the OP families. The remaining six seed parents had significant contamination issues which are summarized in the full report.

GENETIC RELATEDNESS AMONG RESISTANT PORT-ORFORD-CEDAR TREES

US Forest Service – Region 5. NFGEL Project # 280

Project Background and Objectives

A plot was established to test for *Phytophthora lateralis* resistance in a diseased area. Testing resulted in identifying at least 5 different young *Chamaecyparis lawsoniana* (Port Orford-cedar, POC) trees showing high levels of resistance in both stem dip and root dip testing. All of these resistant plot trees are with 200 feet of each other and may have come from the same female parent.

The POC plot is showing zero mortality on 5 different trees from the stem and root dip testing. If each of these trees is unrelated then they could all be candidates for inclusion into an orchard /clone bank or a breeding program. If a resistant parent tree is identified then that parent tree could be used for collecting resistant seed for reforestation or restortation projects.

Project objectives include: (1) determine if any or all of the resistant young trees are related to each other, (2) genotype nearby older (seed bearing trees) to possibly identify the resistant parent tree or trees, and (3) verify that the clones at DGRC actually match their 'parents' in the field.

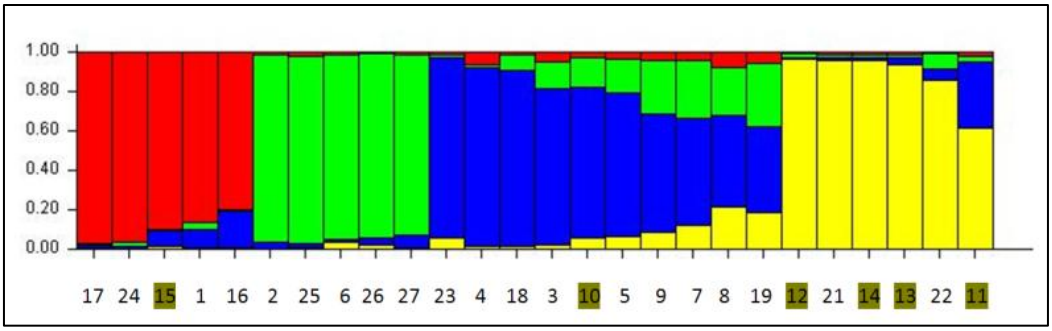
Summary

Objective 1: Genetic data at eight SSR loci showed that there is some potential relatedness within this group of six trees. Trees 11, 12, 13, and 14 are indicated as being a group of related trees. Trees 12, 13, and 14 form a group of related trees with putative half or full-sib relationships. Tree 11 shows a putative half-sib relationship with tree 14, but is seemingly unrelated to the other two trees. Trees 10 and 15 are unrelated to any tree in this group.

Although the significance values in the sibship analysis were moderately low, the combination of all analyses indicates that trees 12, 13, and 14 share enough relatedness (potentially as high as half or full sib relationships) that caution should be taken in including all three trees in future orchard/clone bank or breeding programs.

Objective 2: Genetic data indicate that trees 26 and 27 (the two seed bearing old growth trees) can not be a parent to any of the resistent trees (#10 – 15), or to any other tree in the plot.

Objective 3: Each of the five trees submitted from Dorena matches its respective 'parent' from the field, indicating that the Dorena clones are labeled correctly and do match their respective field 'parent'.



IDENTIFICATION OF WESTERN WHITE PINE CLONES AT BEAVER CREEK SEED ORCHARD

US Forest Service – Region 6. NFGEL Project # 272

The Beaver Creek Seed Orchard was established with western white pine selections from the blister rust resistance program. Unfortunately, many of the establishment records have been lost. Some of the clones do not have tags and some may be ungrafted rootstock. There are 57 identified clones, 51 of which have survivors. Some individuals at Beaver Creek have multiple stems; one stem might be a surviving graft or they may all be rootstock. This project will enable us to rogue this orchard and pollinate more efficiently.

Project objectives include:

1. Confirm that all ramets that are putatively the same clone are the same clone.
2. Confirm that ramets match the original ortet.
3. Match any of the untagged trees to an identified tree.
4. Determine if the any stem from a tree with multiple stems can be matched with a 'known' individual.

Results Summary

Genetic Markers

Data was obtained for six SSR and 64 SNP loci. The SSR data were able to resolve 78 unique genotypes among the 242 samples. With the addition of the SNP data, we were able to distinguish an additional 13 genotypes yielding a total of 91 unique genotypes among all submitted samples. The Probability of Identity (PI) (the average probability that two unrelated individuals, drawn from the same population, will by chance have the same multilocus genotype) is 3.7×10^{-3} and 3.4×10^{-15} for SSR and SNP data, respectively. For the combined data, the PI is 1.2×10^{-17}, indicating ample discriminatory power of the data for distinguishing unique genetic individuals.

Objective 1. Confirm that all ramets that are putatively the same clone are the same clone.

Of the 51 designated clones, 16 of them had some level of genotype mismatches among samples. These discrepancies included non-matching genotypes between ramets of a clone, mismatching genotypes among clones located at different orchards, or matches of genotypes between two different designated clones (suggesting mislabeling of some trees). When two different clones have the same genotype, it is either due to chance, or there is mislabeling as to correct clonal identity of one or more of the trees.

Objective 2. Confirm that ramets match the original ortet.

Twelve trees representing 6 ortets were received from the Dorena and Horning orchards. Four of the Dorena ortets matched their corresponding Beaver Creek ramets. One Dorena ortet did not match the corresponding Beaver Creek ramets. Another Horning ortet also did not match its corresponding Beaver Creek ramet.

Objective 3. Match any of the untagged trees to an identified tree.

Of the 242 samples analyzed, 116 of them were untagged and had no clonal designation indicated. The genotypes of 56 of these matched up with known clones. The remaining 60 samples matched with no known clone in the study. Some of these untagged trees matched each other while 18 of the trees were unique and matched no other sample in the study. These may be unidentified clones, overgrown root stock, or the inability of the data to discriminate samples.

Objective 4. Determine if the any stem from a tree with multiple stems can be matched with a 'known' individual.

Multiple stems were collected from 21 trees resulting in a total of 53 samples. Genotypic comparisons among stems within trees is provided in the full report.

CLONAL IDENTIFICATION OF QUAKING ASPEN IN THE BALD MOUNTAIN PROJECT AREA

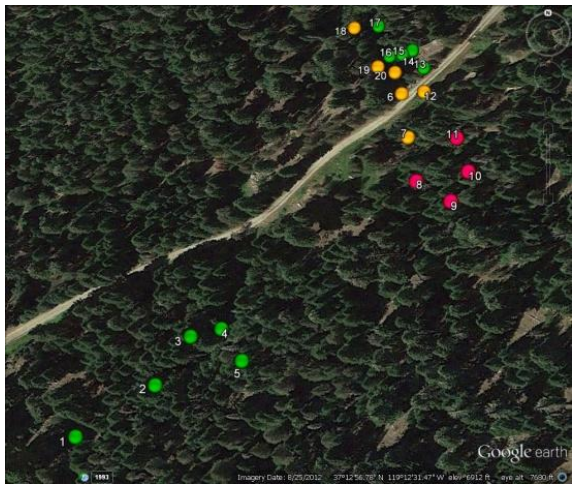
US Forest Service – Region 5. NFGEL Project # 283

The aspen stands in the Bald Mountain project are limited in size, distribution and quantity. Although aspen does reproduce sexually through seed, it is known for its ability to reproduce vegetatively by root suckers, resulting in groups of trees that are genetically identical. These asexually formed stems, or ramets, comprise a single clone. The goal of this project was to provide genetic data to address three objectives:

1. What are the existing genotypes of quaking aspen in the Bald Mountain project aspen restoration areas?
2. Are the aspen clones in the Bald Mountain project related?
3. How genetically similar/different are the stands?

Methods

A total of 93 aspen samples were genotyped at twelve microsatellite (SSR) loci following NFGEL SOPs. PCR products were separated using an ABI3130xl capillary electrophoresis system and visualized using GeneMarker software (v 1.6).



Summary of Results

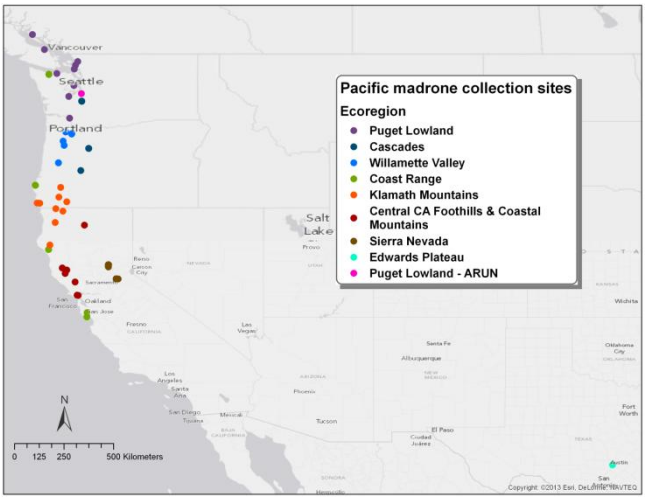
Objective 1: There were 12 different genotypes (or clones) among the 93 trees analyzed. No genotype was shared among the five stands. Two of the five stands were monoclonal; the remaining three stands each contained two or more clones. The Probability of Identity (the average probability that two unrelated individuals, drawn from the same population, will by chance have the same multilocus genotype) is 2.2×10^{-10}.

Objective 2: Sibling relationships were identified using ML-RELATE software (Kalinowski et al 2006) with allele frequencies corrected for the presence of null alleles. Also, relatedness (r) was calculated via GenAlEx using the Ritland and Lynch estimator (Peakall and Smouse 2012). When calculating relatedness, r values vary by the degree of the relationships. Genetic data at twelve SSR loci showed that individuals between stands are not related. Values of 'r' between stands varied from -0.120 and -0.052 (average of -0.079). Results from ML-RELATE also indicate that there are no detected relationships between trees that are located in different stands. In some instances, trees within stand may be related.

Objective 3: The five aspen stand in the study are quite genetically dissimilar. No clone is shared between stands, the average Nei's unbiased genetic identity equals 0.559, and Fst equals 34.6%. Although there is high genetic differentiation among stands, there is a positive correlation between genetic and geographic distance (R^2=0.356, p=0.01). This means that stands located more closely to each other are more genetically similar than stands located far apart.

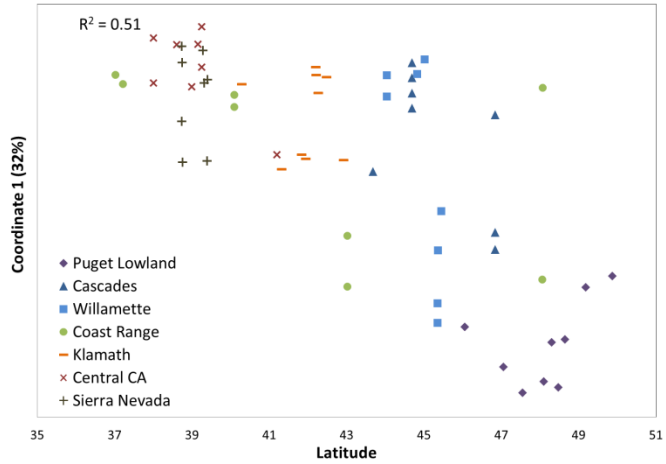
GENETIC STRUCTURE OF PACIFIC MADRONE (*ARBUTUS MENZIESII*)

US Forest Service – Region 6. NFGEL Project # 284



Pacific madrone (*Arbutus menziesii* Pursh) is an evergreen tree with a range from southern British Columbia to southern California. A range-wide collection of trees are currently the subject of a fully replicated common garden experiment to examine fitness and pathogen resistance traits in the species. The availability of neutral genetic markers would benefit the analysis and interpretation of the adaptive genetic variation. This study served as a preliminary assessment of genetic variation in a subset of Pacific madrone from the common garden experiment. Initial tests conducted at NFGEL using DNA based markers failed, likely due to the distance of *Arbutus* from the species for which the markers were developed. Here, isozyme markers were used to assess diversity and differentiation in 58 samples of *A. menziesii* from the Pacific coast, plus one sample from Texas and one sample of *A. unedo*, as an outgroup. The objective of the study was to determine if isozymes resolve sufficient genetic variation to justify a larger study examining half-sib families and intra-population structure.

Low levels of genetic diversity as measured by isozymes was observed (40% loci were polymorphic, a maximum of 3 alleles were observed at a locus, and mean heterozygosity was 0.16). Significant allele frequency variation was detected, indicating ecoregions are genetically differentiated across the landscape (F_{ST} = 0.16, $P < 0.001$). Tests of isolation by distance and correlation with latitude indicate the genetic differentiation occurs across a latitudinal cline. The low level of allelic variation indicates isozyme markers are likely insufficient to address fine-scale questions of genetic structure (e.g. family-level analyses).



Isozymes revealed significant differentiation and a latitudinal cline across the sampling area of Pacific madrone, indicating multiple genetic groups are included in the common garden experiments. However, given the low number of alleles per locus and low variation observed within many ecoregions, isozyme markers likely resolve insufficient variation for analyses of intra-population patterns. It is unlikely that these markers would provide enough power to examine fine-scale differentiation, much less within-family variation.

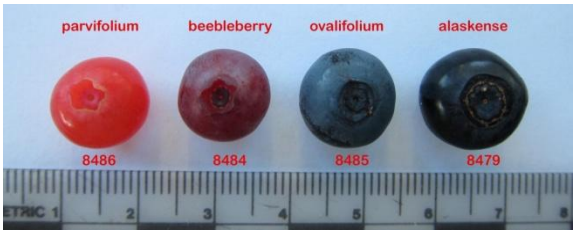
DNA CONTENT VARIATION IN ALASKA *VACCINIUM* SPP

US Forest Service – Region 10. NFGEL Project # 287

Vaccinium species are important understory shrubs described as being morphological variable. In Flora of North America, *Vaccinium alaskaense* has been subsumed into *V. ovalifolium*. However, morphological fruit and flower characters suggest that these are separate entities. Understanding species boundaries in closely-related groups is critical to managing for biodiversity, especially in context of a changing climate. Ploidy distinctiveness in either Beebleberry or putative *V. alaskaense* will provide evidence of the need for additional genetic or systematic analyses of these populations.

Four taxa were included in the analysis: *Vaccinium parvifolium*, *V. ovalifolium*, putative *V. alaskaense* (currently subsumed into *V. ovalifolium*), and a putative hybrid, "Beebleberry". The DNA content was examined for each sample in order to address the two project objectives:

1. Is *V. alaskaense* distinct from *V. ovalifolium*?
2. Is "Beebleberry" a hybrid between *V. parvifolium* and *V. ovalifolium*?



The relative DNA content of each sample was determined using a Partec Ploidy Analyzer. The mean DNA value was consistent in Beebleberry, *V. ovalifolium*, and *V. parvifolium*, but was significantly greater in *V. alaskaense*. These results provide evidence that the putative *V. alaskaense* is distinct from *ovalifolium* in either ploidy level or genome size. Since there is no evidence of genome content variation between the putative hybrid (Beebleberry) and potential parent species, these data provide no evidence of a hybrid origin of Beebleberry. However, flow cytometry data cannot rule out a hybrid origin as it is possible the two parental species are close enough related to produce functional diploid (homoploid) hybrid offspring.

IDAHO FESCUE (*Festuca idahoensis*) PLOIDY LEVEL IN THE NORTHERN REGION AND THREE AGRICULTURAL RELEASES [revised report]

US Forest Service – Region 1. NFGEL Project # 233

Project Goals: Idaho fescue has been identified as one of the core species in the native plants program in the Northern Region. Central to an effective seed collection and seed increase endeavor is an increased understanding of the genetics of the species. Specific project objectives include: (1) Does Idaho fescue have a similar chromosome count as compared to related Festuca spp? (2) Is the ploidy level consistent across the Region or variable? If variable, can source origin information be linked to a specific ecotype? (3) Is the ploidy level among three agricultural releases comparable to native collections? The NFGEL Lab Report was completed in October 2009.

Summary: The original report was revised in April 2013 to include comments and information correlating leaf blade color to DNA content. A trend of greater DNA content in greener leaves was noted.

BIG LEAF MAPLE TIMBER THEFT

US Forest Service – Region 6, International Programs. NFGEL Project # 275

Planks of Big Leaf Maple (*Acer macrophyllum)* were seized by US Forest Service Law Enforcement and Investigation as part of an alleged illegal timber harvest. Genetic markers are being used to match the planks to the stumps in the harvest area. Genetic testing is a tool that can be used to combat illegal logging and support the US Lacey Act. The investigation is pending.

TAXONOMIC IDENTITY OF PUTATIVE TAYLOR'S CHECKERSPOT (BUTTERFLY) POPULATIONS

US Forest Service – Region 6. NFGEL Project # 274

There are some genetics questions about a species of butterfly located in the Pacific Northwest. The Taylor's Checkerspot is a Federal Candidate Species, and there have been three new populations identified on the Olympic National Forest at elevations considerably higher than other known populations (~3000' vs. sea level). Managers would like to know if these populations are the same species (or varieties) as the low elevation populations, and also whether these populations differ genetically. NFGEL extracted DNA from approximately 415 samples of butterflys and larvae, and amplified each sample at 8 SSR loci. Data was scored and binned, and sent to project partners for analysis and reporting.

Summary (provided by Paul Severns and Andy Bower)

- We used DNA markers to assess patterns of genetic diversity between 9 populations of *Euphydryas editha taylori* (Taylor's checkerspot) and 2 populations of the closely related *Euphydryas editha colonia* (Hurricane Ridge, Olympic National Park, WA and Fairview Peak, Lane Co. OR).
- There was strong DNA marker evidence for 5 genetically distinct Taylor's checkerspot populations: 1) Olympic National Forest sites, 2) Dan Kelly and Eden Valley, 3) Sequim, 4) Range 76, and 5) Cardwell Hills. Both populations of *Euphydryas editha colonia* were genetically distinct from one another and levels of genetic differentiation between some populations of Taylor's checkerspot were as great as the differences between *E. e. colonia* and Taylor's checkerspot.
- Within the genetically distinct population groups, the 3 Olympic National Forest sites showed no genetic differences among sites, indicating that these sites are a single population. These were the only sites that showed this genetic similarity among sites.
- Estimates of within population allelic diversity suggest some inbreeding but we refrain from formally estimating the within population fixation values because missing data and null alleles render this estimate biologically inaccurate with the current data.
- Genetic groupings could be used as management units but the occuppied ecological niches, habitat association and patterns of host plant use should also be considered within each management unit.
- DNA markers suggest the potential for more than one taxon to exist within the populations currently considered *E. e. taylori* and *E. e. colonia*.

Budget

NFGEL receives an annual allocation from the National Forest System's Forest Management staff group. From FY09 – FY13, NFGEL received $480,000 each year. In addition to these funds, NFGEL expended $59,190 individual partner program dollars collected for non-NFVW projects in FY13. These dollars were used for additional salary, chemical, supply, equipment, repair needs, and travel.

FY13 NFGEL BUDGET

ALLOCATION		
	WO - Forest Management	$480,000
	USFS-R5 (NFGEL Project 280)	$1,945
	USFS-R6 (NFGEL Project 274)	$11,479
	BLM (NFGEL Projects 228 and 232)	$38,863
	USFS International Programs (travel)	$5,053
	Greenwood Resources (NFGEL Project 285)	$1,850
	TOTAL	$539,190
EXPENDITURES		
	Salary - Permanent Employees	$241,527
	Salary - Temporary Employees	$113,383
	Site Utilities and Rents	$16,800
	Chemicals and Supplies	$76,076
	Equipment and Repair	$54,438
	Computer and Office Supplies	$11,477
	Postage	$1,092
	Administrative Costs (page charges, lynx passes)	$2,894
	Hazardous Waste Removal	$957
	Vehicle	$5,082
	Travel and Training	$15,683
	TOTAL	$539,409
BALANCE		$-219

Staffing and Organization

NFGEL STAFF

During FY 2013, NFGEL was staffed with 2.5 permanent FTEs, and multiple staff on temporary tours. Temporary employees accounted for 3.2 FTEs for the reporting year.

EMPLOYEE	POSITION	TOUR (% FTE)	DATES
Valerie Hipkins	Director	Permanent (100%)	10/1/12 – 9/30/13
Jennifer DeWoody	Geneticist	Permanent (92%)	11/5/12 – 9/30/13
Randy Meyer	Lab Biotechnician	Permanent (56%)	10/1/12 – 9/30/13
Courtney Owens	Lab Biotechnician	Temp-NTE (100%)	10/1/12 – 9/30/13
Jody Mello	Lab Biotechnician	Temp-Pathways (81%)	10/1/12 – 9/30/13
Rosanna Hanson	Lab Biotechnician	Temp-NTE (100%)	10/1/12 – 9/30/13
Michael Benedict	Lab Aid	Summer Temp-Pathways (17%)	6/24/13 – 8/23/13
Garrett Short	Lab Aid	Summer Temp-1039 (6%)	7/1/13 – 8/2/13
Steffen Mahnke	Lab Aid	High School Volunteer (2%)	8/12/13 – 9/30/13
Andrew Jackson	Lab Aid	High School Volunteer (2%)	8/12/13 – 9/30/13
Keenan Raleigh	Lab Aid	High School Volunteer (2%)	8/12/13 – 9/30/13
Garrett Short	Lab Aid	High School Volunteer (10%)	8/10/12 – 5/24/13

NFGEL STEERING COMMITTEE

NFGEL is guided by a Steering Committee made up of Agency professionals with an interest in the genetic assessment of our nation's resources. Steering Committee members:

1. oversee and ensure the accomplishments of the agreed upon work of NFGEL,
2. assist in setting national priorities for NFGEL workload, and
3. assist in securing necessary resources to accomplish the program of work.

Member	Position	Location
Kara Chadwick, Chair	Assistant Director - Forest Management	Washington Office, Washington DC
Tom Blush	Regional Geneticist	Region 5, Placerville CA
Barbara Crane	Regional Geneticist	Region 8, Atlanta GA
Randy Johnson	National Program Leader, Genetics and Global Change Research - Forest Management Sciences	Washington Office, Washington DC
Gary Man	Acting Assistant Director - Cooperative Forestry – Urban and Community Forestry Program	Washington Office, Washington DC
Dave Merritt	Riparian Plant Ecologist - Watershed, Fish, Air, and Rare Plants	STREAM (RMRS), Ft. Collins CO
Larry Stritch	National Botanist - Rangelands Management & Vegetation Ecology	Washington Office, Washington DC

Staff Activities

PUBLICATIONS

Potter, KM, VD Hipkins, MF Mahalovich, and RE Means. 2013. Mitochondrial DNA haplotype distribution patterns in *Pinus ponderosa* (Pinaceae): Range-wide evolutionary history and implications for conservation. American Journal of Botany 100(8):000-000 [10.3732/ajb.1300039].

Vargas-Hernández, JJ, DL Rogers, and V Hipkins. 2013. Restoration of threatened *Pinus radiata* on Mexico's Guadalupe Island. *In*: Bozzano M., Jalonen R., Thomas E., Boshier D., Gallo L., Cavers S., Bordacs S., Smith P., and Loo J. (eds). Genetic considerations in ecosystem restoration using native tree species. A thematic study for the State of the World's Forest Genetic Resources. United Nations Food and Agriculture Organization, Rome, Italy.

INTERNAL ACTIVITIES

Presentations

"Plant DNA and Forensic Science". Presentation given in support of International Programs effort in Lacey Act enforcement (illegal harvesting/exportation/importation of timber). Hamburg Germany, December 2012. (Hipkins)

"Molecular Genetics and Resource Management". US Forest Service Region 6 National Resources meeting, Hood River OR. December 2012. (Hipkins)

Informal presentation, technology transfer from the Plant and Animal Genome Meetings, "PAG XXI: Notes from the not-too-distant future", February 22, 2013. (DeWoody)

"Using DNA to Combat Illegal Logging in the Forest Service". Presentation given in support of International Programs effort in Lacey Act enforcement (illegal harvesting/exportation/importation of timber). Washington DC., April 2013. (Hipkins)

"Climate Change: Managing lands in an uncertain future". Youth Environmental Leadership Conference. Sponsored by the US Forest Service, Eldorado National Forest. May 19, 2013. (DeWoody)

"Integrating Molecular Genetics into Resource Management". National Advanced Silviculture Program, Genetics and Nursery modules. June 2013. (Hipkins)

Participated in briefing on reforestation and NFGEL provided to the USDA Deputy Undersecretary for Natural Resources, Butch Blazer (also in attendance were Steve Hart, Dave Atkins, Kara Chadwick, Jim Pena). June 2013. (Hipkins)

"Genetic differentiation among species of *Fritillaria* in Northern California". Botanical Society of America joint meetings, New Orleans, LA. July 31, 2013. (DeWoody)

Attendance

US Forest Service, Washington Office, Forest Management Retreat. Washington DC. February 2013. (Hipkins)

Forest Genetics Conference. Whistler, British Columbia. July 2013. (Hipkins)

Technical Review

Reviewed McIntire-Stennis/Hatch Act proposal for University of Wisconsin – Madison, October 2012. (Hipkins)

Reviewer for Tree Genetics and Genomes. (Hipkins)

Team Participation

Participant in multiple Deputy Area Forest Service effort to assess and prioritize forest trees at risk ("Conservation Assessment and Prioritization of Forest Trees Under Risk of Extirpation"). (Hipkins)

Member of ENF Workforce Planning Team. (Meyer)

Member of USDA Forest Service National Safety Committee (January 2001 - Current). (Meyer)

Member of the PSW-RS Safety Committee January 2002 – Current. (Meyer)

Member of the PSW-RS Placerville Safety Committee January 2013 – Current. (DeWoody, Meyer)

Member of the PSW-RS Community Enhancement and Diversity (civil rights) Team – January 2003 – Current. (Meyer)

Provided to the Albany PSW Headquarters - 100 Valley Oaks for their Earth Day Celebration. (Meyer)

NFFE Forest Service Council, Union Representative on USDA Strengthening Services Administrative Solutions (SSAS) Homeland Security Workgroup. (Meyer)

Technology-Transfer

Visited Pacific Northwest Research Station and the University of Oregon (Corvallis) to discuss high-throughput sequencing techniques and gather information for tech-transfer to NFGEL, May 20-22, 2013. (DeWoody)

Assisted with a genetic study of clonal variation in aspen in isolated mountain habitats in Texas led by Jerritt Nunneley and Oscar Van Auken, University of Texas at San Antonio. (DeWoody)

Plant Sample Collections

Collected big leaf maple plant samples for Project 275. Washington. December 2012. (Hipkins)

Collected *Vaccinium* plant samples for Project 282. June-July 2013. El Dorado County. (DeWoody)

Union Activities

Union President – Pacific Southwest Research Station (PSW-RS), and Union Steward Eldorado National Forest (ENF) – Region 5. (Meyer)

NFFE Forest Service Council, Safety (Meyer)

NFFE Forest Service Council, Safety Committee Chair (2006 – Current).

NFFE Forest Service Council, Union Representative, Check-in Check-out.

NFFE Forest Service Council, Union Representative, Work Group, ATV/UTV Handbook update.

NFFE Forest Service Council, Union Representative on Accident Investigations.

- Learning from a Traumatic Event, Pikes Peak Suicide.
- Schoolhouse Fire ATV Fatality, Team Member.

NFFE , Union Representative, You Will Not Stand Alone, Presentation on the Union's Role during the Investigation Process.

NFFE , Union Representative, Federal Advisory Council on Occupational Safety and Health (FACOSH) on Field Federal Safety and Health Council (FFSHC) Improvements.

NFFE Forest Service Council, Union Representative on USDA Strengthening Services Administrative Solutions (SSAS) Homeland Security Workgroup.

NFFE Forest Service Council, Union Representative, NLC Safety Core Next Steps – Safety Journey.

NFFE Forest Service Council, Union Representative, Coordinated Response Protocol

HOSTED

NFGEL hosts a variety of visitors throughout the year. Tours of the facility and operation are provided that range from simple walk-through visits of the laboratory (usually 30 – 60 minute duration) to more extensive experiences where visitors get hands-on opportunities to extract DNA, work with liquid nitrogen, pipette liquids, dissect owl pellets, and explore other forest conservation and restoration efforts including soil stability, bark beetle biology, and forest tree disease pathology (1 – 6 hour duration).

TOUR DATE	TOUR GROUP	NFGEL GUIDES
10/2/12	Charter School. 30 students. Grades 7th – 11th.	Hipkins
10/3/12	Intern from Russia (sponsored by Lake Tahoe group) with Doug Leise and Kathy Hardy.	Hipkins
10/15/12	Chinese Delegation. 25 people.	Hipkins
10/29/12	Charter School. 9 students. 10th graders.	Hipkins
3/08/13	Minorities in Agriculture, Natural Resources, and Related Sciences (MANNRS). 2013 Sacramento Conference. Two students.	Meyer, Hipkins, DeWoody
3/12/13	(UCCE) UCD Irrigation Specialists	Meyer
4/11/13	Pro-Teens El Dorado County Office of Education School-to-Career Exposure Program. High School.	Meyer
4/22-23/13	Region 5 Tree Climbing (Re) Certification	Meyer
4/25/13	California Association of Resource Conservation Districts. 30 people.	Hipkins, DeWoody, Meyer
5/29/13	Chico Genetic Resource Center student intern.	Hipkins
6/02/13	Eldorado National Forest (ENF) Desolation Wilderness Volunteer Training	Meyer
6/5/13	Tahoe Expedition Academy. 19 2nd/3rd graders, 8 parents-teachers-staff	Hipkins, DeWoody, Mello, Owens.
6/21/13	National Advanced Silviculture Program, Genetics and Nursery module. 15 employees.	DeWoody
9/3/13	Boy Scout troop. Boy Scout troop. 11 troop members, many with special needs, Two Scout Leaders, Tour to complete Forestry Merit Badge.	Meyer

Contact Information

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