National Forest Genetics Laboratory (NFGEL)

ANNUAL REPORT, FY14

USDA Forest Service, Washington Office, Forest Management

Contents

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ANNUAL REPORT, FY14

NFGEL Overview

This report covers laboratory activities and accomplishments during Fiscal Year 2014 (October 1, 2013 through September 30, 2014).

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. All Laboratory information can be found at www.fs.fed.us/NFGEL/.

Valerie D Hipkins, NFGEL Director
October 6, 2014

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 7 (Provide Science-Based Applications and Tools for Sustainable Natural Resources Management)

NFGEL Projects

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. Fifteen project summaries are included in this Annual Report. The lab currently has an additional 21 open projects at various stages of data collection, analysis, or reporting.

NFGEL Project #	Cooperator	Project Title
232	US Forest Service, Regions 1 - 6; Bureau of Land Management	Phylogeographic, taxonomic and conservation implications across a rangewide sampling of ponderosa pine (*Pinus ponderosa*)
251	US Forest Service, Region 5	Genetics of *Fritillaria* species. Addendum #2: 2013 Collections
254	US Forest Service, Regions 1 - 6; Bureau of Land Management	Phylogeographic, taxonomic and conservation implications across a rangewide sampling of ponderosa pine (*Pinus ponderosa*)
279	Center for Natural Lands Management	Isozyme data indicate variation in ploidy and significant genetic structure in *Acanthomintha ilicifolia* (Gray) Gray, San Diego thornmint
281	Private Company	Ramet identification and parental verification in slash and loblolly pine clones
282	US Forest Service, Region 5	Genetic differentiation in *Vaccinium* from northern California: resolving differences between Lake Shasta and Sierra populations
286	US Forest Service, Region 9	Identifying butternut (*Juglans cinerea*) and Japanese walnut (*J. ailantifolia*) hybrids
288	US Forest Service, Region 5	Verification of controlled mass pollination (CMP) in two ponderosa pine seedlots from the 2012 pollen year
289	US Forest Service, PSW Research Station	DNA extraction of poplar samples
291	University of California-Davis	Confirmation of the haploid nature of Douglas-fir megagametophyte samples
292	University of California-Davis	Fine scale spatial genetic structure in Douglas-fir: understanding how climate change will impact California forests
293	US Forest Service, PSW Research Station	DNA extraction of poplar samples
296	US Forest Service, Region 5	Archiving genetic material for Giant Sequoia conservation
297	Private Company	Clonal identity of Douglas-fir ramets
305	US Forest Service, All Regions	Technology transfer of allozyme markers for species identification in *Botrychium* (moonworts)

Phylogeographic, taxonomic, and conservation implications across a rangewide sampling of ponderosa pine (*Pinus ponderosa*)

US Forest Service, Regions 1 - 6; Bureau of Land Management (BLM). NFGEL Projects #232 and 254

Beginning with Project #103, NFGEL has been building a ponderosa pine DNA database to accomplish several goals: (1) identify off-site plantations, (2) understand the genetic relationships of isolated, disjunct stands in order to prioritize conservation efforts, and (3) predict potential and realized ecological niches of genetic variants under future climates. This work is captured within NFGEL Project numbers 103, 228, 232, and 254. Projects 103 and 228 were reported on in NFGEL Annual Reports FY05 and FY08, respectively. Results and implications from all four of these NFGEL projects are summarized within the following two publications. In addition, eleven genetic assessment management reports were written in FY14 for BLM and National Park Service partners detailing the management and silvicultural conditions and recommendations for their submitted stands.

Ponderosa pine at Lava Beds National Monument. Photo by the National Park Service.

MITOCHONDRIAL DNA HAPLOTYPE DISTRIBUTION PATTERNS IN *PINUS PONDEROSA* (PINACEAE): RANGE-WIDE EVOLUTIONARY HISTORY AND IMPLICATIONS FOR CONSERVATION
Kevin M. Potter, Valerie D. Hipkins, Mary F. Mahalovich, and Robert E. Means
American Journal of Botany 100(8): 1–18. 2013.

- *Premise of the study*: Ponderosa pine (*Pinus ponderosa* Douglas ex P. Lawson & C. Lawson) exhibits complicated patterns of morphological and genetic variation across its range in western North America. This study aims to clarify *P. ponderosa* evolutionary history and phylogeography using a highly polymorphic mitochondrial DNA marker, with results offering insights into how geographical and climatological processes drove the modern evolutionary structure of tree species in the region.
- *Methods*: We amplified the mtDNA *nad1* second intron minisatellite region for 3,100 trees representing 104 populations, and sequenced all length variants. We estimated population-level haplotypic diversity and determined diversity partitioning among varieties, races and populations. After aligning sequences of minisatellite repeat motifs, we evaluated evolutionary relationships among haplotypes.
- *Key results*: The geographical structuring of the 10 haplotypes corresponded with division between Pacific and Rocky Mountain varieties. Pacific haplotypes clustered with high bootstrap support, and appear to have descended from Rocky Mountain haplotypes. A greater proportion of diversity was partitioned between Rocky Mountain races than between Pacific races. Areas of highest haplotypic diversity were the

southern Sierra Nevada mountain range in California, northwestern California, and southern Nevada.

- *Conclusions*: *Pinus ponderosa* haplotype distribution patterns suggest a complex phylogeographic history not revealed by other genetic and morphological data, or by the sparse paleoecological record. The results appear consistent with long-term divergence between the Pacific and Rocky Mountain varieties, along with more recent divergences not well-associated with race. Pleistocene refugia may have existed in areas of high haplotypic diversity, as well as the Great Basin, Southwestern United States/northern Mexico, and the High Plains.

NUCLEAR GENETIC VARIATION ACROSS THE RANGE OF PONDEROSA PINE (*PINUS PONDEROSA*): PHYLOGEOGRAPHIC, TAXONOMIC AND CONSERVATION IMPLICATIONS
Kevin M. Potter, Valerie D. Hipkins, Mary F. Mahalovich, and Robert E. Means
Submitted to Tree Genetics and Genomes, August 2014

Ponderosa pine (*Pinus ponderosa*) is among the most broadly distributed conifer species of western North America, where it possesses considerable ecological, esthetic, and commercial value. It exhibits complicated patterns of morphological and genetic variation, suggesting that it may be in the process of differentiating into distinct regional lineages. A robust analysis of genetic variation across the ponderosa pine complex is necessary to ensure the effectiveness of management and conservation efforts given the species' large distribution, the existence of many isolated disjunct populations, and the potential susceptibility of some populations to climate change and other threats. We used highly polymorphic nuclear microsatellite markers and isozyme markers from 3,113 trees in 104 populations to assess genetic variation and structure across the geographic range of ponderosa pine. The results reveal pervasive inbreeding and patterns of genetic diversity consistent with the hypothesis that ponderosa existed in small, as-yet-undetected Pleistocene glacial refugia north of southern Arizona and New Mexico. The substructuring of genetic variation within the species complex was consistent with its division into two varieties, with genetic clusters within varieties generally associated with latitudinal zones. The analyses indicate widespread gene flow among genetic clusters within varieties, but not between varieties. Isolated disjunct populations had lower genetic variation by some measures and greater genetic differentiation than main-range populations. These results should be useful for decision-making and conservation planning related to this widespread and important species.

Genetics of *Fritillaria* species. Addendum #2: 2013 Collections

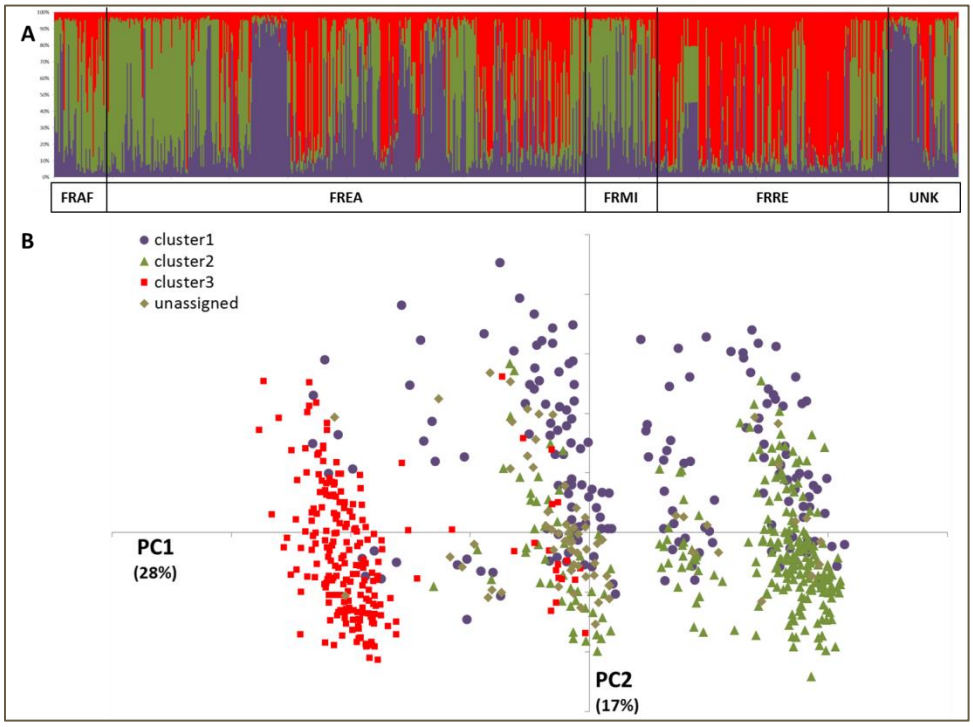
US Forest Service, Region 5. NFGEL Project #251

Background:
Fritillaria eastwoodiae (Liliaceae) is of management and conservation concern due to its restricted range. The initial genetic study (NFGEL Project 251, NFGEL FY12Annual Report) was designed to determine whether the two geographic groups of populations (north and south) correspond to genetic groups that may warrant individual management plans. However, isozyme analyses indicate that not only is there no geographic distinction between northern and southern populations, but that *F. eastwoodiae* is not genetically distinct from three congeners: *F. micrantha*, *F. recurva*, and *F. affinis*.

Full interpretation of the initial study was limited by the number of populations of the common congeners included in the design. Only a single population was confirmed to be *F. micrantha* based on morphological variation, while two other populations originally listed as putative *F. micrantha* are better described as *F. eastwoodiae* based on morphology.

Objectives:
To determine if *F. eastwoodiae* represents a distinct genetic unit or a hybrid swarm related to *F. micrantha* and *F. recurva*, and possibly *F. affinis*. This addendum reports analyses that include samples from six new populations: two of pure *F. affinis* and four of *F. recurva*. One population of *F. recurva* was collected within the range of *F. eastwoodiae*; the other five populations occur outside of that area.

Conclusions:
The addition of six congeneric populations, most of which were collected from outside the range of *F. eastwoodiae*, failed to significantly improve the resolution of the isozyme data set. Multivariate analysis of the isozyme data incompletely distinguished between *F. recurva* and *F. micrantha* individuals, but failed to distinguish *F. eastwoodiae* or *F. affinis* from the other species. Individual assignment tests indicated individuals and populations to be highly admixed. No evidence was observed that the northern populations of *F. eastwoodiae* were distinct from the southern region of populations; all populations were differentiated without correlation to geographic distance.



Multivariate analyses reveal genetic structure in *Fritillaria* samples that does not follow species designation.

This lack of resolution in the isozyme data may be a consequence of any combination of four processes. First, isozyme markers may be insufficient to distinguish between species due to the low level of variation observed at many loci. Second, the low level of isozyme variation may reflect the recent divergence and possible incomplete differentiation of individual species.

Recent studies of slow-evolving sequence data (plastid and ITS loci) failed to resolve differences between *F. eastwoodiae, F. micrantha,* and *F. affinis*. However, ongoing but unpublished plastid data do appear sufficient to resolve species relationships. Third, the species may not be distinct at all but represent a hybrid swarm with ongoing gene flow in sympatric locations. Fourth, the species designations based on field observations may have some level of error due to variable morphology, sufficient to complicate genetic interpretation.

Quantitative analysis of morphological variation in the 30 populations of *Fritillaria* will be critical to further resolving the objectives of this study. The isozyme variation provides no evidence of geographic patterning in *F. eastwoodiae*, but does not rule out a possible hybrid origin of this species. Morphological and genetic data may be compared to further resolve the relationship between populations and species.

Additional DNA-based genetic analyses may resolve greater variation within populations and species, but the interpretation of DNA sequence data may be complicated by the highly repetitive and polyploid nature of the *Fritillaria* genomes. If current research succeeds in resolving fixed variation between these four species at either plastid or ITS loci, these markers may provide sufficient power to re-examine these collections. DNA was extracted from every sample and is stored at NFGEL.

Isozyme data indicate variation in ploidy and significant genetic structure in *Acanthomintha ilicifolia* (Gray) Gray, San Diego thornmint

Center for Natural Lands Management. NFGEL Project #279

Summary
This study examined genetic variation at 18 isozyme loci in San Diego thornmint collected from 15 sites to address four objectives:

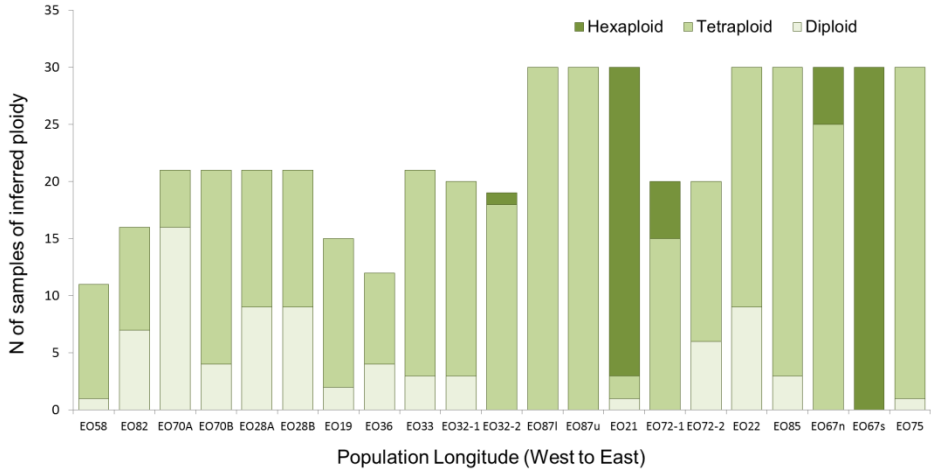
1. Is there evidence of polyploidy in isozyme banding patterns, and if detected, does the frequency of putative polyploids vary among populations?

   Isozyme banding patterns revealed two, four, and six alleles at one locus, indicating diploid, tetraploid, and hexaploid plants may be present in the San Diego thornmint collections. The frequency of each ploidy level varied among populations, but the majority of plants were observed to have a putative tetraploid banding pattern in at least one locus. The possibility that all plants may be of a higher ploidy (e.g. hexaploid) cannot be ruled out, and DNA content analyses (e.g. flow cytometry) are advised to resolve this variation.

Center for Natural Lands Management

2. If polyploidy is indicated, are banding patterns more consistent with autopolyploid or allopolyploid origin?

   The banding patterns observed in putative polyploid individual were consistent with autopolyploid origin, and were not consistent with allopolyploid origin arising through hybridization. No evidence of fixed heterozygosity was observed within a thornmint population as would be expected in an allopolyploid species. Unbalanced heterozygous banding patterns (i.e. dosage effects) were observed at more than one locus, consistent with the full assortment of alleles expected through autopolyploidy. Finally, the significant positive fixation indices observed in all populations is not consistent with a hybrid origin (allopolyploidy) of the complex banding patterns.



Three levels of ploidy were identified from isozyme banding patterns. Ploidy tends to increase with latitude (from west to east), which also roughly corresponds with increasing elevation.

3. What level of genetic diversity is observed within individuals and populations?

   Sixteen of the 18 isozyme loci examined were polymorphic (89%), with a maximum of seven alleles observed at one locus. Over the entire study a mean of 3.5 alleles were observed per locus, and averages were lower within a single population, ranging from 1.29 to 2.28. Individual fixation indices were positive and significantly greater than zero, with an average $F = 0.316$ ($P<0.0001$) over the entire study, indicating a deficit of heterozygotes.

4. How is genetic diversity distributed among populations?

   Populations of San Diego thornmint were highly genetically differentiated. A one-level model of differentiation (among all populations) identified moderate allele frequency variation ($F_{ST} = 0.154$*). A three-level model tested for differentiation among populations separated by less than 20 km ($F_{ST\,(<20)} = 0.139$*), between 20 and 50 km ($F_{ST\,(20\text{-}50)} = 0.167$), and more than 50 km ($F_{ST\,(>50)} = 0.259$*) (* $P<0.05$). These values were consistent with gene flow decreasing as a function of geographic distance. Significant isolation by distance was also detected ($R_{XY} = 0.365$, $P<0.001$).

   Differentiation also tended to follow a geographic cline. Principal coordinate analyses identified two axes that explained 85% of the differences among populations. The first coordinate varied with longitude and (to a lesser extent) latitude. The frequency of hexaploid banding patterns also tended to increase across longitude from west to east. These patterns may reflect phylogenetic signals among populations, or they may indicate some adaptive variation or cline among populations. Combined analysis of the isozyme data and common garden data may test the latter hypothesis.

Ramet identification and parental verification in slash pine (*Pinus elliottii*) and loblolly pine (*P. taeda*) clones

Private Company. NFGEL Project 281

Samples

A combination of branch tips and wood plugs from a total of 1,645 slash and loblolly pine trees were received. Ramets were either from 34 uniquely labeled clones, from 15 individual families, or one of 10 blind samples. Each of the 1,645 trees were collected and packaged separately. However, 1,538 of the trees were analyzed for ramet identification and were therefore bulked for purposes of DNA extractions and microsatellite (SSR) analysis. Between 1 and 5 ramets were provided per bulked sample (average of 4.5 ramets per sample) from a total of 339 consolidated samples. Wood samples usually had to be extracted individually and were not always part of the bulked DNA extractions because of technical laboratory constraints. Trees from families and blind samples were extracted and analyzed individually.

Genetic Analysis

Primer sequences and methodology for sixteen microsatellite loci were provided by Dr. Craig Echt and Sedley Josserand, US Forest Service, Southern Institute of Forest Genetics, Saucier, MS. A final subset of six loci was chosen to assess DNA variability among these slash and lobolly pine study samples (ript-0968, nzpr-0143, pttx-4092, nzpr-0563, sifg-0561, and sifg-4218).

Objective 1. Confirm clonal identity of submitted ramets.

A total of 354 bulked extractions were analyzed for potential mislabeling among ramets within a consolidated sample. Each consolidated sample should contain only one genotype if all the ramets are correctly labeled (i.e., each ramet is from the same clone). If there is mislabeling of one or more ramets in a consolidated sample (therefore, two or more genotypes), we will detect that by observing the presence of three or more alleles in at least one locus.

A total of 6.8% of the consolidated samples contained mislabeling as identified by three or more alleles in a locus. To identify which specific ramet(s) in a consolidated sample were mislabeled, we went back to the 106 individual ramets from the 24 consolidated samples, extracted DNA, and genotyped each tree individually.

The 34 known clones each had a unique genotype. Seventeen other genotypes were detected that were the result of mislabeling. Of the 31 mislabeled trees, 16 had unique genotypes and matched no other genotype in the study, 7 trees matched a clone different from its designated label, and 8 trees shared a unique genotype that matched no other gentoype in the study.

Objective 2. Confirm parentage of 15 families.

Genotype data at six SSR loci confirm that the parentage is correct in five of the 15 families. Ten of the families contained various levels of parentage mislabeling (or mis-identity).

Genetic differentiation in *Vaccinium* from northern California: Resolving differences between Lake Shasta and Sierra populations

US Forest Service – Region 5. NFGEL Project #282

Background

Populations of putative *Vaccinium parvifolium* in inland northern California display berry and leaf morphology and growth habits distinct from the type morphology observed in coastal populations (NFGEL Project 238, DeWoody *et al.* 2012). The typical *V. parvifolium* morphology is distinguished by bright red berries, and an upright growth habit. Typical *V. parvifolium* is also characterized with a narrow, very short calyx scar, and nonciliate, entire or toothed leaves. Populations of *Vaccinium* from the Shasta Lake region display a dark blue berry color with a broader and longer calyx ring on the fruit, and ciliate leaf margins, otherwise entire or rarely toothed. The Shasta Lake *Vaccinium* also has a decumbent and rhizomatous, to an upright, shrubby habit. Further south, *Vaccinium* from the central Sierra Nevada also display a dark blue berry color, a calyx ring similar to the Shasta lake plants, and ciliate leaf margins. Their growth habit is similar, though overall these populations are more decumbent and rhizomatous than upright and shrubby.

The morphological differences motivated previous genetic studies of *Vaccinium* from three geographic regions: coastal northern California, the Shasta Lake region, and the southern and central Sierra Nevada (NFGEL Projects 171 and 238, DeWoody *et al.* 2012). In addition to putative *V. parvifolium*, a single population of *V. deliciosum* was included as an outgroup for comparison. Analysis of five putatively neutral, bi-parentally inherited microsatellite markers indicated the Shasta Lake and Sierra Nevada populations were significantly differentiated from the coastal collections of typical *V. parvifolium*, consistent with the samples being genetically isolated.

Due to sampling design of the previous study, the relationship between the Shasta Lake and Sierra Nevada populations could not be fully resolved. While the two regions appeared distinct in the multivariate analyses, populations of *Vaccinium* in the northern Sierra Nevada were not included in the study. Additional samples were required to confirm whether the Shasta and Sierra regions are genetically differentiated or represent two ends of a genetic cline. This study sampled six additional populations from the northern Sierra Nevada to clarify the relationship between the inland regions. The same five microsatellite markers were applied to these new collections with the goal of determining whether the Shasta Lake populations are differentiated from the Sierra Nevada specimens, or whether they form a cline across the northern latitudes of California.

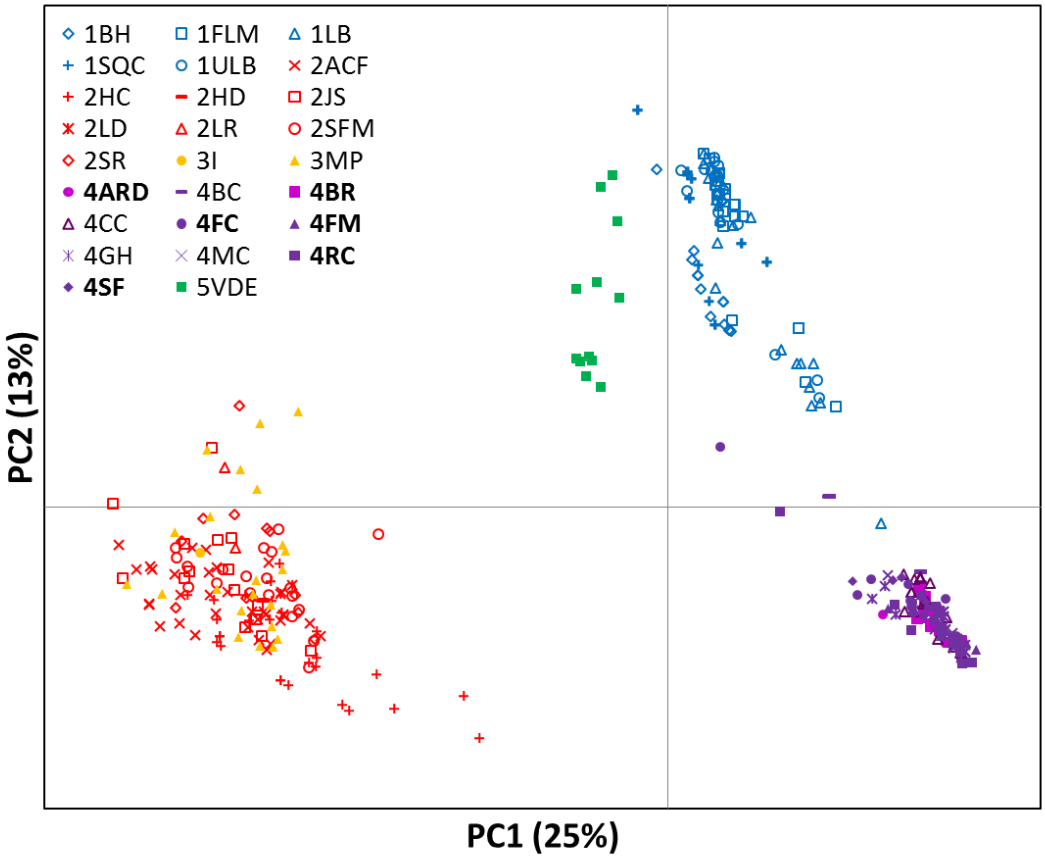
Summary

1) Is the Shasta huckleberry different genetically from the Sierra Nevada populations?

Samples from six populations of *Vaccinium* were sampled from the northern and central Sierra Nevada and examined for genetic variation at the same five microsatellite loci previously reported in NFGEL Project #238, "Genetic Structure of *Vaccinium* (Huckleberry) near Shasta Lake, Northern California". Multivariate analyses identified significant genetic differentiation between the Shasta Lake and Sierra Nevada populations. The new collections clustered with the previous samples from the southern Sierra Nevada, and did not appear intermediate to the southern Sierra and Shasta Lake populations. Both the Sierra and Shasta groups were distinct from the typical red-berried, coastal *V. parvifolium* collections. Individual assignment tests revealed greater differentiation within the Sierra Nevada samples than between the Shasta Lake populations and one population of *V. deliciosum*, but this conclusion may be affected by the small number of loci assayed.

2) Does their genetic relationship support recognition of two new taxa, or just one?

Genetic differentiation based on five microsatellite loci is insufficient evidence for taxonomic revision. That said, the fact that the Sierra Nevada populations are distinct from the Shasta Lake populations indicates that gene flow is restricted between these geographic regions. These data resolved no evidence of a genetic cline linking the Shasta Lake populations in the north and the southern Sierra Nevada sites collected previously.



Principal coordinates analyses based on pairwise individual genetic differences indicate genetic differences correspond to geographic location in *Vaccinium* from Northern California.

Indentification of Butternut (*Juglans cinerea*) and Japanese Walnut (*J. ailantifolia*) hybrids

US Forest Service – Region 9. NFGEL Project #286

Background

This work used three molecular markers to assess the species and hybrid identity of submitted putative butternut samples. Methodology followed Zhao and Woeste (2010) using protocols for markers *trnT-F*, ITS, and 22-5.

Methods

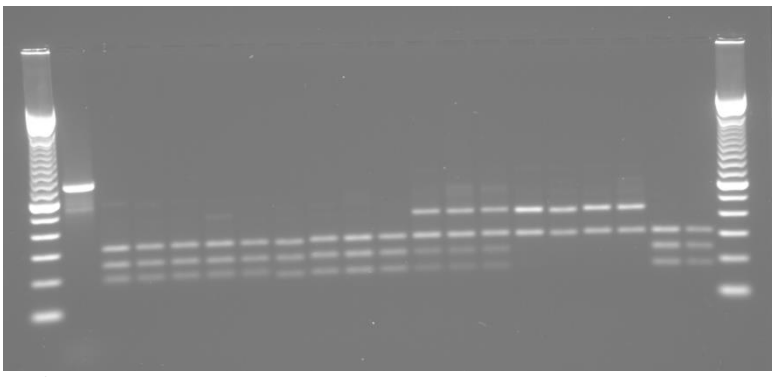
Leaf tissue was received from 137 putative butternut trees. Eighteen DNA samples from NFGEL Project #198 were used as positive controls: four samples of butternut, six samples of Japanese walnut, five samples of black walnut (*J. nigra*), and three samples of *J. cinerea* x *J. ailantifolia* hybrids.

DNA was extracted from all samples using the Qiagen DNEasy 96-well standard protocol, following the NFGEL liquid nitrogen extraction procedure. Yields were estimated using a Gemini XPS 96-well plate fluorometer and pico green fluorescence and ranged from 2.4 to 13.7 ug per sample (average yield = 5.2ug).

All samples were amplified using three markers (Zhao and Woeste 2010): 22-5 (sequence characterized amplified region (SCAR) of nuclear DNA), *trnT-F* (a chloroplast marker), and ITS (internal transcribed spacer region of nuclear DNA).

Results and Discussion

Analysis of the DNA data was completed by assigning a haplotype or pattern per locus to each individual based on the presence of specific bands. Amplification of the *trnT-F* locus followed by digestion with MboII produced two haplotypes: haplotype 1 which contained one band at 460bp, and haplotype 2 which contained one band at 390bp. The amplification of the ITS region and subsequent digestion with BSiEI produced two distinct patterns: pattern A was distinguished by two bands at 430 bp and 305 bp, and pattern B contained three bands at 305 bp, 240 bp, and 195 bp . And finally, amplification of the 22-5 locus produced up to three patterns: pattern A contained a single fragment at 651bp, pattern B contained a single fragment at 698bp, and pattern C contained one 702bp fragment.

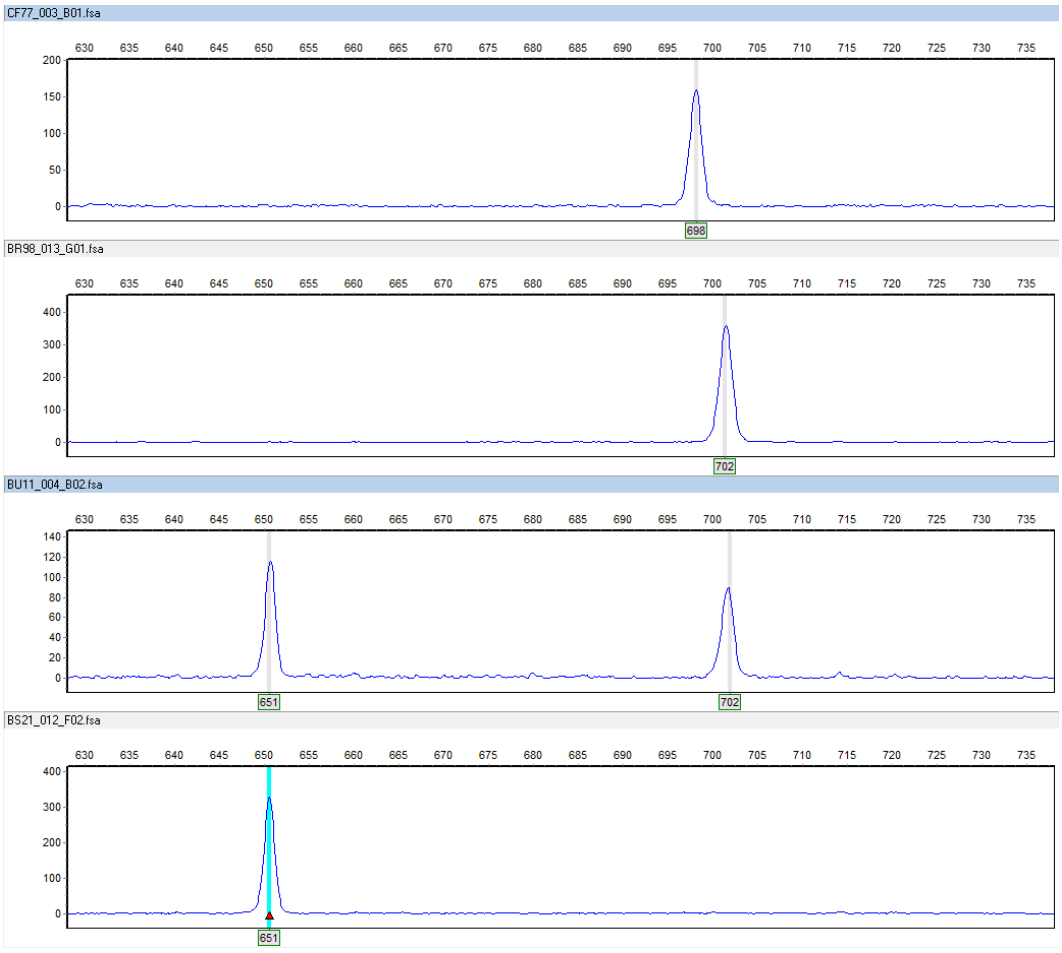


ITS/BSiEI banding patterns indicative of taxonomy in *Juglans*. Lanes 1 and 21 contain a 100bp size ladder; lane 2 = uncut PCR product from *Juglans*; lanes 3 – 7 = black walnut; lanes 8 – 11, 19, and 20 = Japanese walnut samples; lanes 12 – 14 = butternut x Japanese walnut hybrids; and lanes 15 – 18 = butternut samples.

Using control DNA from the prior NFGEL butternut identification project, we confirmed that all three molecular markers revealed fixed differences between *Juglans cinerea*, *J. ailantifolia*, and *J. nigra*. All *J. cinerea* individuals contained the *trnT-F* haplotype 1, ITS pattern A, and 22-5 pattern A; all *J. ailantifolia* individuals contained *trnT-F* haplotype 2, ITS pattern B, and 22-5 pattern C. The *J. cinerea x ailantifolia* individuals contained *trnT-F* haplotype 2 (as did *J. ailantifolia)*, an AxB pattern for the ITS marker, and an AC 22-5 pattern. *J. nigra* contained *trnT-F* haplotype 1, the B pattern at the ITS marker, and a B pattern at 22-5.

Summary

The 137 submitted samples were all genotyped at the three molecular markers. Of the total number of submitted trees, 97.8% (133 trees) had genotypes matching the butternut controls. The remaining four trees had genotypes that matched that of the *J. cinerea x J. ailantifolia* (butternut x Japanese walnut) hybrid controls.



Electropherograms of four *Juglans* samples at the 22-5 genetic marker. Each peak is a DNA fragment measured in base pairs. Four samples are presented for illustration: control black walnut (CF77), control Japanese walnut (BR98), control Japanese walnut x butternut hybrid (BU11), and a control butternut (BS21).

Verification of controlled mass pollination (CMP) in two ponderosa pine seedlots from the 2012 pollen year

US Forest Service – Region 5. NFGEL Project #288

Project background and objectives

NFGEL Project #263 (Verification of Controlled Mass Pollination (CMP) in Two Ponderosa Pine Seedlots) identified the pollen contamination levels in two seedlots: A and B. In the first cross, 5% of the pollen could not have come from the indicated male parent; in the second cross, only 1% of the pollen was not contributed by the indicated male. The pollen year (2010) producing these seedlots was light to moderate in the areas outside the orchard. The question arose as to how a heavy pollen year in areas outside the orchard would effect controlled mass pollination success. It happens that year 2012 was a very heavy pollen production year outside the orchard. The same two crosses made in 2010 (A and B) were made again in 2012. Also, these 2012 controlled pollinations may have been a bit late for some females (so late pollinations and heavy outside orchard pollen loads in 2012). The 2010 & 2012 outside pollen & pollination conditions should give us the range of what to expect from the impact of outside pollen with a CMP program without bags. The project objective was to determine the percent pollen contamination in two 2012 seedlots which used the same parents that were crossed in NFGEL Pj 263.

Summary

Female Parent	Male Parent	Year	NFGEL Pj #	# of Seed Analyzed	# Seed that Cannot be Produced by Male Parent	% Contamination
1	3	2010	263	181	9	5.0%
1	3	2012	288	190	55	28.9%
2	3	2010	263	190	2	1.1%
2	3	2012	288	190	64	33.7%

- Seedlot 1 x 3: Pollen contamination was 5% in 2010, and increased to 28.9% in 2012.
- Seedlot 2 x 3: Pollen contamination was 1.1% in 2010, and increased to 33.7% in 2012.
- Contamination levels were significantly greater in 2012 than they were in 2010. Contamination increased by 6-fold in seedlot 1 x 3, and by 30 fold in seedlot 2 x 3. The data is unable to distinguish the cause of the increased contamination. Both the late pollinations and the heavy outside orchard pollen loads in 2012 may be contributing to the higher contamination levels observed in this project

DNA extraction from poplar samples

US Forest Service – PSW Research Station. NFGEL Projects 289 and 293

The core goal of poplar biomass breeding is the creation of novel genotypes with superior performance. The poplar clones with the highest biomass yields are created through hybridization between species. Altered gene dosage relationships are believed to play a causative role in hybrid performance. The goal of our project is to identify, generate and characterize dosage-dependent variation and to facilitate its manipulation to breed poplar for biomass production. Our objectives are: (1) survey genomic composition in commercial pedigrees of *Populus* F1 hybrids, (2) manipulate gametic contribution for functional genomic and germplasm enhancement, and (3) correlate variation in karyotype, gene dosage, and transcriptional modules with superior biomass production.

NFGEL supported these goals by extracting high quality DNA from 1,056 poplar samples provided to the lab. DNA isolation was achieved using Qiagen DNeasy 96 format plant kits with the liquid nitrogen and proteinase-k modifications. DNA was quantified using pico-green. Project 289 consisted of 96 samples of small amounts of frozen fresh tissue; the average DNA yield per sample was 2.2ug. Project 293 consisted of 960 desiccated leaf tissue samples; average yield per sample equaled 8.2ug.

Confirmation of the haploid nature of Douglas-fir megagametophyte samples

University of California – Davis. NFGEL Project #291

This work was performed in support of a USDA-funded project to develop a Douglas fir reference genome. The project objective was to genotype submitted Douglas-fir DNA samples at six SSR loci to determine if a particular megagametophyte sample was haploid or instead a mosaic or contaminated (diploid).

Methods

Each megagametophyte DNA sample was amplified twice, and the putative mother tree DNA was amplified four times at each locus. Amplifications at each locus also included four negative controls and all samples were randomized so as to identify and eliminate any source of contamination among samples.

Results

(1) Is sample MGP_29 haploid? MPG_29 is supposed to be haploid megagametophyte DNA from an individual seed collected from tree 412-2.
No, MGP_29 is not haploid, it is diploid. Two alternate alleles were observed in this sample at 5 of the 6 loci analyzed. In four of the loci, one of the two alleles at each locus could not have originated from tree 412-2. At all loci, MGP_29 does have one allele shared with tree 412-2. This is suggestive of the MGP_29 sample being comprised of DNA from a single meg from tree 412-2, along with some source of contaminating DNA (DNA from either the embryo of that seed or some other source of contamination). A 'mosaic' diploid (O'Malley et al, Silvae Genetica 37, 3-4 (1988)) would result in up to four copies of the maternal alleles

being present in a megagametophyte (up to two alternate alleles visible in a single meg). Therefore, contamination of the MGP-29 meg DNA with outside or embryonic DNA seems a more plausable explanation of the sample diploidy.

(2) Confirm the haploid condition of three additional DNA samples: MGP_41, MGP_44, and MGP_49.
Samples MGP_41, MGP_44, and MGP_49 are haploid as determined at six SSR loci. All three DNA samples can originate from single megagametophytes from tree 412-2.

Fine scale spatial genetic structure in Douglas-fir: understanding how climate change will impact California forests

University of California – Davis. NFGEL Project # 292

The overall objective of this work is aimed at understanding how climate change will impact California forests. The project objective was to genotype submitted Douglas-fir DNA samples at up to six SSR loci to conduct a preliminary analysis to assess fine-scale spatial within stand genetic structure.

Roughly one and a half plates containing 140 samples of extracted genomic Douglas-fir DNA was submitted for analysis. Each DNA sample was run twice at six SSRs. Final data was sent to the UC Davis cooperator for analysis.

Archiving genetic material for Giant Sequoia conservation

US Forest Service – Region 5. NFGEL Project #296

The objective of this work was to archive material in support of giant sequoia (*Sequoiadendron giganteum*) conservation. DNA was isolated from each submitted seedling, and needle tissue from each seedling was also lyophilized and stored.

A total of 165 living seedlings (33 locations * 5 seedlings/location) were submitted. DNA was isolated using the Qiagen DNeasy 96 format (lq N benchtop with proteinase K modification). DNA was quantified using picogreen. DNA yield ranged from 3.7ug to 15.3ug per sample, with an average yield of 8.0ug. All DNA is stored on NFGEL DNA Plates 574 and 575 at -80C.

One to three small branch tips per seeding were placed in individual coin envelopes labeled with sample NFGEL #s and lyophilized to dryness. Samples are stored at room temperature under desiccation at NFGEL. Additional DNA can be extracted from the desiccated needle tissue in the future.

Paul Bolstad, University of Minnesota, Bugwood.org

Clonal identity of Douglas-fir ramets

Private Company. NFGEL Project #297

Objective of Work

Compare foliage from five putative Douglas-fir ramets to check for a potential clonal match.

Materials and Methods

One to two branch tips, including needles and dormant buds, from each of five individual Douglas-fir trees were submitted to NFGEL for genetic analysis. DNA was isolated from all samples using the Qiagen DNEasy-96 well format kit with the liquid nitrogen procedure. DNA concentrations were determined by fluorometry using Pico Green. Six SSR markers were used in the clonal identification of these Douglas-fir samples: 2G12, 2C3, 3B2, 4A7, 1C3, and 3G9. PCR conditions followed NFGEL Standard Operating Procedures, and amplified fragments were analyzed on an ABI-3100xl instrument with ROX as the size standard. Alleles were visualized using the GeneMarker software.

Results

All five trees had matching genotypes at the six SSR loci, indicating that the five trees can be ramets of the same clone. Given that these six loci are highly variable in Douglas-fir, the matching genotypes do suggest there is no mislabeling among these ramets and they are all trees of the same clone.

Technology transfer of allozyme markers for species identification in *Botrychium* (moonworts)

US Forest Service – All Regions. NFGEL Project #305

W. Carl Taylor, hosted by the USDA-NRCS PLANTS Database / USDA NRCS. 1992. *Western wetland flora: Field office guide to plant species*. West Region, Sacramento

Ferns in the genus *Botrychium* are frequently of local conservation concern or of federal protected status, making accurate classification critical to forest planning. Several species of *Botrychium* are difficult to distinguish morphologically in the field, especially when specimens are small or immature, and other taxa may naturally hybridize in sympatric locations. The inability to positively identify species from morphological observations complicates conservation efforts and necessitates molecular genetic analyses. In the past, Forest Service Botanists across the continental US and Alaska have contracted with Donald Farrar (Prof. Emeritus, Iowa State University) to positively identify samples of *Botrychium* using allozyme markers. Dr. Farrar is retired and planning to decommission his allozyme lab, but NFGEL is uniquely positioned to provide this service to National Forests. An informal survey of Regional and Forest Botanists from across the Forest Service found interest in this service from at least nine Botanists from forests in five Regions.

Because morphological traits may be insufficient to positively distinguish species regardless of the training or expertise of the botanist, Forest Service personnel and managers will continue to need genetic data for species identification indefinitely.

During more than 20 years of work examining the taxonomy and genetics of *Botrychium* species (moonwort ferns) using morphological and allozyme characteristics, Dr. Farrar has provided specimen identification to the NFS, National Park Service, and other collaborators. His large genetic database (22 allozyme loci) were the focus of his visit to NFGEL in August 2014, with the intention of transferring the laboratory methods and knowledge required for NFGEL to independently identify *Botrychium* specimens to species based on allozyme banding patterns. NFGEL Project 305 was designed as a technology transfer effort to successfully transfer from Dr. Donald Farrar to NFGEL the knowledge and skills required to use allozyme analyses to identify species of *Botrychium*. Ultimately, future *Botrychium* projects will ask:

1. Is the sample a known species of *Botrychium*? Which taxon?
2. If the sample is not a previously described species, is it a hybrid between known species? Which taxa are likely parents?
3. If the sample is not a hybrid of known species, does it qualify as a new taxon given previous marker data?

Results and Interpretations

A total of 187 sample preparations were made during the course of the technology transfer. Twenty-nine initial preps were made for the first allozyme runs. The same plants were re-ground during D. Farrar's visit and used in the subsequent tests. Finally, up to five replicate grindates were prepared from the remaining 23 healthy samples after Dr. Farrar's visit. These final preparations were stored at -80ºC for use as "positive controls" in future *Botrychium* studies.

Allozyme runs 1 and 2 tested the technology translation of the Farrar methods to the NFGEL lab by verifying all buffers and methods worked to produce active isoenzyme bands. In addition, run 2 reused the same grindate as run 1, testing the effect of a freeze-thaw on enzyme activity. Both run days occurred prior to Dr. Farrar's visit. Results were positive in both runs, with all enzyme stains producing banding activity and no significant decrease in band intensity observed in run 2. Several significant observations were made regarding the migration rate of the gel fronts. All gels ran very slowly, with some loci not fully migrating even after 6 hours. There was also a discrepancy in the voltage observed in each system compared to that expected from the run notes (provided by C. Skelton and D. Farrar) for the same constant amperage.

Allozyme runs 3 and 4 were conducted during Dr. Farrar's visit. The first (run 3) repeated the protocols of runs 1 and 2 with new preparation of the same samples from runs 1 and 2, plus additional species collected from California. The objective of this run day was to determine if the NFGEL process was producing the same banding patterns as described by Dr. Farrar. A slight modification of procedure was also tested by *not* de-wicking half of the gels to determine if that step can be omitted for *Botrychium* (gels are not de-wicked in the standard Farrar method). The same slow migration and voltage discrepancy was noted as in the previous runs. The slow run of the gels and lack of migration even after 6 h indicates an increase in amperage may be needed. Further, a distinct waviness was observed on the gels *not* dewicked, possibly due to less consistent current through the gels. It was agreed to continue to dewick the gels following normal NFGEL protocol. The overall results of these tests were negative, with few stains migrating sufficiently to resolve the same banding patterns as expected by Dr. Farrar.

After consideration of all protocol variables, two possible explanations for the difference in migration rate and banding patterns were considered: the difference in gel set up (NFGEL uses towels to conduct the current while the Farrar gel molds have feet that set in the buffer directly) and the difference in gel starch content (11% gels poured by NFGEL vs. 13% starch used by Farrar). While NFGEL owns "footed" gel molds, and these supplies could be tested in the future, differences in starch content and pouring method were tested in run 4 and gels were poured at 13% following the detailed method provided by C. Skelton. A higher portion of the buffer than used by NFGEL was boiled in a volumetric flask on a hotplate, the remaining cold buffer was added to the starch to create a slurry, and the boiling buffer was added to the slurry in one motion and swirled to combine. Unlike the NFGEL method which further cooks the starch in a microwave, the starch suspension was not cooked further and was instead immediately de-gassed using a water tap vacuum adapter (not the centralized vacuum pump).

As with the 11% gels, a discrepancy in voltage was noted with the 13% starch gels, though this time a lower voltage was observed than expected. In addition, the gels were very fragile, to the point that even if the allelic resolution was better using this method, the gels would be impossible to handle on a large scale. The migration was still much reduced compared to that expect from Dr. Farrar's experience, and most loci failed to migrate sufficiently to resolve all alleles. However, Dr. Farrar indicated the banding pattern was more compatible with his previous observations, indicating that greater migration distance may fully resolve all reported alleles. Given the lack of migration and the reduced voltage compared to expected, it was agreed that future runs should increase the amperage of the current in order to increase the migration distance and improve allelic resolution.

The final test (run 5) was conducted after Dr. Farrar's visit to test the different gel pouring methods: NFGEL's protocol that cooks the slurry in the microwave and the Farrar method that does not. Both sets of gels used 13% starch. A subset of stains (those known to be the most informative and having shown high discrepancy in previous runs) were chosen to assess allelic spread and resolution: System 7: PGI-2, TPI-1,-2; System 9: MDH-1,-2,-3,-4; System 11: SKDH, DIA-1,-2,-3,-4. In addition, a greater current was applied to systems 7 (50 mA) and 11 (55 mA). Higher voltage was noted in systems 7 and 9, but not system 11. After 5.5 h of electrophoresis, the system 7 gel had migrated the full distance, but the system 9 and system 11 gels had migrates approximately three-quarters and one-half of the full distance, respectively. The reduced migration still resulted in a reduced ability to distinguish all alleles at those loci examined. For MDH examined in system 9, while the separation was sufficient, the banding pattern did not reach the same level of separation depicted in Dr. Farrar's gels despite running to the NFGEL end point.

Accurate and consistent interpretation of allozyme gels is required for species identification, and relies both on the technical aspect of electrophoresis and on a sufficient database of known *Botrychium* allelic patterns. As part of the technology transfer, Dr. Farrar provided three types of data for use as a database for identification of future samples:

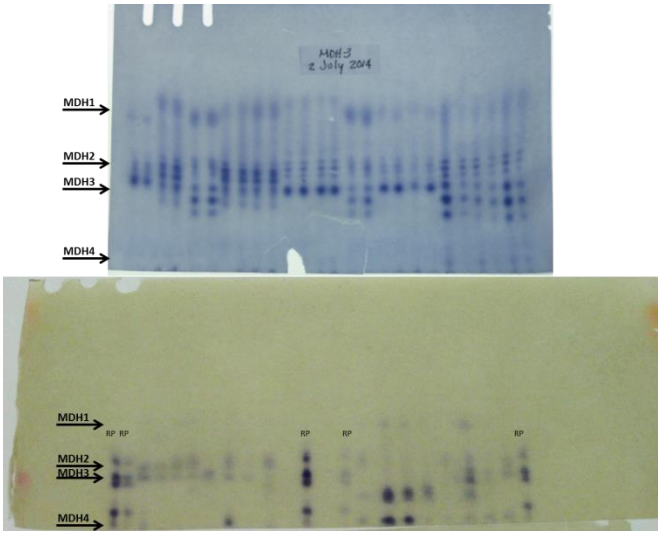
1. Photos of three allozyme runs conducted by D. Farrar and C. Skelton in 2014, plus the associated score sheets depicting Dr. Farrar's interpretation of each sample.
2. Lab notes, allele maps, and published descriptions of banding patterns for all species of *Botrychium* examined by Dr. Farrar. These documents include all alleles observed in each species or species complex, and interpretation of tetraploid banding patterns in context of putative parental (diploid) species.
3. A simplified guide listing all scores (genotypes) observed at each locus for all *Botrychium* species examined by Dr. Farrar.

These data should be sufficient for comparison and identification of *Botrychium* samples of uncertain identification if: 1) the botanists submitting the samples provide putative identifications or a list of suspected species; and 2) the new samples display alleles previously observed by Dr. Farrar. If novel alleles are observed at one or two loci a putative identification may be possible using data from the other loci. The data shared by Dr. Farrar do not represent his full database of samples (meaning the 15,000+ sample identifications and genotypes collected over 20 years of study). The full database remains the intellectual property of Dr. Farrar as a number of manuscripts based on the work are in preparation. While access to the full data could eventually provide additional power to conduct multilocus assignment tests for species identification, the three data sets provided should prove sufficient for basic taxonomic questions.

Additional Requirements to Complete the Transfer

Successful transfer of the ability to use allozyme markers to identify species of *Botrychium* will require additional knowledge and skills by NFGEL staff, and J. DeWoody in particular. The pattern and variation of allozyme diversity in the genus is not more complex than other species studied by NFGEL, and the consistent interpretation of *Botrychium* is likely given additional study and practice by J. DeWoody. Sufficient practice and skill will be obtained using the gel photos provided by Dr. Farrar and the samples of *Botrychium* maintained by NFGEL. The goal will be to continue practicing scoring photos and then re-run known samples immediately prior to 2015 collections of *Botrychium*.

Final confirmation of successful technology transfer will require at least one additional run day as the exact banding patterns reported by Dr. Farrar have not all been reproduced by NFGEL. The additional test will need to involve longer run times, higher current, or a combination of both changes in order to increase the migration of alleles to match that observed by Dr. Farrar. These tests will be conducted in FY2015 before any new *Botrychium* samples are received by NFGEL.



Requests for Assistance

In addition to accepting and processing Projects, NFGEL also fulfills requests for assistance. A Project is characterized by (1) the presence of a project plan with defined management objectives and sampling design, (2) the receipt of plant material needed to meet objectives, (3) a strategy for the laboratory methodology needed to meet objectives, and (4) the design for the interpretation and reporting of results. Significant work done by NFGEL Staff outside the Project definition is captured by the use of "Requests for Assistance (RAF)" numbers.

14-RAF009: Genetic diversity in morphologically distinct *Lewisia* populations

There is interest by Sierra Pacific Industries to investigate the taxonomy of unknown *Lewisia* located in northern California that appears to fall into the *L. kelloggii* group. Specifically, they need to know whether there are two subspecies within *L. kelloggii*, no subspecies, or that this is a new subspecies within the *Lewisia kelloggii* group.

A detailed project proposal has been developed and an agreement to contract the work is in progress. If the work proceeds as a project, collections may take place over as many as three growing seasons. NFGEL has received some opportunistic collections which could serve as a start to a full project examining the genetic diversity in morphologically distinct *Lewisia* populations. Until a contract is in place, this work does not have a project number.

NFGEL has received 55 *Lewisia* samples from three locations. All samples were processed in three ways upon receipt at the lab: (1) isozyme extracts were prepared and frozen at -80C, (2) leaf material from each sample was dissected into collection tubes and placed at -80C for possible future DNA extraction, and (3) remaining leaf material was frozen for possible lyophilization in the future.

14-RAF010: SNP development for the selective breeding of Douglas-fir

DNA needed to be isolated from 1,920 desiccated Douglas-fir needle samples as part of an Oregon State Univeristy (OSU) effort to selectively breed Douglas-fir for traits amenable for biofuel use while retaining wood value for traditional uses. The original plan by OSU was to have a UC-Davis laboratory extract DNA from these samples. However, shortly before samples were due to be processed, the UC-Davis facility was unable to perform the work. NFGEL was requested to step in and perform the DNA isolation. Because of a staffing shortage at NFGEL, we could not commit to processing samples in the time frame needed (within a month of the request). However, we agreed to make our lab available to a visiting Oregon State University (OSU) scientist, where we would (1) orient him to our lab processes and setup, (2) train him to extract DNA using the Qiagen DNeasy 96-well format kits, and (3) offer technical guidance during his stay. After training, the visiting scientist would perform the remaining extractions.

The OSU scientist arrived at NFGEL on August 25, 2014 and departed on September 4, 2014. Desiccated needle tissue had been minced by hand at OSU, placed in specialized 96-well S-block type plates, and transported to the lab on August 25th. Four DNEasy 96-well kits were shipped to NFGEL from the UC-Davis lab. Modification to the extraction had to be made because we found that the S-block plate wells holding the tissue would crack or break out when homogenized or centrifuged after freezing in liquid nitrogen per standard NFGEL operating procedures. Therefore, tissue had to be homogenized without a liquid nitrogen freezing step before the addition of AP1 lysis buffer. We also added a proteinase-k modification step to increase DNA yield and quality. The volume of tissue per well varied, leading to some extraction difficulties with samples characterized by large pellets. It was not possible to inspect pellets in the S-block style plates (unlike the Qiagen Collection Tube Plates that NFGEL typically uses), making some pipetting steps during extraction problematic. Also, the S-block style plates could not be capped and shaken (as can Collection Tube Plates), making mixing of solutions during the extraction process difficult. Approximately 200 samples with low DNA yields after the initial extraction were re-dissected and extracted a second time, resulting in the processing of 22 plates of samples. DNA concentrations were quantified using a Gemini XPS Microplate Spectrofluorometer (Molecular Devices, Sunnyvale, California, USA) with PicoGreen dsReagent (Invitrogen, Carlsbad, California, USA). The average yield per sample was 4.2ug.

Budget

NFGEL receives an annual allocation from the Washington Office, National Forest System's Forest Management staff group. From FY09 – FY13, NFGEL received $480,000 each year. The FY14 allocation was decreased to $473,500. In addition to these funds, NFGEL expended $242,304 individual partner program dollars collected for non-NFVW projects in FY14. These dollars were used for additional salary, chemical, supply, equipment, repair needs, and travel.

FY14 NFGEL BUDGET

ALLOCATION	
WO - Forest Management	$473,500
USFS-R8 (NFGEL Project 294)	$9,308
Center for Natural Lands Management (NFGEL Project 279)	$24,134
BLM (NFGEL Projects 232 and 254)	$208,862
TOTAL	$715,804
EXPENDITURES	
Salary - Permanent Employees	$297,080
Salary - Temporary Employees	$79,688
Salary - Contracts	$8,182
Site Utilities and Rents	$20,786
Chemicals and Supplies	$92,176
Equipment and Repair	$46,017
Computer and Office Supplies	$6,576
Postage	$516
Administrative Costs (awards, lynx passes)	$4,375
Vehicle	$4,785
Contract – Hendrix College (southern pine taxonomy)	$30,000
Contract – USFS/SRS (sugar pine SSR development)	$10,000
Contract – USFS (Gene Conservation of Forest Trees Workshop)	$10,000
Donation – Establish WCF Special Project	$80,500
Travel and Training	$12,845
TOTAL	$703,526
BALANCE	$12,278

NFGEL Staff

During FY 2014, NFGEL was staffed with 2.65 permanent FTEs, and multiple staff on temporary tours. Temporary employees accounted for 2.17 FTEs for the reporting year.

EMPLOYEE	POSITION	TOUR (% FTE for year)	DATES
Valerie Hipkins	Director	Permanent (100%)	10/1/13 – 9/30/14
Jennifer DeWoody	Geneticist	Permanent (100%)	10/1/13 – 9/30/14
Randy Meyer	Lab Biotechnician	Permanent (65%)	10/1/13 – 9/30/14
Courtney Owens	Lab Biotechnician	Temp-NTE (30%)	10/1/13 – 1/7/14
Jody Mello	Lab Biotechnician	Temp-Pathways (64%)	10/1/13 – 7/25/14
Rosanna Hanson	Lab Biotechnician	Temp-NTE (88%)	10/1/13 – 8/7/14
Courtney Canning	Lab Aid	Temp-Pathways (1%)	8/14 – 9/14
Garrett Short	Lab Aid	Summer Temp-1039 (9%)	6/14 – 8/14
Steffen Mahnke	Lab Aid	High School Volunteer (3%)	10/1/13 – 5/13/14
Andrew Jackson	Lab Aid	High School Volunteer (3%) Contract	10/1/13 – 5/13/14 7/21/14 – 9/19/14
Keenan Raleigh	Lab Aid	High School Volunteer (3%) Contract	10/1/13 – 5/13/14 6/10/14 – 7/25/14
Morgan Linville	Lab Aid	High School Volunteer (1%)	8/11/14 – 9/30/14
Laura Polkinghorn	Lab Aid	High School Volunteer (1%)	8/11/14 – 9/30/14
Tyler Francis	Intern	American River College (6%)	1/24/14 –5/16/14
Jian Alsarraj	Intern	American River College (6%)	1/24/14 –5/16/14
Brendan Aherns	Intern	American River College (2%)	8/29/14 –9/30/14

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
John Crockett, 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
Keith Woeste	Acting National Program Leader, Genetics and Global Change Research - Forest Management Sciences	Northern Research Station, West Lafayette, IN
Gary Man	Acting Assistant Director - Cooperative Forestry – Urban and Community Forestry Program	Washington Office, Washington DC
Dave Levinson	Program Leader, National Stream & Aquatic Ecology Center	STREAM, Ft. Collins CO
Larry Stritch	National Botanist - Rangelands Management & Vegetation Ecology	Washington Office, Washington DC
David Gwaze	National Silviculturist and Genetics Lead	Washington Office, Washington DC

Staff Activities

Publications

Potter, KM, VD Hipkins, MF Mahalovich, and RE Means. 2014. Nuclear genetic variation across the range of ponderosa pine (*Pinus ponderosa*): phylogeographic, taxonomic and conservation implications. *Submitted to* Tree Genetics and Genomes.

Presentations

Using Laboratory Genetics as a Tool in Silviculture. Placerville Nursery Clients Open House and Sowing Orders Workshop. Placerville, CA. November 7, 2013. (Hipkins)

DeWoody, J., V.D. Hipkins, J.K. Nelson, and D.L. Rogers. Inferring ploidy variation in natural populations, and its implications for species conservation. Poster presentation, Joint Botany Meetings, Boise, Idaho. July 27-30, 2014. (DeWoody)

Integrating Molecular Genetics into Resource Management. Tree Crop Tour for delegation of scientists and foresters from India. Placerville, CA. September 8, 2014. (Hipkins)

From Timber Theft to Climate Change: Incorporating Molecular Genetics into Forest Management. Invited speaker for the Foreign Specialist Seminar series, Korea Forest Research Institute. Seoul, South Korea. September 21 – 26, 2014. (Hipkins)

Attendance

US Fish and Wildlife, Forensics Laboratory. Tour and presentations covering the legal processing of laboratory samples for timber theft. Ashland, OR. February 26 – 28, 2014. (Hipkins, DeWoody, Meyer, and Mello)

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"). Lied Lodge, Nebraska City, Nebraska. March 24 – 28, 2014. (Hipkins)

Contracting Officer's Representative (COR) Training – Level II (40 hours). US Forest Service, Placerville, CA. March 31 – April 4, 2014. (Hipkins)

NFGEL Steering Committee Meeting. Washington Office, Washington DC. May 19 – 23, 2014. (Hipkins)

IUFRO Joint Conference: Challenges and opportunities in (1) genetics of five-needle pines and (2) rusts of forest trees research: Conservation, evolution and sustainable management in a changing climate. Ft. Collins, CO. June 17 – 20. (Hipkins)

US Forest Service, Washington Office, Forest Management Staff Retreat. Washington DC. August 19 – 21, 2014. (Hipkins, DeWoody, Meyer)

Technical Review

Provided review and guidance for the publication (DeWoody):

Nunneley, J., O.W. Van Auken, and J. Karges. 2014. Outlier stands of quaking aspen in the Davis Mountains of west Texas: Clone or clones? American Journal of Plant Sciences 5: 2298-2311.

Member of American River College Business, Computer Science & Work Experience advisory committee (Work Experience focus). December 2013 – present. (DeWoody)

Member of North Valley Biotechnology Center advisory committee. December 2013 – present. (DeWoody)

Team Participation

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

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

Co-Lead (with David Gwaze) of organizing committee for "Gene Conservation of Forest Trees – Banking on the Future" workshop being planned for spring/summer 2016. (Hipkins)

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

Technology-Transfer

Organized and participated in technology transfer to use allozyme electrophoresis to identify species of *Botrychium* (moonwort ferns), working with Donald Farrar from Iowa State University (DeWoody (lead), Hipkins, Meyer)

Plant Sample Collections

With collaborators from USFS-Region 5, collected *Vaccinium* and *Botrychium* for genetic analyses. (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). (Meyer)

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

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

NFFE Forest Service Council, Union Representative on Accident Investigations. (Meyer)

- Schoolhouse Fire ATV Fatality, Team Member.
- Klamath FLA, Team Member

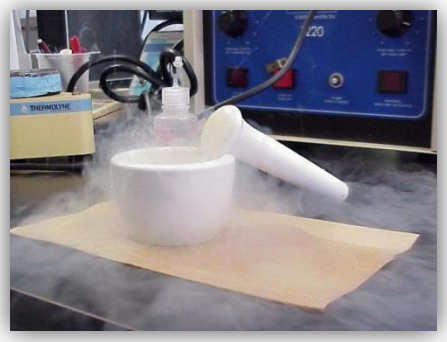
NFFE, Union Representative, Federal Advisory Council on Occupational Safety and Health (FACOSH) on Field Federal Safety and Health Council (FFSHC) Improvements. (Meyer)

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

NFFE Forest Service Council, Union Representative, Coordinated Response Protocol (CRP). (Meyer)

NFFE Forest Service Council, Union Representative, Emergency Medical Services (EMS). (Meyer)

Hosted



NFGEL continues to host 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
October 23, 2013	Charter Connections Academy, Pro-Teens Program, grades 9-12	Meyer
November 11, 2013	Jessica O'Brien, Sierra Pacific Industries	DeWoody
November 14, 2013	UC Berkeley student	Hipkins
November 15, 2013	American River College Biotechnology Club, 15 students and professor	DeWoody
November 16, 2013	El Dorado National Forest Interpretive Assoc.	Meyer
November 21 – 22, 2013	Kathelyn Paredes, Universidad Autonoma Gabriel Rene Moreno, Santa Cruz, Bolivia	Hipkins, DeWoody
November 27, 2013	Brook Milligan, New Mexico State University	Hipkins
January 7, 2014	USFS 2013 Middle Leader Program	Meyer, Hipkins
March 6, 2014	El Dorado High School, student tour	Hipkins
March 18, 2014	Shadow Day, El Dorado High School, 2 high school Juniors	Hipkins, DeWoody, Hanson
March 20, 2014	Sons in Retirement	Meyer
April 1, 2014	ENF Fire Management Fuels Program	Meyer
April 10, 2014	Shadow Day, El Dorado High School, 1 high school Senior	Hipkins, DeWoody
April 10, 2014	Camino Environmental School, 50 students plus parents and teachers	Meyer, Hanson, DeWoody, Hipkins
April 16, 2014	Local public (3 people)	Hipkins
May 30, 2014	Ellen Mackey, Council for Watershed Health	Hipkins
June 23, 2014	Dr. Choonsig Kim, Gyeongnam National University of Science and Technology, Dept of Forest Resources, Korea	Hipkins
September 8 - 12, 2014	Tree Crop Study Tour from India (12 people)	Hipkins, DeWoody
September 26, 2014	Rescue School, 85 5th graders plus parents and teachers	Meyer

Contact Information

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