NATIONAL FOREST GENETICS LABORATORY (NFGEL)

USDA Forest Service
Washington Office
Forest Management

ANNUAL REPORT, FY12

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.

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INTRODUCTION

This report covers laboratory activities and accomplishments during Fiscal Year 2012.
October 1, 2011 through September 30, 2012

Background

NFGEL was established in 1988 as part of the National Forest System of the USDA-Forest Service. The Lab is located at the Institute of Forest Genetics (IFG) in Placerville, California and is administrated by the Washington Office (WO) Forest Management staff. The focus of the lab is to address genetic conservation and management of all plant species using a variety of laboratory techniques including DNA analyses. NFGEL services are provided to managers within the Forest Service, other government agencies, and non-government organizations for assessing and monitoring genetic diversity.

Purpose of Laboratory

The purpose of the Laboratory 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.

Alignment to National Strategic Plans

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:

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

PROJECTS

Overview

NFGEL projects were processed to meet a variety of management objectives. Project results were used to guide restoration and conservation projects, and assist in silviculture and tree improvement activities. Seventeen project reports are included in this Annual Report.

NFGEL uses molecular genetic data to help managers with adaptive planning by:

- identifying endangered and invasive plant species
- identifying the effectiveness of conservation efforts
- maintaining species and genetic diversity
- assisting in the breeding and production of new genotypes and seed sources for "assisted migration" in the face of changing climate
- identifying refugia that can be used as sources of seed for species and population recovery
- identifying species, populations, and communities that are sensitive to increased disturbance
- identifying genetic variation in climatic tolerances of tree and other plant species

List of 17 Projects completed at NFGEL during FY12, organized by Cooperator.

NFGEL PROJECT	COOPERATOR	SPECIES	PROJECT TITLE
210	US Forest Service, Region 4	Cottonwoods	Clonal structure and genetic diversity in *Populus angustifolia, P. fremontii*, and *P. trichocarpa*
238	US Forest Service, Region 5	*Vaccinium* species	Genetic structure of *Vaccinium* (huckleberry) in California reveals potential systematic distinctions
250	US Forest Service, Region 5/Sierra Pacific Industries	Ponderosa Pine	Genetic analysis of relationship among Sierra Pacific Industries Super Trees near Stirling City
251	US Forest Service, Region 5	*Fritillaria eastwoodiae*	Population genetics study of *Fritillaria eastwoodiae*
255	US Forest Service, Region 5/Sierra Pacific Industries	*Lewisia kelloggii*	Genetic diversity in putative *Lewisia kelloggii* from northwestern California
263	US Forest Service, Region 5	Ponderosa Pine	Verification of controlled mass pollination (CMP) in two ponderosa pine seedlots
266	US Forest Service, Region 5	Sugar Pine	Ramet identification and marker development in sugar pine
267	US Forest Service, Region 6; Carex Working Group	*Sedum* species	Taxonomic boundaries in *Sedum oregonense* and *S. obtusatum retusum*
173	US Forest Service, Region 6	Port Orford Cedar	Genetic markers and the assessment of variation in Port-Orford Cedar

List of 17 Projects completed at NFGEL during FY12, organized by Cooperator.			
NFGEL PROJECT	**COOPERATOR**	**SPECIES**	**PROJECT TITLE**
262	US Forest Service, Region 6	Quaking Aspen	Determining clonal identity of aspen stands
242	US Forest Service, Rocky Mountain Research Station	Ponderosa, Douglas-fir, Lodgepole, Subalpine Fir, AR Longleaf Pine, W Redcedar, Grand Fir, W Larch	Quantifying gene flow and adaptive variation in conifers across the Western states to predict effects of climate change on forest ecosystems
246	Bureau of Land Management	Douglas-fir, Sugar Pine, Noble Fir	Ramet identification in Douglas-fir, Noble fir, and Sugar pine
165	University of CA, Davis	Monterey Pine	Genetic diversity and structure of an endangered population of a binational species of concern: Monterey pine (*Pinus radiata*)
235	Western Nebraska Resources Council	Quaking Aspen	Genetic make-up of aspen colonies across Nebraska
249	Colorado State University	Lodgepole Pine	The role of genetic differentiation and local adaptation on the morphological variation of *Pinus contorta*
257	Archangel	Giant Sequoia, Coast Redwood	Clonal identity in giant sequoia and coast redwood
261	GreenWood Resources, Inc.	Poplar	DNA fingerprinting elite Populus clones

Project Summaries

Clonal structure and genetic diversity in *Populus angustifolia*, *P. fremontii*, and *P. trichocarpa* located in Nevada (Project #210; Partner: FS – Region 4)

Project Goals: The purpose of this project was to determine the clonal structure and species identity of submitted individuals of *Populus* using laboratory genetic markers. The submitted cottonwoods were not producing viable pollen and genetic degradation of the clones was suspected to be a factor.

Summary

- One hundred eight *Populus* trees from three species (*P. angustifolia*, *P. fremontii*, and *P. trichocarpa*) were genetically tested.
- No mis-labeling of ramets per clone was detected.
- *Populus trichocarpa* clone POTR-F-10-6 was the only clone that appeared to be polyploid (all other clones of all species appeared to be diploid). However, there were two alternate genotypes detected among the 6 polyploid trees, indicating possible mutation.

- A total of five genotypes (clones) were detected in *P. angustifolia*, 10 genotypes in *P. fremontii*, and 14 genotypes in *P. trichocarpa* (therefore, 29 clones were identified among the 108 samples tested).
- Levels of genetic diversity within species was moderate, with *P. trichocarpa* containing the most variation and *P. angustifolia* and *P. fremontii* containing similar, lower levels of diversity.
- There were clear genetic differences among the three species, and all individuals appeared to be classified correctly.

Genetic structure of *Vaccinium* (huckleberry) in California reveals potential systematic distinctions (Project #238; Partner: FS – Region 5)

Vaccinium species (Ericaceae) are important understory shrubs in conifer forests in North America. Populations putatively classified as *V. parvifolium* Sm. in northern California display a dark berry color undescribed in the species. In order to inform conservation guidelines, *Vaccinium* populations were characterized via molecular genetic analyses. Plants of typical *V. parvifolium* morphology from the coastal areas of northwest California, western Oregon and Washington, atypical plants from Shasta

County and the central Sierra Nevada, and one population of *V. deliciosum* Piper, a congener, were assessed at five nuclear microsatellite loci. Analyses of differentiation, admixture, and phylogenetic relationships indicated that populations displaying atypical morphology were more similar to *V. deliciosum* than to the typical *V. parvifolium*. Although additional data are required to determine whether these differences warrant taxonomic treatment within *Vaccinium*, management plans should consider three distinct gene pools among these *Vaccinium* populations.

Genetic analysis to determine relationships between ponderosa pine super trees (Project #250; Partners: FS – Region 5 and Sierra Pacific Industries)

Genetic relationships were assessed between pairs of ponderosa pine parent trees using two different laboratory based genetic markers: isozymes and DNA-based microsatellites. Neither marker system was ideal for estimating relatedness (lack of variation and/or limited number of loci available). However, some trends in the general level of pairwise relatedness (none, low, moderate, or high) were seen in the data when using both marker systems. When calculating relatedness, r values vary by the degree of the relationships. In general, non-relatives have a relatedness value r of zero or less; half-sibs have a value of 0.25; full sibs = 0.5, 1st cousins = 0.125, and so forth. As r increases between two individuals or groups, relatedness is greater.

Pairwise relatedness estimates presented in this report have only moderate to low confidence because of the high sampling variance in the relatedness calculations. Therefore, this data should be used to look at trends (i.e., high vs. low relatedness -- high being potentially related pairs of trees), and not put too much emphasis on the specific r scores between any one pair. For example, if two trees have an r value of 0.5, I would urge this be interpreted as that these two trees may be notably related, not that they may actually be half-sibs per se.

Genetic Diversity: The parent trees as a group are less diverse than the comparison trees at standard measures of genetic diversity when using isozymes to characterize diversity levels. The DNA-based microsatellite data was much more variable than the isozyme data overall, and diversity levels between parent and comparison trees were roughly the same with the parent group showing just slightly lower levels (non-significant) than the comparison trees. It would be expected that there would be less diversity in a group of related individuals than compared to an unrelated group of individuals. The data could therefore support the idea that the parent trees are more related as a group than are the comparison trees. However, it should be strongly noted that the diversity differences between groups were non-significant.

Relatedness: Relatedness calculations show that the parents (or some of the parents) may be more related overall than are the comparison trees. In particular there are 13 pairwise comparisons involving 10 of the 12 parent trees that yield higher than average r values. Specifically, trees *a*, *b*, c, and *d* form a 'related' group; tree *e* shows higher than average r values with trees *a*, *b*, and *d*; trees *f*, *g*, and *h* form a 'related' group; and trees *i* and *j* form a pairwise comparison with a moderate/high r value.

Population genetics study of *Fritillaria eastwoodiae* (Project #251; Partner: FS – Region 5)

This study used isozyme markers to investigate the genetic variation within and among *Fritillaria* species occurring on National Forests in northern California. The study was designed to assess the genetic differentiation between *F. eastwoodiae* and three congeners occurring in the same area: *F. micrantha, F. recurva,* and *F. affinis*. Of particular interest is the possible hybrid origin or ongoing introgression between *F. eastwoodiae* and *F. recurva* in the northern populations and *F. eastwoodiae* and *F. micrantha* in the southern range. Specifically, this study asks: How is genetic diversity partitioned within and among populations? Is there genetic substructuring within the collections? Does *F. eastwoodiae* represent one or multiple taxonomic entities? These questions were addressed by assessing genetic variation at 15 isozyme loci in *Fritillaria* samples collected from 22 populations. A suite of analyses reveal a complex pattern of genetic differentiation among populations broadly corresponding to differences between *F. recurva*-like genotypes, *F. micrantha*-like genotypes, and putative hybrids (admixed genotypes). In addition, some evidence of vegetative reproduction and polyploidy were observed, with both phenomenon occurring at a single site.

- How is genetic diversity partitioned within and among populations?
  The majority of genetic variation measured was contained within populations, though populations were still significantly differentiated across the collection (i.e., populations were genetically different from each other; F_{ST}=0.15, P<0001). Gene flow does not appear to decrease as a function of distance, particularly when only *F. eastwoodiae* populations were examined (meaning populations growing in close proximity are not necessarily more genetically similar). Geography is likely not a barrier to gene flow, and other factors likely drive differentiation among populations.

- Is there genetic substructuring within the collections?
  Significant differentiation was observed among species and the pattern of differentiation was greatest between *F. micrantha* and *F. recurva*. In addition, the single population of *F. affinis* sampled was highly similar to *F. micrantha* but distinctive from *F. recurva*.

Collections were made from two geographic areas: region 1 in the north encompassing Shasta and Tehama counties, and region 2 to the south, including Butte, Yuba, and Nevada couties in northern California. Evidence of differentiation between these two regions was mixed (polyploid data: F_{RT}=0.01, P<0.001; diploid data: F_{RT}<0.001, P=0.236). The restriction of polyploid individuals to region 2 likely drives the significant differentiation observed when examining the full polyploid data set. The lack of differentiation observed in the reduced diploid analyses may more accurately reflect the distribution of alleles across the landscape and indicates that there is not a significant genetic difference between the two regions.

- Does *Fritillaria eastwoodiae* represent one or multiple taxonomic entities?
  Multivariate analyses revealed *F. eastwoodiae* populations to be either similar to *F. micrantha* or *F. recurva*, or intermediate to the two. Both the population assignment and the principal coordinate analyses indicated the *F. eastwoodiae* populations were not distinct from the other species in the collection.

  Population assignment tests identified two anonymous genetic clusters in this study. Principal coordinate analyses identified three (or possibly five) broad genetic groups. When individual plants were assigned to one of three groups (cluster 1, cluster 2, or admixed), the pattern of the population assignment tests roughly corresponded to the three genetic groups identified by the principal coordinate analyses.

  These anonymous genetic groups did not correspond to geographic regions. *Fritillaria eastwoodiae* samples were assigned to either one of the two genetic clusters or were identified as genetically admixed (possible hybrids). The pattern of genetic variation observed in *F. eastwoodiae* is consistent with a putative hybrid swarm, with populations being more similar to either presumptive parental species (*F. micrantha* or *F. recurva*) or genetically intermediate to the two groups. These data are insufficient to determine if gene flow continues to occur between *F. eastwoodiae* and the two parental species, or if populations constitute a stable hybrid zone. Additional studies of DNA-based genetic data and morphological differentiation among populations will be required to resolve the evolutionary history of these populations.

- What are the implications for management and conservation?
  Polyploid individuals were observed in population 8 (*F. eastwoodiae*) and population 18 (*F. recurva*). These populations occur sympatrically. The polyploid *F. recurva* samples displayed high levels of vegetative reproduction (inferred from matching multilocus genotypes) and were more similar to *F. micrantha* than the other *F. recurva* populations. The *F. eastwoodiae* population contained both diploid and polyploid samples, and did not display a high rate of vegetative reproduction. The polyploid nature and tendancy towards vegetative reproduction make this sympatric population unique.

  Germplasm collection efforts will require a more complex plan than focusing on geographic regions. The pairwise genetic similarity of *F. eastwoodiae* populations did not correspond to geographic separation. Instead, defining genetically similar populations based on the population assignment tests (cluster 1, cluster 2, or admixed) may provide a

convenient guide for seed or bulb collections. In addition, population 8 displayed unique variation in ploidy, and three alleles unique to *F. eastwoodiae* were observed, each allele in at most two populations (populations 1, 5, 9, and 11). Evidence of phenotypic variation would complement these genetic analyses in resolving the taxonomic structure and evolutionary history of these populations.

Genetic diversity in putative *Lewisia kelloggii* from northwestern California (Project #255; Partners: FS – Region 5 and Sierra Pacific Industries)

In summer 2010, an unknown Lewisia was observed in three separate regions in northwestern California on Six Rivers National Forest and Sierra Pacific Industries land. The unknown Lewisia appears to fall within the *L. kelloggii* group. The Six Rivers National Forest observed the Lewisia northwest of the town of Orleans at 3900 ft on ultramafic substrate. Sierra Pacific Industries observed one region on the northeast end of Trinity Lake in the Trinity Mountains on Bragdon Shale Formation substrate at an average elevation of 3794 ft. The second region SPI observed was on Panther Rock west of Castle Crags on ultramafic substrate at an average elevation of 6554 ft. The Six-Rivers NF and Panther Rock collections look more similar to each other than to the Trinity collection.

Sierra Pacific Industries and the Six Rivers National Forest were interested in knowing whether this species is indeed a new rare species or subspecies, a range extension of *L. kelloggii* ssp. *hutchisonii* (Rare Plant Rank 3.3), or a range extension of the common taxon *L. kelloggii* ssp. *kelloggii*. We conducted an isozyme analysis to address project objectives.

SUMMARY

- As a group, the California Lewisia's showed great isozyme variation.
- Overall, populations are differentiated genetically (meaning populations are moderately to highly genetically different from each other).
- The Six-Rivers population consists of 29 plants with the same genotype. Seven of the 24 isozyme loci measured (30%) in this genotype are fixed for a heterozygous condition. This is likely indicative of the polyploid nature of this plant. Additionally, high levels of fixed heterozygosity in the only detected genotype in this occurrence could be the result of vegetative reproduction (although the presence of vegetative reproduction in *L. kelloggii* has not been documented to our knowledge). It should be strongly noted that some of the genetic interpretations made about the Six-Rivers NF population may be questionable because we're making 'population level' statistical inferences using only a single genetic score. If this species can reproduce asexually, this population may be made up of a single individual (or a single clone).
- Panther Rock and the three Plumas populations cluster together genetically. The three Plumas populations share 94.9% genetic similarity to each other; Panther Rock shares an average of 91% genetic similarity to the Plumas populations. Since the Plumas populations have been identified with some certainty as *L. kelloggii* ssp. *hutchisonii*, it is certainly plausible that the Panther Rock population may be ssp. *hutchisonii* as well. Even though the Panther Rock collection looks similar to the Six-River plants by field observation, this pair of populations share only 76.2% genetic similarity (Fst = 51%) and do not cluster together genetically.

- Trinity A and B populations share high genetic similarity (94%), but are quite diverged from other sampled populations. Surprisingly, the population that is most genetically similar to the Trinity populations is the Shuteye Peak collection that is the southern-most collection in this study.
- Genetic and geographic distances among populations are not strongly correlated (meaning it is not necessarily true that as population are located farther and farther apart, they become less and less genetically similar).
- Genetic data do not indicate that these population collections are a species other than *Lewisia kelloggii*.
- Due to the high population divergence, management may want to focus on populations or genetic groups, not necessarily on subspecies identity (regardless of subspecies identity, people may want to manage for distinct populations or genetic groups).
- In this California *L. kelloggii* study, the genetic differences among populations are large and there is some clustering of populations into genetic groups. However, these groups don't seem to be correlated to any geographic-type patterning, and with the absence of voucher specimens, we cannot associate genetic variation with morphological traits. Molecular genetic variation is extremely useful when it is coupled with morphological and field data to achieve a full taxonomic understanding of a species group. We used this approach in our prior study of *L. kelloggii* (NFGEL Project #74 and Wilson et al. 2005). This California Lewisia genetics work perhaps should be followed up with a morphological analysis of variation found throughout the range of *L. kelloggii* to accurately define potential subspecies structure within the species.

Verification of controlled mass pollination (CMP) in two ponderosa pine seedlots (Project #263; Partners: FS – Region 5 and Sierra Pacific Industries)

Project Objectives

A young clonal ponderosa pine orchard (planted Fall 2005) has been producing some females & no male catkins over the last couple of years. Elite pollen from the same breeding zone orchard in 2009 was collected from another orchard to use for pollinations & they have been pollinating the females since 2009 with successful, viable seed resulting from the 2010 cones collected. Females cones were not bagged to eliminate outside pollen contamination. The project objective is to see how this version of Controlled Mass Pollination is working out & check for outside pollen contamination (calculated as % pollen contamination per seedlot).

Summary

- No mislabeling was detected among parental ramets.
- There was 5% pollen contamination detected in Seedlot *a* x *b* (9 out of 181 seed); 95% of the seed (embryos) could be produced by the *b* pollen source.
- There was 1% pollen contamination detected in Seedlot c x *b* (2 out of 190 seed); 99% of the seed (embryos) could be produced by the *b* pollen source.

Ramet identification and marker development in sugar pine (Project #266; Partner: FS – Region 5)

Project Objectives

(1) To determine if the two submitted samples are the same clone.
(2) To extract and store ample amounts of DNA from both ramets (if ramets are correctly identified, both are ramets of the clone that Dave Neale is using for his work).
(3) Use these DNAs (among others) to start developing better DNA markers for the species.

Results

(1) Determine if the two submitted samples are the same clone.
Each of the two samples was genotyped twice at 20 isozyme loci. Both samples had matching genotypes as expected if they were two ramets of the same clone. Data was scored identically in both preparations of each sample indicated that scoring error rate is close to zero.
(2) To extract and store ample amounts of DNA from both ramets.
Each sugar pine tree was extracted four times on NFGEL Plate #502 (Tables 2 and 3). DNA is stored at -80C. DNA yields ranged from 12 to 65 ug per extraction.
(3) Use these DNAs (among others) to start developing better DNA markers for the species.
These DNAs will be held at -80C until marker development and assessment occurs.

Taxonomic boundaries in *Sedum oregonense* and *S. obtusatum retusum* (Project #267; Partners: FS – Region 6 and Carex Working Group)

Taxonomic/identification issues exist in *Sedum* species growing in the Pacific Northwest. Current taxonomic boundaries seem to be based on chromosome counts. Ploidy information can help managers sort samples into these existing taxonomic groups. Managers will compare the ploidy to morphology and see if morphological and ploidy boundaries seem to coincide. NFGEL conducted a ploidy analysis on submitted *Sedum* plants.

These submitted *Sedum* samples were generally characterized by broad DNA histograms and shifting peak positions. Poorly formed, instable histograms are often the result of the presence of secondary metabolites that may be interfering with the binding of the fluorochromes to the DNA, or oxidation which is leading to DNA degradation. Some samples did resolve well, but the shifting of peak position was still common when the sample was re-run on a different date (relative to control). We highly suspect that *Sedum* contains interfering compounds in abundance which is leading to the difficulty in obtaining clear ploidy results

Regardless, we detected no ploidy difference among samples. When comparing the diploid control runs to those of the other samples, peak positions do not greatly differ. The large window of shifting peak position can make this determination difficult, however the diploid controls show the same range of position as do the other samples. We see no evidence of varying genome content levels relative to the diploid control.

Genetic markers and the assessment of variation in Port-Orford Cedar (Project #173; Partner: FS – Region 6)

Overall goals for the Port-Orford Cedar (POC) genetic study include:

1. To compare the genetic structure and diversity among pre-epidemic, post-epidemic, and *Phytophthora lateralis* resistant Port-Orford Cedar orchard populations.
2. To screen a pedigree for any contaminates (ramets or F1 progeny); this pedigree will be used for a F2 mapping population to search for resistant gene(s).
3. To determine the mode of organelle inheritance in Port-Orford Cedar; this knowledge will have direct application for marker selection for other POC genetic efforts.

Prior POC isozyme work performed at NFGEL indicated that overall genetic diversity within the species was low. Sniezko and Kolpak began working with Richard Cronn, US Forest Service, Pacific Northwest Research Station (PNW) in approximately 2003 to develop molecular markers better able to address project objectives. NFGEL's role in Project #173 was to extract DNA and isozymes from submitted samples. We were to share DNA with Cronn's lab as requested, and to run the isozymes if DNA marker development was unsuccessful. DNA aliquots from various samples were shipped to PNW throughout 2006 and early 2007. All DNA (from approximately 1,135 trees) were shipped to PNW in February 2009.

Based on the latest Special Technology Development Program progress report (Development of DNA tools to aid genetic conservation and restoration in Port-Orford-cedar and enhance the *Phytophthora lateralis* resistance breeding program; Kolpak, Sniezko, and Cronn; October 2011), the DNA work is just about completed and a final report is expected this fiscal year (FY12). DNA marker development was successful, and these new markers were, and can be, used to address project objectives. It was determined in December 2011 that isozyme extracts were, therefore, not needed and could be discarded (concurred by Sniezko, Cronn, and Vicky Erickson via email). Plates of all isozyme extracts were discarded in January 2012.

Determining clonal identity of aspen stands (Project #262; Partner: FS – Region 6)

The goal of this project was to provide a 'clonal identity' of aspen stands on the Walla Walla Ranger District, Umatilla National Forest. Region 6 was interested in finding out more about the genotype of several clones. One tree of interest is the clone that produced the previous national champion aspen. The champion tree has fallen down but there are still a number of very big aspen at that site. The problem is that there is virtually no regeneration. The Forest is trying to get a CE put together that will let them clear some brush and build a fence around these trees. Having the genetic info to determine if that clone was unique on the district would help.

Data at seven SSR loci from this study indicate that each aspen stand analyzed is made up of a single unique clone. Therefore, all four stands studied are monoclonal for a different clone. Because of the monoclonal nature of the samples, we effectively genotyped only four genetically different trees (one clone/stand). Even given the small sample size, 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 2.1×10^{-5}

(meaning the variation detected among the four clones is high). None of these four stands were genotyped in any prior aspen genetic studies conducted by NFGEL. It is noteworthy that stand POTR50080 contains an apparent polyploid clone (three alleles/locus were detected in three of the seven loci). This may be an example of true polyploidy (triploidy) or of duplication of chromosomal segments.

Quantifying gene flow and adaptive variation in conifers across the Western states to predict effects of climate change on forest ecosystems (Project #242; Partner: FS - RMRS)

NFGEL extracted DNA from 2,140 conifer samples from 14 species. Samples were provided to the lab as one to three desiccated needles per tree. Species included mainly PIPO (*Pinus ponderosa*, ponderosa pine), PSME (*Pseudotsuga menziesii*, Douglas-fir), PICO (*Pinus contorta*, lodgepole pine), ABLA (*Abies lasiocarpa*, subalpine fir), PIEN (*Pinus engelmannii*, Arizona longleaf pine), THPL (*Thuja plicata*, western redcedar), ABGR (*Abies grandis*, grand fir), and LAOC (*Larix occidentalis*, western larch). Each sample (one to a partial needle per tree) was extracted for DNA using the 96-well DNeasy benchtop Iq N protocol with proteinase K modification. DNA quantity was assessed by fluorometry using picogreen, and quality was checked by amplifying the DNA using a 'barcoding' fragment with a fluorescently labeled forward primer for the trnL intron P6 loop and visualizing fragments on an ABI-3130xl.

Overall Purpose (provided by S. Cushman)

An ongoing collaborative effort between RMRS, PNW, PSW, and Interior-west FIA is co-locating networks of inexpensive temperature and relative humidity sensors with collection of conifer genetic samples from approximately 5000 locations distributed across 8 western states. From these data, we will build a statistical microclimate model for the Rocky Mountains correcting temperature and relative humidity estimates for local topographic effects. We will model tree species distributions, growth rates, regeneration rates and landscape genetics of gene flow and local adaptation as functions of spatial and climate gradients. We will then parameterize our microclimate model to downscale Global Climate Model (GCM) predictions to explore fine scale climate change impacts on productivity, species distributions, potential species migrations, and evolution of local ecotypic variation across a 60 million acre area of the Rocky Mountains.

Ramet identification in Douglas-fir, Noble fir, and Sugar pine (Project #246; Partner: Bureau of Land Managment)

Submitted ramets of clones were genetically tested to verify if ramet genotypes are the same. The project objective is to verify the identity of material going into new conifer seed orchards.

<u>Ramet Identification in Noble Fir (*Abies procera*)</u>

A total of 311 samples were submitted for a genetic analysis using 19 isozyme loci. An average of five ramets per clone were tested (ranging from 1 to 7 ramets per clone) from a

total of 61 clones. Of the 61 clones analyzed, four clones contained ramet mis-labeling (6.6% mislabeling).

Ramet Identification in Sugar Pine (*Pinus lambertiana*)

A total of 326 samples were submitted for a genetic analysis using 19 isozyme loci. An average of three ramets per clone were tested (ranging from 2 to 5 ramets per clone) from a total of 90 clones.

Ten clones contained ramet mis-labeling (11.1% mislabeling among clones). Unique genotypes were identified among the 108 total clones analyzed (90 clones with multiple ramets + 8 clones submitted as single ramets + 10 mislabeled ramets of unknown identity). There were a total of 107 unique genotypes among this group with only two clones sharing the same genotype. The average probability that two unrelated individuals with this genotype could be drawn from the same randomly mating populations is 1 out of 314 trees. Therefore, this is a fairly common genotype and these two clones likely share this genotype just out of random chance. Furthermore, mis-labeled ramets did not match any other genotype in the data set.

Ramet Identification in the Douglas-fir (*Pseudotsuga menziesii*) Provolt Orchard

A total of 1,496 samples were submitted for genetic analysis. An average of four ramets per clone were tested (ranging from 2 to 13 ramets per clone) from a total of 385 clones. DNA from all trees was extracted and characterized at three microsatellite loci. Of the 385 clones analyzed, 32 clones contained ramet mis-labeling (8.3% mis-labeling in the clones).

Ramet Identification in the Douglas-fir (*Pseudotsuga menziesii*) Horning Orchard

A total of 1,658 samples were submitted for genetic. An average of four ramets per clone were tested (ranging from 2 to 18 ramets per clone) from a total of 385 clones. DNA from all trees was extracted and characterized at three microsatellite loci. Of the 385 clones analyzed, 21 clones contained ramet mis-labeling (5.4 % mislabeling in clones). Almost every clone contained a unique genotype. 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 1.6×10^{-7} (making the possibility of having matching genotypes by chance between clones in this orchard very rare). However, in eight cases, trees identified as being different clones had the same genotype. These matches either indicates that there is further mislabeling among some clones, or that these are just examples of random data matches between clones (and if further variation was assessed, we would start to detect differences.)

Ramet Identification in the Douglas-fir (*Pseudotsuga menziesii*) Tyrell Orchard

A total of 1,055 samples were submitted for genetic analysis. An average of five ramets per clone were tested (ranging from 2 to 9 ramets per clone) from a total of 213 clones. DNA from all trees was extracted and characterized at three microsatellite loci. Of the 213 clones analyzed, 11 clones contained ramet mis-labeling (5 % mislabeling in clones). Every clone contained a unique genotype. 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 1.3×10^{-7} (making the possibility of having matching genotypes by

chance between clones in this orchard very rare). However, in four cases, trees identified as being different clones had the same genotype.

Use of native species in biodiversity restoration and management (Project #165; Partners: University of CA, Davis and Colegio de Postgraduados, Mexico)

Monterey pine (*Pinus radiata* D. Don) is a forest tree species of great economic importance worldwide, with a native range restricted to the coastal zone of central California and northern Baja California. The island of Guadalupe, in the Pacific Ocean off the coast of Baja California, hosts one of the five remnant natural populations of this species. The current population size on the island is down to about 220 adult trees growing isolated or in small patches. Because of the fast and presumably massive loss of pines, causing both fragmentation and drastic reduction in population size, several genetic impacts have likely occurred on the island, including loss of genetic diversity and increase in inbreeding. If genetic diversity has been drastically reduced in the population, it might be necessary to re-introduce genetic material from earlier *ex-situ* collections. Similarly, if inbreeding is an issue, actions might be required to promote cross pollination and seed dispersal between patches to reduce relatedness among parental trees in the next generation. Based on a germplasm sample from about 35% of the current population distributed along the patches, an analysis of genetic diversity, as well as its spatial structure and inbreeding level, was done using microsatellite markers. The sampling structure allowed comparing the genetic diversity and inbreeding level in the progeny (seed) in relation to that in the maternal generation (remnant trees). Results showed that despite the drastic reduction in population size, an adequate level of genetic diversity remains, both in the remnant trees and their open-pollinated progeny. The data also indicated a minimum of 45% cross-pollination in the population. Thus, the genetic information obtained does not support the need for a genetic intervention to restore this population other than to move seed among resident trees to increase dispersion distance and accelerate connectivity between patches.

Genetic make-up of quaking aspen colonies across Nebraska (Project #235; Partner: Western Nebraska Resources Council)

The goal of this project was to provide a 'genetic make-up' or 'clonal identity' of quaking aspen (*Populus tremuloides*) colonies growing across Nebraska. A total of 207 trees from 18 colonies were included in the study. 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. Studies have shown that aspen in the Pacific West can grow in large stands that are made up of multiple genetic types (so the number of stems per clone is small, with multiple clones per stand). Aspen growing in eastern North America tends to grow in stands that are small in size, where clones size is also very small (a low number of stems per clone). Finally, aspen in the Intermountain West often grow in large stands with correspondingly large clonal sizes. Data at six microsatellite (SSR) loci from this study indicate that the aspen stands (colonies) located in Nebraska are genetically diverse (100% polymorphic loci, 9.7 alleles/locus, He = 0.784), and stands are genetically different from each other, with 78.5% of all genetic variation measured being the differences among stands. Seventeen of the 18

stands studied were monoclonal (contained a single clone). Therefore, aspen stands growing in Nebraska are usually comprised of just one clone per stand.

The role of genetic differentiation and local adaptation on the morphological variation of lodgepole pine (*Pinus contorta)* (Project #249; Partner: Colorado State University)

DNA was extracted from 508 conifer needle samples and shipped back to the project cooperator. The overall goals driving the need for DNA are to: investigate the role of genetic differentiation and local adaptation on the morphological variation of *Pinus contorta*. The primary focus of the project is to determine the extent of genetic variation within and among the four distinct subspecies of *Pinus contorta* (ssp. *murrayana,* ssp. *latifolia,* ssp. *contorta,* and ssp. *bolanderi*) and uncover how specific ecological site characteristics may elicit variation in morphological characteristics.

Clonal identity in giant sequoia and coast redwood (Project #257; Partner: Archangel)

Project objectives included:

(1) To identity effective marker systems for ramet/clonal ID in Giant Sequoia and Coast Redwood. Initial work will be to develop isozyme protocols and test SSR primers on Redwood and Giant Sequoia DNA obtained from trees on site.
(2) To perform ramet IDs on four submitted samples of Giant Sequoia

Results from three DNA microsatellite loci and ten isozyme loci indicate that the ramet/scion pairs of Giant Sequoia submitted for analysis have matching genotypes and the two pairs are distinct from each other. Therefore, sample A-ramet matches A-scion, and sample B-ramet matches B-scion. The A samples do not match the genotype of the B samples.

To assess the overall variability of the markers, the four samples were compared to eleven Giant Sequoia trees that are located here at our site. Of the 15 samples analyzed, we detected 13 unique genotypes: each of the local trees had a unique genotype + the two genotypes from the project samples. Even though each tree did have a unique DNA genotype, the overall variability of the markers was rather low. 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 9.4×10^{-3} (so roughly 9 trees out of every 1000 will, by chance, have the same genotype when using these markers). The microsatellite markers used were actually developed for Coast Redwood and are not very variable in Giant Sequoia. Most of the discriminating power of the data comes from the isozyme loci.

DNA fingerprinting elite *Populus* clones (Project #261; Partner: Greenwood Resources)

Project Objective: Fingerprint elite poplar clones using DNA technology.

Results from nine microsatellite loci indicate distinct genotypes among all 72 poplar trees analyzed. 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 1.84×10^{-10}.

Total genomic DNA was isolated from three hole punches of desiccated leaf tissue per tree using the Qiagen® DNEasy-96 plant kit following the liquid nitrogen procedure with the addition of proteinase-K. DNA concentrations were quantified using fluorometry. The average DNA yield per tree was 9.7 ug. Primer sequences are available from the International Populus Genome Consortium (http://www.ornl.gov/sci/ipgc/ssr_resource.htm) and Dayanandan et al, 1998. The forward primer for each locus was labeled with a fluorescent tag, and PCR products were separated using an ABI-3130xl capillary electrophoresis system and visualized using GeneMarker software (v 1.6). The results provided here were confirmed by replicating amplification of all loci for all samples from two to four times each. All genotypes of these replicate samples were identical, indicating the error rate for these data to be near zero.

Although there was no initial intent to compare NFGEL derived genotypes to those obtained from the genotyping done by GenServe Laboratories, we noted that GenServe did genotype 66 of the same trees submitted to NFGEL for analysis (at 8 of the 9 loci). Upon comparing the data from the two labs, the concurrence of scores was quite high. There are, of course, instances of shifts (for example, NFGEL scores may by one basepair greater than GenServe scores at a given locus) and rounding (binning) differences (for example, NFGEL may have called a certain allele 190 (bp), whereas GenServe may have called it a 191 (bp)). Many of these issues can be simply explained by the differences in laboratory reagents and equipment (primarily NFGEL's use of a capillary-based electrophoresis system versus GenServe's use of a gel-based system). In fewer instances did we actually genotype a tree differently than did GenServe. When this did happen, most of these cases involved GenServe calling a homozygous condition at a locus that we observed to be a heterozygote with two alleles that varied by only two basepairs.

STATUS OF ON-GOING PROJECTS

The following projects are in various stages of activity in the lab.				
PROJECT	**COOPERATOR**	**SPECIES**	**PROJECT TITLE**	**STATUS**
128	FS - SRS	Loblolly Pine	Characterize genetic variation in the Founder Tree Project population	Prepped for analysis; holding due to low priority.
147	FS - R8/R9	*Panicum virgatum, Schizachyrium scoparium, Elymus virginicus*	Distribution of genetic variation across the population range of grass species used for restoration	Assessing whether to drop project or get further needed samples collected and submitted.
155	FS - R9	Eastern White Pine	Genetic analysis of an Eastern White Pine seed orchard for the Lake States	Analysis complete; partial report complete; assessing possibility to run additional markers to better address one objective.
188	FS - PSW	*Picea chihuahuana*	Genetic analysis of *Picea chihuahuana*	Analysis complete; in reporting.
207	FS - PSW	Southwestern White Pine	Family structure in WPBR *Pinus strobiformis* (south western white pine) family 564	Isozyme analysis complete; more variable markers needed to address objectives.
232	BLM	Ponderosa Pine	Genetic testing of disjunct Ponderosa Pine stands on BLM lands in Wyoming and throughout the west	mtDNA dataset in analysis. Nuclear data in reruns, QA checks, and final scoring.
234	American Chestnut Foundation	American Chestnut	DNA extraction from American Chestnut (*Castanea dentata*)	DNA extracted; being held until shipped to FS-R&D cooperator needs them.
244	FS - RMRS	Rocky Mountain Bristlecone Pine	Genetic diversity in *Pinus aristata* samples from the St Mary's Glacier site on the Roosevelt National Forest, Colorado	Samples prepared and being held in freezers.
248	FS - R6	Golden Chinquapin	Genetic structure of Golden Chinquapin (*Chrysolepis chrysophylla*)	Samples prepared for isozymes. Isozymes ready to run. Samples extracted for DNA. Holding for possible marker development.
254	BLM / FS - R6	Ponderosa Pine	Genetics relationships of isolated, disjunct ponderosa pine stands	Samples prepared (isozymes, DNA and needle counts). Isozymes complete (in analysis). SSRs and mtDNA analysis on-going.
256	FS - R5	*Astragalus webberi*	Genetic variation of *Astragalus webberi* (Webber's milkvetch)	Data complete, in analysis and reporting.

The following projects are in various stages of activity in the lab.				
PROJECT	**COOPERATOR**	**SPECIES**	**PROJECT TITLE**	**STATUS**
258	FS - R6	*Sisyrinchium sarmentosum*	Hybridization and species identitity in *Sisyrinchium sarmentosum*	Prepped and in analysis (ploidy complete; SSR marker development unsuccessful; DNA sent to R. Cronn, FS-PNW, for next-gen sequencing approach).
259	FS - R8	Longleaf Pine	Is there a genetic difference between the traditional coastal sources and the piedmont sources of longleaf pine?	SSR data complete, and sent to C. Echt (FS-SRS) for analysis. Isozyme data complete and is in analysis.
264	Sierra Pacific Industries; FS - R5	Ponderosa Pine	Check parental identity of two controlled cross seedlots.	Project is in reporting.
265	FS - R6	Port-Orford Cedar; Western White Pine	Can DNA be extracted from pollen? (Being able to use DNA from pollen will help to confirm parent identity or contamination in controlled crosses.)	Report complete. (Will be in FY13 Annual Report).
268	FS - R6	Oregon White Oak (*Quercus garryana)*	Oregon white oak genetic diversity and geographic differentiation. Data will help develop a restoration strategy for the species.	Samples still arriving. Samples received have been prepared for isozymes and extracted for DNA.
269	FS - R6	Baker Cypress (*Cupressus bakerii*)	Genetic diversity and population structure of Baker Cypress. Data will be used to assist in gene conservation efforts.	Samples have been prepared for isozymes and extracted for DNA. Isozymes are ready to run, and DNA markers will need to be developed.
270	Oregon State University / FS - RMRS	Douglas fir	SNP marker development in Douglas-fir.	56 DNA Douglas-fir samples are ready to send to UC Davis for SNP development after we receive 40 more Doug-fir samples to extract and include.
271	FS - R6	Port-Orford Cedar	Confirmation of family identities and diversity for Port-Orford-cedar Inbreeding Depression Study. Data will aid in disease resistance breeding of the species.	Samples extracted for DNA. SSR analysis is on-going.
272	FS - R6	Western White Pine	Identification of western white pine clones at Beaver Creek Seed Orchard. To aid in rust resistance breeding efforts.	Samples extracted for DNA. SSR analysis is on-going.

The following projects are in various stages of activity in the lab.				
PROJECT	**COOPERATOR**	**SPECIES**	**PROJECT TITLE**	**STATUS**
273	FS - R1-6/BLM	Ponderosa Pine	Genetics relationships of isolated, disjunct ponderosa pine stands	Samples arriving. A continuation of Projects 232 and 254.
274	FS - R6	Butterfly	Taxonomic identity of putative Taylor's Checkerspot (butterfly) populations.	DNA extraction and SSR analysis is on-going.

BUDGET

NFGEL receives an annual allocation from the National Forest System's Forest Management staff group. From FY09 – FY12, NFGEL received $480,000 each year. In addition to these funds, NFGEL receives between $50,000 to $250,000 annually of individual partner program dollars collected for non-NFVW projects. These program dollars are used for additional salary, chemical, supply, equipment, and repair needs.

ALLOCATION	
WO - Forest Management	$480,000
WO - Forest Management (carry-over)	$100,000
BLM (NFGEL Project 246)	$66,748
BLM (NFGEL Projects 228 and 232)	$4,793
Colorado State University (NFGEL Project 249)	$3,200
Greenwood Resources (NFGEL Project 261)	$1,200
TOTAL	$655,942
EXPENDITURES	
Salary - Permanent Employees	$171,762
Salary - Temporary Employees	$96,451
Salary - Contract Employees	$54,000
Site Utilities and Rents	$16,896
Chemicals and Supplies	$93,299
Equipment and Repair	$138,782
Computer and Office Supplies	$2,793
Travel and Training	$2,323
Vehicle	$3,000
Administrative Costs (cell phones, awards, building improvements)	$13,692
TOTAL	$592,998
BALANCE	$62,944

STAFFING

During FY 2012, NFGEL was staffed with 1.6 permanent FTEs, and multiple staff on temporary tours.

EMPLOYEE	EMAIL ADDRESS	POSITION	TOUR (% FTE)
Valerie Hipkins	vhipkins@fs.fed.us	Director	Permanent (100%)
Randy Meyer	rmeyer@fs.fed.us	Lab Biotechnician	Permanent (60%)
Courtney Owens	cowens03@fs.fed.us	Lab Biotechnician	Temp-NTE (100%)
Jody Mello	jmello@fs.fed.us	Lab Biotechnician	Temp-STEP (60%)
Rosanna Hanson	rosannahanson@fs.fed.us	Lab Biotechnician	Temp-NTE (100%)
Sarah Trujillo		Lab Aid	Summer Temp-STEP (70%)
Dayna Hightower		Lab Aid	High School Volunteer (10%)
Garrett Short		Lab Aid	High School Volunteer (10%)

STAFF ACTIVITIES

Publications

- DeWoody, J, L Lindstrand III , VD Hipkins, and J Kierstad Nelson. 2012. Population genetics of *Neviusi cliftonii* (Shasta snow-wreath): patterns of diversity in a rare endemic. Western North American Naturalist (in press).
- DeWoody, J, VD Hipkins, J Kierstad Nelson, and L Lindstrand III. 2012. Genetic structure of *Vaccinium parvifolium* (Ericaceae) in northern California reveals potential systematic distinctions. Madroño 59(4): 196-210.
- Schoettle, AW, BA Goodrich, V Hipkins, C Richards, and J Kray. 2012. Geographic patterns of genetic variation and population structure in *Pinus aristata*, Rocky Mountain bristlecone pine. Canadian Journal of Forest Research 42: 23-37.
- Potter, KM, RM Jetton, WS Dvorak, VD Hipkins, R Rhea, and WA Whittier. 2012. Widespread inbreeding and unexpected geographic patterns of genetic variation in eastern hemlock (*Tsuga canadensis*), an imperiled North American conifer. Conservation Genetics 13(2): 475-498.

Internal Activities

- V. Hipkins spent the week of March 12, 2012 in the Washington Office discussing NFGEL mission and purpose, and the integration of the lab within the Forest Management program of work. Meetings occurred with staff from: NFS-Forest Management, NFS-Range Management, NFS – Directors, R&D – Genetics, SPF – Forest Health Protection, International Programs, and Natural Resources Conservation Service (NRCS).
- Presentation (V. Hipkins): DNA and Timber Theft. Forest Law Enforcement to Address Illegal Logging: An Exchange Program to Build Partnerships in Forestry and Law Enforcement in Russia and the U.S. Seattle, WA. September 28, 2012.
- Hosted Project Learning Tree module on Biotechnology and Risk. June 26, 2012. (C. Owens, R. Hanson, R. Meyer, V. Hipkins)
- Reviewed PhD dissertation on *Botrychium* species for Region 9. A review of the genetic testing methods used was needed to help the Forest assess the soundness of the approach. (V. Hipkins)
- Determined protocol required to dry down and store Pongamia seed (tropical species used for biofuels). (R. Meyer)
- Union President – Pacific Southwest Research Station, and Union Steward Eldorado NF – Region 5. (R. Meyer)
- Member of Eldorado NF Workforce Planning Team. (R. Meyer)
- Member of USDA Forest Service National Safety Committee (January 2001 - Current). (R. Meyer)
- Member of the PSW Research Station Safety Committee January 2002 – Current. (R. Meyer)
- Member of the PSW Research Station Community Enhancement and Diversity (civil rights) Team – January 2003 – Current. (R. Meyer)
- NFFE Forest Service Council, Safety Committee Chair (2006 – Current). (R. Meyer)
- NFFE Forest Service Council, Union Representative on Accident Investigations. (R. Meyer)
- National Leadership Council (Safety Core Team) (2008 – December 2011). (R. Meyer)
- Safety Engagement Facilitator, October and November (x2) 2011. (R. Meyer)

- NFFE , Union Representative, Federal Advisory Council on Occupational Safety and Health (FACOSH) Occupational Exposure Limits by Federal Agencies. (R. Meyer)
- NFFE , Union Representative, Federal Advisory Council on Occupational Safety and Health (FACOSH) Uniform Training Guidelines Workgroup. (R. Meyer)
- NFFE Forest Service Council, Union Representative on USDA Strengthening Services Administrative Solutions (SSAS) Homeland Security Workgroup. (R. Meyer)

Hosted

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

CONTACT INFORMATION

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