National Forest Genetics Laboratory (NFGEL)

FY12 2nd Quarter Report

January – March 2012

Project Reports Completed

Clonal structure and genetic diversity in *Populus angustifolia*, *P. fremontii*, and *P. trichocarpa* located in Nevada (Project #210; Partner – 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 analysis to determine relationships between ponderosa pine super trees located near Stirling City, CA (summary) (Project #250; Partners – R5 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.

Ramet identification in Douglas-fir, Noble fir, and Sugar pine (Project #246; Partner - BLM)

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.

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.

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

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.

In addition to providing the genotype data for the poplar trees within this report, we are also sending you an excel spreadsheet containing genotype scores and comparisons. The excel spreadsheet includes (1) the genotype data for the 72 poplar trees at nine loci, (2) comments for each of the nine loci, (3) a listing of genotype scores that differed between NFGEL and GenServe (so these are cases where the two labs scored different genotypes at a locus for a given tree), and (4) a listing of genotype scores that varied between NFGEL and GenServe due to rounding differences (so both labs essentially are seeing the same genotype, but due to rounding differences, the scores between the labs vary). For comparisons, NFGEL scores were adjusted to match the GenServe's scoring system.

Verification of controlled mass pollination (CMP) in two ponderosa pine seedlots (Project #263; Partners - R5 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.

Quantifying gene flow and adaptive variation in conifers across the Western states to predict effects of climate change on forest ecosystems (Pj242; Partner - RMRS)

Project Objectives

Extract 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 lq 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.

Genetic structure of *Vaccinium* (huckleberry) in California reveals potential systematic distinctions (Pj238; Partner – R5)

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.

Project Priorities (Planned order of analysis in lab; several projects may overlap at once)

Isozymes

Project	'Region'	Species
257	non-profit	Giant Sequoia
254	BLM	Ponderosa Pine *(samples still arriving)*
248	R6	Golden Chinquapin *(samples still arriving)*
259	R8	Longleaf Pine *(samples still arriving)*
244	FS-RMRS	Rocky Mountain Bristlecone Pine
128	FS-SRS	Loblolly Pine

DNA

Project	'Region'	Species
232	BLM	Ponderosa Pine (re-runs and QA checks on nuclear data)
257	non-profit	Giant Sequoia
264	SPI (private)	Ponderosa Pine (controlled cross check)
262	R6	Quaking Aspen
254	BLM/R6	Ponderosa Pine *(samples still arriving)*
259	R8	Longleaf Pine *(samples still arriving)*
249	Colorado State U	Lodgepole Pine (DNA extractions)

Marker/Procedure Development

Project	'Region'	Species
155	R9	Eastern White Pine (DNA marker development)
258	R6	*Sisyrinchium sarmentosum* (DNA marker development)
207	FS-PSW	Southwestern White Pine (DNA marker development)
248	R6	Golden Chinquapin (DNA marker development)
265	R6	Port Orford Cedar; Western White Pine (DNA extraction from pollen procedural development)

In Analysis and Reporting

Project	'Region'	Species
232	BLM/R1-4	Ponderosa Pine (mtDNA dataset)
251	R5	*Fritillaria* ssp
256	R5	*Astragalus* ssp
255	R5	*Lewisia* ssp

Administrative News

- V. Hipkins spent the week of March 12th 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).
- NFGEL's status as a detached unit is still pending the acceptance of signed WO - Forest Management organizational charts.
- NFGEL administrative services are being obtained through the WO administrative support group BASS (Business Administrative Support Services).
- Potter, KM, RM Jetton, WS Dvorak, VD Hipkins, J 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 13:475-498.
- 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.
- DeWoody, J, VD Hipkins, JK Nelson, and L Lindstrand III. 2012. Genetic structure of *Vaccinium* (huckleberry) in California reveals potential systematic distinctions. Madrono (in press).

Budget

NFGEL FY12 Budget is $480,000 (NFVW) before administrative overhead and rents/utilities.

Staffing

The temp-NTE GS-404-05 vacancy (2 vacancies) request was submitted 9/13/2011; vacancy opened 11/3/2011; vacancy closed 11/14/2011; referral list generated 12/21/2012; start dates for two hire was 1/15/2012. These are one-year positions with the ability to extend for a second year (1/15/2012 – 1/15/2014).

Permanent GS-440-11/12 position description remains in classification (since September 2011).

Valerie Hipkins (vhipkins). GS-426-13. Permanent (100%)
Randy Meyer (rmeyer). GS-404-07. Permanent (40%)
Courtney Owens (cowens03). GS-404-05. Temp-NTE (100%)
Jody Mello. GS-401-05 (jmello). Temp-STEP (60%)
Rosanna Hanson (rosannahanson). GS-404-05. Temp-NTE (100%)

Status of On-Going Projects

PROJECT	REGION	SPECIES	CONTACT	PROJECT TITLE	STATUS
128	FS-SRS	Loblolly Pine	F.Bridgewater	Characterize genetic variation in the Founder Tree Project population	Prepped for analysis; holding due to low priority.
147	R8/R9	*Panicum virgatum, Schizachyrium scoparium, Elymus virginicus*	P.Berrang	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	R9	Eastern White Pine	P.Berrang	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.
173	R6	Port Orford Cedar	R.Sniezko	Port-Orford Cedar sampling stradegy to compare the genetic structure and diversity among pre-epidemic, post-epidemic, and *Phytophthora lateralis* resistant orchard populations	DNA extracted from about 2000 trees and shipped to FS-PNW for DNA marker development. Isozymes prepped and being held if needed. <u>It was determined that the isozymes were not needed. All isozyme preps were thrown away in January 2012.</u>
188	FS-PSW	*Picea chihuahuana*	P.Hodgskiss	Genetic analysis of *Picea chihuahuana*	Analysis complete; in reporting.
207	FS-PSW	Southwestern White Pine	A.Mix	Family structure in WPBR *Pinus strobiformis* (south western white pine) family 564	Analysis complete; more variable markers needed to address objectives.
232	BLM	Ponderosa Pine	R.Means; MF Mahalovich	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.

PROJECT	REGION	SPECIES	CONTACT	PROJECT TITLE	STATUS
234	Amer. Chest. Found.	American Chestnut	B. Monahan	DNA extraction from American Chestnut (*Castanea dentata*)	DNA extracted; being held until shipped to FS-R&D cooperator needs them.
242	FS-RMRS	Ponderosa Pine, Douglas-fir, Lodgepole Pine, Subalpine Fir, Arizona Longleaf Pine, Western Redcedar, Grand Fir, Western Larch	S. Cushman	Quantifying gene flow and adaptive variation in conifers across the Western states to predict effects of climate change on forest ecosystems	Project complete; there is interest in sending more samples, and also in sharing samples with Oregon State Univ to develop SNP markers for Douglas-fir.
244	FS-RMRS	Rocky Mountain Bristlecone Pine	A. Schoettle	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	R6	Golden Chinquapin	A. Bower	Genetic structure of Golden Chinquapin (*Chrysolepis chrysophylla*)	Samples still arriving (starting arriving in July 2010); prep and hold until all samples collected.
251	R5	*Fritillaria eastwoodiae*	J. Nelson	Population genetics study of *Fritillaria eastwoodiae*	In analysis and reporting.
254	BLM/R6	Ponderosa Pine	B. Means	Genetics relationships of isolated, disjunct ponderosa pine stands	Samples still arriving; prep and hold until all samples collected.
255	R5	*Lewisia kelloggii*	J. O'Brien	Putative new taxon of *Lewisia* from northwestern California	Data complete, in analysis and reporting.
256	R5	*Astragalus webberi*	C. Rowe	Genetic variation of *Astragalus webberi* (Webber's milkvetch)	Data complete, in analysis and reporting.
257	Archangel	Giant Sequioa, Coast Redwood	B. Walraven	Clonal identity in giant sequioa and coast redwood	Prelim analysis complete; awaiting DNA and isozyme analysis for 4 giant sequoia samples
258	R6	*Sisyrinchium sarmentosum*	A. Bower	Hybridization and species identitity in *Sisyrinchium sarmentosum*	Prepped and awaiting analysis (ploidy complete; SSR marker development unsuccessful; may look for appropriate loci to sequence)

PROJECT	REGION	SPECIES	CONTACT	PROJECT TITLE	STATUS
259	R8	Longleaf Pine	B. Crane	Is there a genetic difference between the traditional coastal sources and the piedmont sources of longleaf pine?	Awaiting further collections (all samples are isozyme prepped and DNA extracted upon arrival)
262	R6	Quaking Aspen	V. Erickson	Determining if aspen regeneration is sexual (seedlings) or asexual (clonal)	Samples in DNA analysis.
264	SPI (R5) (private)	Ponderosa Pine	G. Lunak	Check parental identity of two controlled cross seedlots.	Samples in DNA analysis.
265	R6	Port Orford Cedar; Western White Pine	R. Sniezko	Can DNA be extracted from pollen? (Being able to use DNA from pollen will help to confirm parent identity or contamination in controlled crosses.)	Samples in lab; awaiting procedure development.