

National Forest Genetics Laboratory (NFGEL)

FY12 1st Quarter Report

October – December 2011

Project Reports Completed

Use of native species in biodiversity restoration and management (Project #165)

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)

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, $H_e = 0.784$), 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.

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

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 Noble Fir (*Abies procera*)

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.)

The role of genetic differentiation and local adaptation on the morphological variation of lodgepole pine (*Pinus contorta*) (Project #249)

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.

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

Isozymes

Project	'Region'	Species
251	R5	<i>Fritillaria eastwoodiae</i>
256	R5	<i>Astragalus webberi</i>
255	R5	<i>Lewisia kelloggii</i>
244	FS-RMRS	Rocky Mountain Bristlecone Pine
263	SPI/R5	Ponderosa Pine
254	BLM/R6	Ponderosa Pine (<i>samples still arriving</i>)
128	FS-SRS	Loblolly Pine

DNA

Project	'Region'	Species
246	BLM	Douglas-fir, Tyrell Orchard
232	BLM	Ponderosa Pine
261	Greenwood Res.	Poplar
250	SPI/R5	Ponderosa Pine
263	SPI/R5	Ponderosa Pine
262	R6	Quaking Aspen
254	BLM/R6	Ponderosa Pine (<i>samples still arriving</i>)
259	R8	Longleaf Pine (<i>samples still arriving</i>)

DNA Marker Development

Project	'Region'	Species
155	R9	Eastern White Pine
258	R6	<i>Sisyrinchium sarmentosum</i>
256	R5	<i>Astragalus webberi</i>
207	FS-PSW	Southwestern White Pine

Administrative News

- NFGEL's status as a detached unit is pending the acceptance of signed WO - Forest Management organizational charts.
- NFGEL is receiving its administrative support through the Washington Office.
- FY11 Accomplishment Reporting was completed in October 2011.
- Potter, KM, RM Jetton, WS Dvorak, VD Hipkins, J Rhea, and WA Whittier. 2011. Widespread inbreeding and unexpected geographic patterns of genetic variation in eastern hemlock (*Tsuga canadensis*), an imperiled North American conifer. *Conservation Genetics* (in press).
- Schoettle, AW, BA Goodrich, V Hipkins, C Richards, and J Kray. 2011. Geographic patterns of genetic variation and population structure in *Pinus aristata*, Rocky Mountain bristlecone pine. *Canadian Journal of Forest Research* (in press).

Budget

NFGEL FY12 Budget is currently set at \$480,000 (NFVW).

Staffing

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

Temp-NTE GS-404-05 vacancy closed on 11/14/2011. We have not yet received a referral list from HRM (as of 12/5/2011).

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

In-the-Lab News Update

The ABI instrument (runs all microsatellite (gene fragment) and sequence samples) has been out of commission from November 19th to December 7th with a broken power button. There has been a delay in repairing as we work out the procurement processes with our new administrative services groups.

NFGEL was unable to micropurchase from October 3th to November 14th due to our cardholders information needing to be realigned with the WO (and not with PSW).

We were not able to run any isozyme samples from August 22nd to December 12th due to backorders of two critical isozyme chemicals (original orders from ~April 2011). The supply of these chemicals is still limited and sporadic, which may cause delays on future projects. Current isozyme projects were delayed by four months.

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	<i>Panicum virgatum</i> , <i>Schizachyrium scoparium</i> , <i>Elymus virginicus</i>	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 strategy to compare the genetic structure and diversity among pre-epidemic, post-epidemic, and <i>Phytophthora lateralis</i> 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 (determination needed).
188	FS-PSW	<i>Picea chihuahuana</i>	P.Hodgskiss	Genetic analysis of <i>Picea chihuahuana</i>	Analysis complete; in reporting.
207	FS-PSW	Southwestern White Pine	A.Mix	Family structure in WPBR <i>Pinus strobiformis</i> (south western white pine) family 564	Analysis complete; more variable markers needed to address objectives.
210	R4	Cottonwoods	J.Richardson	Clonal structure and species determination in cottonwood	Analysis complete; in reporting.
232	BLM	Ponderosa Pine	R.Means	Genetic testing of disjunct Ponderosa Pine stands on BLM lands in Wyoming and throughout the west	In analysis.
234	Amer. Chest. Found.	American Chestnut	B. Monahan	DNA extraction from American Chestnut (<i>Castanea dentata</i>)	DNA extracted; being held until shipped to FS-R&D cooperator needs them.

PROJECT	REGION	SPECIES	CONTACT	PROJECT TITLE	STATUS
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	Analysis complete; in reporting.
244	FS-RMRS	Rocky Mountain Bristlecone Pine	A. Schoettle	Genetic diversity in <i>Pinus aristata</i> samples from the St Mary's Glacier site on the Roosevelt National Forest, Colorado	Samples prepared and being held in freezers (isozymes – priority #4)
246	BLM	Douglas-fir	M.Crawford	Ramet identification in the Douglas-fir Tyrell orchard	In analysis (DNA)
248	R6	Golden Chinquapin	A. Bower	Genetic structure of Golden Chinquapin (<i>Chrysolepis chrysophylla</i>)	Samples still arriving (starting arriving in July 2010); prep and hold until all samples collected.
250	R5	Ponderosa Pine	G. Lunak	Genetic analysis of relationship among Sierra Pacific Industries Super Trees near Stirling City	In analysis (isozymes complete, DNA)
251	R5	<i>Fritillaria eastwoodiae</i>	J. Nelson	Population genetics study of <i>Fritillaria eastwoodiae</i>	In analysis (isozymes – priority #1; ploidy complete)
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	<i>Lewisia kelloggii</i>	J. O'Brien	Putative new taxon of <i>Lewisia</i> from northwestern California	Prepped and awaiting analysis (isozymes-priority #3)
256	R5	<i>Astragalus webberi</i>	C. Rowe	Genetic variation of <i>Astragalus webberi</i> (Webber's milkvetch)	Prepped and awaiting analysis (isozymes-priority #2 and DNA)
257	Archangel	Giant Sequoia, Coast Redwood	B. Walraven	Clonal identity in giant sequoia and coast redwood	Analysis complete, in reporting
258	R6	<i>Sisyrinchium sarmentosum</i>	A. Bower	Hybridization and species identity in <i>Sisyrinchium sarmentosum</i>	Prepped and awaiting analysis (DNA; ploidy complete)

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
261	GreenWood Resources Inc.	Poplar	C. Scott	DNA fingerprinting elite populus clones	In analysis
262	R6	Quaking Aspen	V. Erickson	Determining if aspen regeneration is sexual (seedlings) or asexual (clonal)	Samples prepared and being held in freezers (DNA) (may be waiting further collections?)
263	R5	Ponderosa Pine	G. Lunak	Pollen contamination from outside sources in ponderosa pine	Samples prepared and being held in freezers (DNA).