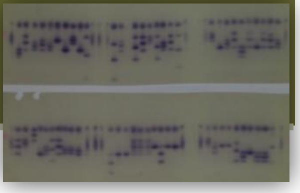
October 2012 - April 2013

FY13 Project Status Update



National Forest Genetics Laboratory (NFGEL), US Forest Service - Forest Management

This report provides a NFGEL project update for the period October 2012 – April 2013.

Valerie Hipkins, NFGEL Director
Placerville, California
530-622-1609
vhipkins@fs.fed.us

INCLUDED

Completed Project Report Summaries 1
Project Publications 4
On-Going Projects 4
Study Ideas in Development 6
Staffing 6
Budget 6

Completed Project Report Summaries

NFGEL PROJECT#	PARTNER	PROJECT TITLE
233	USFS – R1	Idaho fescue (*Festuca idahoensis* Elmer) ploidy level in the Northern Region and three agricultural releases. [Revised Report]
256	USFS – R5	Genetic variation of *Astragalus webberi* (Webber's milkvetch)
264	Sierra Pacific Industries	Determining the validity of parent IDs in a ponderosa pine seed orchard program
265	USFS – R6	DNA extraction from pollen: confirming parent identity and contamination in controlled crosses of Port-Orford-cedar and western white pine.
271	USFS – R6	Confirmation of family identities and diversity for a Port-Orford-cedar inbreeding depression study
275	USFS – International Programs	Big leaf maple timber theft
276	USFS – R6	DNA extraction for purposes of ramet and parental identification in western white pine

Idaho fescue (*Festuca idahoensis* Elmer) ploidy level in the Northern Region and three agricultural releases

[Revised Report] (Project #233)

Project Goals

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

Summary

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

Genetic variation of *Astragalus webberi* (Webber's milkvetch) (Project #256)

Project Goals

To investigate the genetic structure of this rare species, samples were collected from all nine extant occurrences of *A. webberi*, and from one population of *A. pulsiferae*, a common congener. Samples were examined at 18 isozyme loci to quantify measures of genetic variation and differentiation, and to qualitatively examine the genetic structure across the species range.

Summary

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

Determining the validity of parent IDs in a ponderosa pine seed orchard program (Project #264)

Project Goals

The primary project objective is to verify the parents used in two controlled crosses of ponderosa pine. The three parents involved in the two crosses will be genotyped at 8 SSR loci (two ramets per parent clone) using vegetative material, and the two seedlots will be genotyped using 20 seed per lot. Meg/embryo pairs will be genotyped individually to determine the maternal and paternal contribution to each seed, and therefore to check the parents of each controlled cross. A secondary objective is to determine if an additional seedlot is the product of a self.

Summary

The parents are correctly identified in both controlled cross seedlots. However, further analysis showed that the paternal identity of the planted progeny was switched at both planting sites. Since both sites show the same switch, it is likely the labeling error occurred before material was received at the sites for planting. Analysis showed that the additional seedlot tested is not the product of a self, however the putative parent can be the maternal parent of the seedlot.

DNA extraction from pollen: confirming parent identity and contamination in controlled crosses of Port-Orford-cedar and western white pine (Project #265)

Project Goals

Being able to use DNA extracted directly from pollen collections will help in confirming parent identity or contamination in controlled crosses. Knowing the parental identities is key to the success of a breeding program. Efficiency in the breeding program can be improved because one source of contamination can be examined. The project objective is to assess the ability to extract DNA from pollen that is of sufficient quality and quantity for further analysis.

Summary

We were successfully able to extract DNA from submitted pollen samples, and all DNA was of high enough quantity and quality that we were able to amplify samples at a minimum of six SSR loci. DNA yields from the three western white pine extractions ranged from 10.5ug to 14.5ug. DNA yields from the three Port-Orford Cedar extractions ranged from 10.6ug to 28.5ug. We could extract approximately 1 to 2 ug of DNA per 1mg of pollen.

Confirmation of family identities and diversity for a Port-Orford-cedar inbreeding depression study (Project #271)

Project Goals

Through selfing of Port-Orford-cedar (POC) we can more effectively meet two program goals: to develop populations of disease resistant trees and to produce resistant seed for reforestation and restoration. A major drawback to selfing in other species has been inbreeding depression. The evaluation of 19 self-pollinated families will provide a first look of the potential level of contamination in our control crosses as well as confirm that there are 100% S_1 progenies. The examination of diversity in the 19 open-pollinated families will provide some basic information on level of genetic diversity generated in containerized orchard of POC, as well as confirming they provide the proper contrast in the S_1 vs OP test. Specific project objectives include:

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

Summary

There are genotype mismatches among ramets in five of the 19 parent clones tested. Open pollinated families contain similar levels of genetic diversity, though one family in particular did contain less diversity overall compared to the other 18 OP families. There is some contamination in the S_1 and OP progenies of some families. Nine seed parents have no detectable contamination issues within their S_1 or OP families. Four of the seed parents had some minor levels of outcrossing in the S_1 families, or seed contamination in the OP families. The remaining six seed parents had significant contamination issues.

DNA extraction for purposes of ramet and parental identification in western white pine (Project #276)

Project Goals

The purpose of this genetic testing is to confirm identity of western white pine clones. Contamination of a full-sib seedlot was detected in seedlings produced from a 1990 pollination. In 1990, two crosses were made involving a particular seed parent. Both crosses were made on each of 4 ramets. A total of 5 crosses were made in 1990, involving 5 different parents. The project objective is to extract DNA from 18 samples of western white pine—12 pollen and 6 foliage samples. The extracted DNA is to be shipped to a colleague in Victoria, BC, Canada for analysis.

Summary

Up to10ug of each DNA sample was ethanol precipitated and shipped to Canada. Results at five SSR loci revealed that all DNA samples were good for SSR genotyping, and that these markers are able to distinguish five parental trees. The genotypes of multiple ramets from the same clone match except for one clone, suggesting that some ramets may be mislabelled. Limited SSR variation was unable to determine both parents of the full-sib family for Cr2 mapping.

Big leaf maple timber theft (Project #275)

Project Goals

Extract DNA from big leaf maple samples and ship DNA to another lab for further processing. The purpose of the DNA is to build a database to be used in a US Forest Service big leaf maple timber theft case.

Summary

DNA was successfully extracted from twenty-five big leaf maple samples consisting of either leaf or wood tissue. An average of 40ug of DNA was recovered from 1gram of wood tissue. Approximately 15ug of DNA was extracted from 50mg leaf tissue. Ten ug of each sample was ethanol precipitated and shipped to a second lab for further marker development and testing.

Project Publications

Potter, KM, VD Hipkins, MF Mahalovich, and RE Means. 2013. Mitochondrial DNA haplotype distribution patterns in *Pinus ponderosa* (Pinaceae): Range-wide evolutionary history and implications for conservation. American Journal of Botany (in press). [NFGEL Projects 103, 228, 232, and 254]

Vargas-Hernández, JJ, DL Rogers, and V Hipkins. 2013. Restoration of threatened *Pinus radiata* on Mexico's Guadalupe Island. *In*: Bozzano M., Jalonen R., Thomas E., Boshier D., Gallo L., Cavers S., Bordacs S., Smith P., and Loo J. (eds). Genetic considerations in ecosystem restoration using native tree species. A thematic study for the State of the World's Forest Genetic Resources. United Nations Food and Agriculture Organization, Rome, Italy. [NFGEL Project 165]

On-Going Projects

ON-GOING PROJECT#	PARTNER	SPECIES	CONTACT	PROJECT TITLE	STATUS
232	BLM	Ponderosa Pine	R.Means; MF Mahalovich	Genetic testing of disjunct Ponderosa Pine stands on BLM lands in Wyoming and throughout the west	Reporting
248	R6	Golden Chinquapin	A. Bower	Genetic structure of Golden Chinquapin (*Chrysolepis chrysophylla*)	Isozymes extracted; 1/3 of samples run; drop marker due to poor resolution. Developing plan for DNA markers (SSR transfer &/or next-generation sequencing approach).
251	R5	*Fritillaria eastwoodiae*	J. Nelson	Population genetics study of *Fritillaria eastwoodiae*	Report complete and submitted. Additional sample collections received and in isozyme and ploidy analysis. DNA extracted and stored. Information will be re-analyzed and a revised report submitted.
254	BLM/R6	Ponderosa Pine	B. Means	Genetics relationships of isolated, disjunct ponderosa pine stands	Reporting

ON-GOING PROJECT#	PARTNER	SPECIES	CONTACT	PROJECT TITLE	STATUS
258	R6	*Sisyrinchium sarmentosum*	A. Bower	Hybridization and species identitity in *Sisyrinchium sarmentosum*	Ploidy complete; SSR marker development unsuccessful; DNA sent to Rich Cronn (USFS-PNWRS) for next-gen sequencing (cpDNA SNPs)
259	R8	Longleaf Pine	B. Crane	Is there a genetic difference between the traditional coastal sources and the piedmont sources of longleaf pine?	Isozyme data complete. SSR data complete. SSR data sent to Craig Echt (USFS-SRS) for combination with SRS data and full analysis.
268	R6	Oregon White Oak	A.Bower	Oregon white oak genetic diversity and geographic differentiation	Isozyme prepped, DNA extracted, and stored samples that have arrived. Waiting for additional samples to arrive in the summer of 2013.
269	R6	Baker Cyress	A.Bower	Genetic diversity and population structure of Baker Cypress (*Cupressus bakerii*)	Isozyme data complete. Dataset sent to A. Bower for analysis. Report pending.
270	Oregon State Univ.	Douglas-fir, Western White Pine	G.Howe	SNP development in Douglas-fir	DNA extracted and shipped to University of Arizona for SNP development. Report pending.
272	R6	Western White Pine	R.Sniezko	Identification of western white pine clones at Beaver Creek Seed Orchard	SSRs complete. We will need additional variation to meet objectives. Sent DNA to University of Arizona to obtain SNP data (from panel developed as part of Pj 270). Awaiting data. We could look for additional SSRs, and/or run isozymes (which have been prepared and stored).
273	R1-6/BLM	Ponderosa Pine	M.Mahalovich/ R.Means	Genetics relationships of isolated, disjunct ponderosa pine stands	DNA extracted, isozymes prepped, needles counted. Awaiting any additional collections before analysis. These samples will fill in holes in the ponderosa database or be off site stands that need seed source identification.
274	R6	butterfly	A.Bower	Taxonomic identity of putative Taylor's Checkerspot (butterfly) populations.	DNA extracted. Approximately 2/3 complete with SSR data collection.
277	Northwest Tree Improvement Cooperative (NWTIC)	Douglas-fir	K.Jayawickrama	Genetic Quality Control Study in Coastal Douglas-fir	DNA extracted. Approximately ½ complete with SSR data collection (6 loci). We also sent DNA to University of Arizona to obtain SNP data (from panel developed as part of Pj 270). Awaiting data.

ON-GOING PROJECT#	PARTNER	SPECIES	CONTACT	PROJECT TITLE	STATUS
278	UC-Davis	Douglas-fir	D.Neale	Parentage verification in Douglas-fir	SSR analysis complete. In reporting.
279	Center for Natural Lands Management	San Diego Thornmint	D.Rogers	Population Genetics Study of *Acanthomintha ilicifolia*	Samples have arrived and been prepared for isozymes. Awaiting analysis.
280	R5	Port-Orford-cedar	C.Frank	Genetic relatedness among resistant Port-Orford-cedar trees	SSR data collection complete and in analysis.
281	Private Company	Slash Pine, Loblolly Pine	J.Sherrill	Ramet identification in slash and loblolly pine clones	Awaiting the arrival of samples. SSR primer screening will have to occur first to identify 3-6 loci to use for full project.

Study Ideas in Development

PARTNER	SPECIES	CONTACT	PROJECT TITLE
R1	*Festuca* species	M.F.Mahalovich	Species identification and cultivar detection in *Festuca* collections from Montana
Center for Natural Lands Management	Spineflower	D.Rogers	Genetic studies of *Chorizanthe parryi* var. *fernandina* (San Fernando Valley Spineflower)
R5	Quaking aspen	R.Rojas	Genetic testing of at-risk quaking aspen in the Bald Mountain Project
R6/WSU	Pacific madrone	R.Sniezko/G.Chastagner	Genetic variation and structure in Pacific madrone
R6	*Sidalcea*	C.Emerson	Resolving taxonomic confusion around *Sidalcea setosa* and *Sidalcea oregana* ssp. *spicata*
R9	Butternut	P.Berrang	Identification of pure butternut clones

Staffing

Valerie Hipkins (vhipkins, 530-622-1609). GS-426. Permanent (100%)
Jennifer DeWoody (jadewoody, 530-621-6883). GS-440. Permanent (100%)
Randy Meyer (rmeyer, 530-295-3037). GS-404. Permanent (50%)
Courtney Owens (cowens03, 530-295-3028). GS-404. Temp-NTE (100%)
Jody Mello (jmello, 530-295-3038). GS-499. Temp-Pathways (60%)
Rosanna Hanson (rosannahanson, 530-295-3030). GS-404. Temp-NTE (100%)

Budget

NFGEL FY13 Budget is $480,000 (NFVW) that is used to pay for salaries, administrative overhead, rents/utilities, travel, and laboratory equipment, chemicals, and supplies.