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Annual Report 1997-98 (FY98)

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HIGHLIGHTS OF 1997-98

- NFGEL processed six projects in the isozyme lab, two developmental projects in the DNA lab, and began work on two collaborative DNA projects with Forest Service Research. Results were used to guide restoration and revegetation projects, conservation concerns, and silviculture and tree improvement activities.
- NFGEL has added Isoelectric Focusing capabilities as a new lab service to address issues of qualitative genetic identification.
- Laboratory efficiency was enhanced, and long-term consistency and quality were improved, with the purchase and use of a gel imaging system.
- The NFGEL Director position was filled by Valerie Hipkins in October 1998.
- NFGEL has formed collaborations and increased cooperation with Forest Service Research, and other government and non-government entities to better serve our customers.

INTRODUCTION

Background

The National Forest Genetic Electrophoresis Laboratory (NFGEL) was established in 1988 as part of the National Forest System of the USDA-Forest Service. The focus of the lab was to assess the genetic variation in conifers using starch gel electrophoresis. Forest Service Regional Tree Improvement programs submitted conifer material to (1) obtain information on the effects of timber management on genetic diversity and gene pools, (2) validate specific materials, and (3) obtain genetic information about relative amounts and geographic patterns of natural variation. Ten years have passed and many things have changed. We have expanded our scope of activities to address genetic conservation and management of all plant species using a variety of laboratory techniques including DNA analyses. To date, we have studied 20 different gymnosperm and 19 different angiosperm species. Angiosperms studied include woody species (such as aspen, cottonwood, and oaks) as well as grasses, shrubs, and forbs. Land management questions we now study include issues of genetic diversity and structure, taxonomy, and plant identification. Our work supports tree improvement programs, conservation of plant species (particularly threatened, endangered, and sensitive species), and restoration efforts. The Forest Service benefits from NFGEL's service by gathering information on genetic diversity for a variety of forest plant species that can be used to validate management strategies.

This report details Laboratory accomplishments for Fiscal Year 1998, covering the period October 1, 1997 - September 30, 1998.

Mission and Purpose

The mission of NFGEL is to provide state-of-the-art molecular genetic information to the National Forests and other cooperating agencies for the evaluation and protection of our nation's genetic resource. Techniques used will be the minimum necessary to resolve the genetic question at hand.

The purpose of the Laboratory is to analyze molecular genetic markers (proteins 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 the tree improvement program, and contributes information in the support of conservation and restoration programs, especially those involving native and TES (threatened, endangered, and sensitive) species. Our services include project proposal development, sample design and collection strategy formulation, protein and DNA marker electrophoresis, data interpretation and analysis, and reports of results and management implications.

Current Staffing

During FY98, NFGEL was staffed with four permanent and four temporary full-time employees. Just after the end of FY98 (10/11/98), the Director position was filled by Valerie Hipkins, leaving NFGEL with two vacancies (Associate Director and Computer Programmer).

Name	Position	Term	E-mail Address
Valerie Hipkins	Director	PFT	vhipkins/r5_eldorado@fs.fed.us
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Hilary Pallet	Lab Biotechnician	Temp (12/20/97-4/13/98)	
Kathy Bradshaw	Lab Biotechnician	Temp (6/8/98-8/10/98)	--
Paul Skaggs	Data Manager	Temp (6/20/98-present)	pskaggs/r5_eldorado@fs.fed.us
Barbara Wilson	Associate Director	Temp (8/30/98-present)	bwilson/r5_eldorado@fs.fed.us

Future Outlook

NFGEL will continue to meet the broad needs of resource managers by working to address genetic issues that have immediate on-the-ground applications. The scope of our work continues to expand. In addition to aiding tree improvement programs, NFGEL supports the Forest Service Natural Resource Agenda by improving watershed health and restoration, protecting sources of biological diversity, and conserving threatened, endangered, and sensitive species.

In the next year, we expect to:

- develop seed collection strategies to conserve and restore broadleaf lupine,
- make taxonomic determinations to protect the rare plant *Perideridia erythrorhiza*,
- identify parentage of ponderosa pine plantations to eliminate the use of off-site material,
- develop grazing guidelines and conservation strategies for the sensitive plants *Collomia rawsoniana* and *Sisyrinchium sarmentosum* ,
- preserve native cottonwood in riparian areas, and

- determine deployment strategies for blister-rust resistant sugar pine seed.

PROJECTS

Overview

During this report year, we have processed six projects in the isozyme lab, two developmental projects in the DNA lab, and began work on two collaborative DNA projects with Forest Service Research (see Appendices: Lab Production). Five projects processed prior to FY98 were analyzed in this report year. Only one pre-FY98 project remains to be analyzed (Project #50 -- Limber Pine, Region 1).

NFGEL projects were processed to meet a variety of management objectives. In this report, we provide information about plant identity, taxonomy, hybridization, mating systems, and levels and structure of genetic diversity. This genetic information was used to guide restoration and revegetation projects, conservation concerns, and silviculture and tree improvement activities.

Restoration

(1) HABITAT RESTORATION USING THE NATIVE GRASS, *Elymus glaucus* (BLUE WILD RYE

Recent interest in using native grasses for erosion control, forage, and habitat restoration has led to planting *Elymus glaucus* (Blue Wild Rye). This has raised concern about appropriate seed transfer zones and possible deleterious genetic effects of introducing non-local genotypes. To address issues of seed zones and local adaptation, NFGEL, together with Jay Kitzmiller, Regional Geneticist-R5, and Wayne Rolle, Forest Botanist-Rogue River NF, conducted an isozyme analysis using 3344 individuals of *E. glaucus* from 233 populations located throughout southern Oregon and the California Bay Area and Sierra Nevada (Projects #39 and #45).

Our genetic data show that *E. glaucus* is a genetically variable species. Populations appear to be highly differentiated (genetically different from each other). Genetic similarity between populations appears to have little correlation to geographic distance, except that populations collected within 5 km are often more similar than average. Genetic distance between populations could not be predicted from the distance between them, geographic region, foliage pubescence, serpentine substrate, or moist habitat. However, populations from high altitudes are genetically differentiated from those in low elevations. Significantly, *E. glaucus* var. *jepsonii* is found to be a form not worth taxonomic recognition.

<Click to see larger image> <Click to see larger image>

Elymus glaucus
low elevation group

Elymus glaucus
high elevation group

Management implications of this study depend upon management goals. Such goals might include preservation of genetic biodiversity in this species, perpetuating native plant communities, erosion control, and minimizing dollar costs. These goals are not entirely compatible. These goals do share the requirement that the seed must grow where planted. Isozyme studies usually can not predict patterns of adaptation. Reciprocal transplants or common garden studies are recommended. Pending such studies, the following suggestions are offered.

The distribution of isozyme variation suggests that *E. glaucus* seed zones are either tiny (ca. 5 km across) or huge. There are no mid-scale seed zones within the northern California Floristic Province, except that low elevation forms and high elevation forms (with overlapping ranges) exist. In small restoration projects, the number of habitats is low and preservation of local genotypes may be an important goal. Therefore, *E. glaucus* seed collection should be restricted to similar habitats in the immediate area of the project (within 5 km). This maximizes *E. glaucus* genetic diversity by preserving local genotypes. *Elymus glaucus* is common; local seed is usually available. This study suggests that if distant seed is used, its source makes little difference because the genetic similarity of the presumed extirpated local population and the distant source can not be predicted from the distance between them (within the California Floristic Province). Common sense suggests using a source from a similar habitat, until actual patterns of adaptation are

known. In large scale revegetation projects, such as roadside soil stabilization throughout a national forest, matching collection and planting localities is not practical because many microhabitats are involved and minimizing dollar costs is a high priority. On the other hand, using seed from any one genetic line risks seedling failure, while successfully establishing any one genetic line in a large area risks decreasing *E. glaucus* genetic diversity. These contradictory concerns may be resolved by using seeds derived from as many different wild populations as possible. This maximizes the genetic diversity of the seeds and maximizes the diversity of local habitats to which the seeds are adapted. The seed can be increased under cultivation, although maintaining a multilineage seed source in cultivation is far different from developing a cultivar. The inevitable loss of genetic diversity under cultivation can be reduced by minimizing the number of generations between collection of wild seed and production of seed for use.

This study and a common garden study (Snyder 1950) suggest that genetically differentiated high and low elevation ecotypes of *E. glaucus* exist, although they overlap in range. That should be taken into consideration when establishing any cultivated seed source. Planting native grass seeds from mixed sources implies planting seed not native to the site, which can pose risks. For *E. glaucus*, those risks are low. Could non-local genes break up local coadapted gene complexes? No *E. glaucus* is highly self pollinating. Would introducing new genes change the overall pattern of *E. glaucus* gene diversity? Not much; allele frequencies of natural *E. glaucus* populations are already highly divergent, but detected alleles already appear widespread and morphological variation is already detectable within populations (Snyder 1950). Would introduced genotypes die where they are intended to grow? Probably some of them would die; sowing seed from diverse sources maximizes the chance that some of them would also grow. Would introduced lineages outcompete local populations, decreasing *E. glaucus* genetic diversity? This is possible, to the extent seed travels. However, we fail to see how that is worse than Eurasian grasses outcompeting local *E. glaucus*. Would introduced lineages change local community structure? No. However, community structure would be changed by successful establishment of the broadly adapted, inexpensive, invasive, non-native grasses that are often planted, such as *Agropyron cristatum* (L.) Gaertner, *Agrostis castellana* Boiss. & Reuter, *A. capillaris* L., *Dactylis glomerata* L., *Elytrigia pontica* (Podp.) Holub, *Festuca arundinacea* Schreber, *F. ovina* L., *F. trachyphylla* (Hack.) Kraj., *F. valesiaca* Schleicher ex Gaudin, *Lolium multiflorum* Lam., *L. perenne* L., *Poa nemoralis* L., *P. palustris* L., *P. trivialis* L., and introduced taxa of the *Festuca rubra* L. and *Poa pratensis* L. complexes. The harm non-local *E. glaucus* could do is minimal by comparison.

In situations where *E. glaucus* might be planted, some grass certainly will be planted. The argument that planting exotics is preferable to planting non-local natives because the exotics can be identified and removed later is valid for conifers, which can be exterminated with timber sales. However, identifying some of the exotic grasses listed above is challenging, and eradicating them is frequently impossible.

We stress that using local populations of *E. glaucus* as seed sources for revegetation is preferable to using distant ones. However, when using local sources is not practical, we recommend using multiple distant seed sources rather than a single distant source. We also recommend using *E. glaucus* from multiple distant sources (within the California Floristic Province) rather than invasive introduced grasses, in habitats suitable for *E. glaucus* and within its native range.

These project results have been submitted for publication: Barbara L. Wilson, Jay Kitzmiller, Wayne Rolle, and Valerie D. Hipkins. 1999. *Elymus glaucus* isozyme variation, environmental variables, and seed transfer in the California Floristic Province. Ecological Applications (submitted).

Snyder, L.A. 1950. Morphological variability and hybrid development in *Elymus glaucus*. Am.J.Bot

37:628-636.

Conservation

(1) Conservation of Blue-Flowered Showy Stickseed (Hackelia venusta), a TES Species
(2) Gene Conservation and Restoration of Quaking Aspen (Populus tremuloides) in Oregon and Washington

(1) CONSERVATION OF BLUE-FLOWERED SHOWY STICKSEED (*Hackelia venusta*): A TES SPECIES

Hackelia venusta (showy stickseed) is a narrow endemic species found only in Chelan County, Washington. It is currently on the Sensitive Plant Species List for the Wenatchee National Forest, is listed as state endangered by the Washington State Heritage Program (WSHP), and is also a candidate by WSHP for federal listing. Recent work has suggested that high elevation populations of *H. venusta* (characterized by blue flowers) may be taxonomically distinct from lower elevation, white-flowered, *H. venusta* A taxonomic study of *Hackelia venusta* was undertaken (Project #64) to clarify the taxonomic status of the blue vs. white flowered populations. This work was initiated by Richy Harrod, Botanist, and Carol Aubry, Geneticist, USDA Forest Service, Wenatchee National Forest.

Taxon	#individuals/population	#populations	Total # of individuals
H. venusta (white-flowered)	22	1	22
H. venusta (blue-flowered)	12	1	12
H. diffusa var. *cottonii*	25	1	25
H. diffusa var. *diffusa*	25	1	25
H. diffusa var. *arida*	25	7	175
		Total:	259

Leaf tissue from 259 individuals of *Hackelia* species was processed for isozyme analysis. Between 12 and 25 individuals per population were sampled.

Isozyme data was obtained at 17 enzyme stains, and transformed into band pattern scores. Data is currently in analysis with the intent on publishing final results in a peer-reviewed journal. The manuscript will be

first authored by Richy Harrod, with Carol Aubry and Valerie Hipkins as co-authors.

Preliminary results do indicate that *H. venusta* contains levels of genetic diversity comparable to *H. diffusa* var. *arida* populations, and slightly more variation than the *H. diffusa* var. *cottonii* and var. *diffusa* populations. The white flowered *venusta* contains more variation than the blue flowered *venusta* The white flowered group also contains four unique isozyme bands relative to any of the other *Hackelia* material in this study. The isozyme data also show that *H. venusta* is taxonomically distinct from *H. diffusa*. The white and blue flowered *H. venusta* groups are also clearly distinct from each other, and share levels of genetic diversity commonly indicitive of population or varietal differences. If the morphological and reproductive data suggest that blue flowered *venusta* is a separate species than the white flowered taxon, the genetic data would indicate that this is a very recent speciation event. Management directives will be drawn in the final manuscript.

(2) GENE CONSERVATION AND RESTORATION OF QUAKING ASPEN (*Populus tremuloides*) IN OREGON AND WASHINGTON

Quaking aspen is a deciduous hardwood, native to the Blue Mountains of eastern Washington and Oregon. An isozyme project (Project #68) was developed with Vicky Erickson, Area Geneticist, USDA Forest Service, Umatilla National Forest, to investigate the patterns and levels of genetic diversity within and among aspen populations. As written in V. Erickson's project proposal, aspen "plays an important ecological role with respect to wildlife habitat and browse for wild and domestic ungulates. Resource managers have become increasingly concerned over the apparent decline of aspen in the Blue Mountains. Historical data show aspen to be absent on many sites formerly occupied, and many present day stands lack evidence of local regeneration. Factors leading to these vegetative changes include fire suppression (lack of sprout stimulation and/or seedling recruitment), livestock grazing, wild ungulate browsing, and conifer succession. In an effort to maintain and restore aspen communities to the Blue Mountains, projects are underway to reintroduce fire to remnant stands, plant seedlings where appropriate, and build exclosures to protect young seedlings and suckers from herbivory. Gene banks are also planned for establishment to protect at-risk stands, and for use as stool beds for the production of high quality planting materials. Funding for these projects is limited, however, and more efficient strategies must be developed to help stretch scarce resources. At present, information regarding levels and patterns of genetic diversity within and among Blue Mountain aspen populations is not available. This information is essential for the development of gene conservation strategies and plant material development, and could also be extremely valuable in terms of developing restoration prescriptions and prioritizing stands for treatment".

Leaf tissue was processed for isozyme analysis from 547 aspen individuals sampled from 46 stands within 20 drainages in the North Fork John Day Ranger District, Umatilla NF. Tissue from each individual was also frozen at -80C for possible future, fine resolution study using DNA-based techniques. Isozyme data was obtained for all individuals at 18 loci.

The data show that half of the stands sampled each contain a single clone. The other 23 stands each contain between two and fifteen clones. Thirteen of the drainages contains only one stand. In 12 of these single-stand drainages, only one clone per stand was detected. In the thirteenth drainage, the single stand contained two clones. Seven drainages contained between two and eight stands. None of these drainages were entirely clonal. Instead, stands usually contained multiple clones, with each stand in a drainage containing a different set of clones compared to the other stands within the drainage.

At the species level, there is moderate to high levels of diversity (expected heterozygosity = 0.206, percent polymorphic loci = 88.9, and mean number of alleles per locus = 3.3). These values are higher than those for the average plant species. The within stand and within drainage diversity levels are lower than the overall species level, indicating there is substantial genetic differentiation at the stand and drainage level (actually, more at the stand level as many of the drainages are made up of only one stand). For the entire study, 47.4% of the variation was found among stands, and 44.8% was found among drainages. General, preliminary results do indicate that these aspen stands are genetically different from each other, both within and among drainages (with certain exceptions).

Further analysis is currently being performed by Vicky Erickson. Genetic diversity in all stands and drainages is being analyzed in more detail (drainage within geographic area, stands within drainage, etc.). Genetic similarity among stands and drainages is being determined. Clonal occurrences are being mapped on an existing aspen inventory (stand structure, age, condition, degree of browse damage, and other site characteristics). Resulting information will be used to develop restoration prescriptions, devise gene conservation strategies, and prioritize stands for treatment.

Silviculture and Tree Improvement

(1) The Effect of Silvicultural Treatments on the Genetic Diversity of Ponderosa Pine, Bitterbrush, and Idaho Fescue
(2) Genetic Diversity in Longleaf Pine
(3) Jeffrey or Ponderosa Pine? Species Identification Using Genetic Markers
(4) Identification of Local vs Non-local Ponderosa Pine Plantations
(5) Clonal Identification of Douglas-fir
(6) Genetic Diversity in California Sugar Pine
(7) Genetic Diversity in Blister-rust Resistant Sugar Pine Seed Crops
(8) Identifying Longleaf Pine X Slash Pine Hybrids

(1) THE EFFECT OF SILVICULTURAL TREATMENTS ON THE GENETIC DIVERSITY OF PONDEROSA PINE, BITTERBRUSH, AND IDAHO FESCUE

In an effort to assess the effects of silvicultural treatments on the genetic diversity of a conifer, shrub, and grass, as isozyme study (Projects #42, 43, 44) was conducted as part of the USDA Forest Service, Pacific Southwest Research Station, Black's Mountain Interdisciplinary Research Program (Safiya Samman, PI). A manuscript titled, "Comparing genetic variation in outcrossing, community dominant tree, shrub, and grass", by S. Samman, Valerie D. Hipkins, and Barbara L. Wilson, is in preparation and excerpts follow.

Several reviews summarize plant genetic variability by taxonomic group, habitat, life form, and other life history characteristics. This study directly compares genetic diversity in species of three life forms, while holding constant habitat, breeding system, and community dominance. The three plant species chosen for study are Ponderosa Pine *(Pinus ponderosa* Laws.), Bitterbrush *(Purshia tridentata* (Pursh) DC.), and Idaho Fescue *(Festuca idahoensis* Elmer). They represent three life forms, tree, shrub, and grass, respectively. All three are common, widespread, outcrossing, long-lived perennial species. All dominate their respective layers in the plant community at the study site. They do have some life history differences; Bitterbrush alone is insect pollinated while the others are wind pollinated, and Idaho Fescue is tetraploid while the others are diploid. The three species effect one another in a complex web of competitive and commensal relationships. This comparison is part of a long-term study of effects of silvicultural treatments on genetic biodiversity. Genetic and species biodiversity are elements of a healthy ecosystem, but little is known about the effects of forest management on the genetics of forest plants.

The study site is the Black's Mountain Experimental Forest, established in 1934, in the Lassen National Forest, Lassen County, California. More than 60 years of experimentation and careful record keeping make the 10,000-acre forest a uniquely valuable resource for investigating the effects of different timber

management practices on eastside pine type forests. In 1993 the Black's Mountain Interdisciplinary Research Program was established to study the effects of forest management on various ecosystem components including vertebrates, insects, soil organisms, and vegetation.

Four plots similar in topography and vegetation were chosen for this study. After initial samples were collected, three silvicultural treatments, a timber-cutting regime, fire, and grazing, were applied to the plots. Genetic variation in the three selected species was sampled in 1994 and 1995. Cones from 207 individuals of ponderosa pine, and leaf tissue from 404 individuals of bitterbrush and 385 individuals of Idaho fescue were processed for isozyme analysis.

The gymnosperm tree Ponderosa Pine, the dicot shrub Bitterbrush, and the monocot grass Idaho Fescue do not look at all alike, nor are they phylogenetically close. However, they have strikingly similar patterns of electrophoretically detected genetic variation. All are genetically variable, with well over 90% of the variation within, rather than among, populations in the area studied. The high level of genetic variability detected in the three plants is consistent with observed trends in genetic variability. All three are common, widespread, long-lived, perennial, outcrossing species that dominate late successional stages in their communities. Widespread, long-lived, perennial, outcrossing, and late successional plants tend to be more genetically variable than average, and to have their genetic variation within, rather than among, populations. The markedly lower genetic variation in Bitterbrush than in the other two species is consistent with the tendency for dicots to have much less isozyme variation than gymnosperms and monocots. Most measures of genetic variability for Bitterbrush were somewhat high for woody angiosperms.

Black's Mountain populations of Ponderosa and Idaho Fescue were similar. Any effects of burning, grazing, and logging regimes on genetic variability will be detectable. Genetic variation was less evenly distributed among plots in Bitterbrush than in the other two species. Although plots were homogeneous for most measures, there were signfificant differences in the Shannon-Weaver diversity index. Assuming it persists, this slight unevenness could be misinterpreted as a consequence of silivicultural treatments. However, this preliminary study allows more accurate assessment of changes due to treatments.

Genetic variation will be resampled in three to twenty years to detect any effects from silvicultural practices initiated in 1995.

(2) GENETIC DIVERSITY IN LONGLEAF PINE

In order to explore the pattern of genetic variation across the geographic range of longleaf pine, an isozyme study (Project #47) was carried out with Ron Schmidtling, USDA Forest Service, Southern Research Station.

Results have been published: R.C. Schmidtling and V.Hipkins. 1998. Genetic diversity in longleaf pine (*Pinus palustris*): influence of historical and prehistorical events. Canadian Journal of Forest Research 28:1135-1145. Abstract and conclusions from the manuscript follow.

Abstract: Genetic diversity of allozymes at 24 loci was studied in 23 populations of longleaf pine (*Pinus palustris* Mill.), including three seed orchard populations and an old-growth stand. Overall, the mean number of alleles per polymorphic locus was 2.9, the percentage of polymorphic loci was 92%, and the mean expected heterozygosity was 0.105. These values are comparable with diversity measures found in a similar loblolly pine (*Pinus taeda* L.) study. Diversity measures of the seed orchard sources and the old-growth stand were similar to those in the other natural seed sources. *F* statistics indicate very little

inbreeding overall (F_{IS} = -0.002) and low differentiation among populations (F_{ST} = 0.041). All measures of genetic diversity were significantly related to longitude; western sources tended to have more allozyme diversity. Since growth or survival are not related to longitude, and no important climatic variables are related to longitude within the natural range of longleaf, it is proposed that the east-west variation in longleaf pine is a result of migration from a single refugium in the west (south Texas or northeastern Mexico) after the Pleistocene.

Conclusions: Longleaf pine has somewhat less allozyme variability than the other southern pines but, in general, does not appear to have diminished variation due to past logging practices, except in unusual instances, such as source 123. Although tree improvment programs seem to have resulted in less genetic variation in loblolly pine (Williams et al., 1995), this does not appear to be a problem in the first generation longleaf orchard populations included in this study. Allozyme variability does not seem to have much utility in predicting growth, except in cases of greatly diminished genetic variation.

The data in this paper supports the hypothesis that longleaf pine occupied a single, perhaps restricted, refugium in southern Texas or northern Mexico during the Pleistocene. A re-examination of the taxonomic relationship between the pines of northeastern Mexico and the southern pines seems warranted. The data also suggest that population sampling should favor western sources, because of the greater amount of variation in these sources.

(3) JEFFREY OR PONDEROSA PINE? SPECIES IDENTIFICATION USING GENETIC MARKERS

It can be difficult to correctly distinguish ponderosa pine from Jeffrey pine in the field. Together with Dr. Nancy Grulke, USDA Forest Service, PSW Forest Fire Laboratory, Riverside, CA, we developed isozyme markers that may be diagnostic at distinguishing ponderosa from Jeffrey pine.

Isozymes were processed at 23 loci for 9 known Jeffrey samples, 14 known ponderosa, and 9 unknown, morphologically intermediate individuals. A principal component analysis was run by N. Grulke. Eighteen of the loci were found to be informative and six principal components explained 85% of the variance. Two of the pondersoa were mis-identified and are really Jeffrey pine. Of the 9 unknowns, three appear to be Jeffrey, five are ponderosa, and one fits neither profile well and may be a hybrid.

Although 18 loci are informative, six loci (PGI-2, PGM-2, UGPP-1, ADH, 6PGD, AND SKD) by themselves appear able to distinguish these species. It remains to be seen if these loci remain diagnostic when comparing the species across their geographic ranges. Future work may include obtaining reference material from other geographic sources.

(4) IDENTIFICATION OF LOCAL VS NON-LOCAL PONDEROSA PINE PLANTATIONS

Background

In 1910, fires wiped-out millions of acres of ponderosa pine in northern Idaho. Following the burn, plantations were often established using off-site material, usually from the Bitterroot (west-central Montana) (most common source planted), Black Hills (southwest South Dakota), Wenatchee (central Washington), or Colville sources (northeast Washington). Many of these off-site stands have deteriorated and have been subsequently removed.

On the Avery Ranger District of the Idaho Panhandle National Forest, an 80-year-old ponderosa pine stand exists that is performing well (healthy, good reproduction) and is being used as a Seed Production Area (SPA). This stand was established after the 1910 burn, possibly from off-site seed (although no planting record exists) or by natural regeneration. If naturally regenerated, the probable parental material is a 150-year-old stand located down the ridge from the SPA. Several known off-site plantations of the Bitterroot and Colville sources exist within two air miles of the SPA.

The Forest is in need of genetic information that addresses the parentage of this Idaho Panhandle NF SPA. If results indicate that the SPA was established using off-site material, the stand would likely be abandened as a seed production area. However, if genetic tests confirm the stand was naturally regenerated, the Forest will continue using this stand to produce seed for reforestation.

Lab Work

Bud tissue was collected from 30 to 37 individual trees at each of the following sites: the SPA, a nearby native stand (MLO - most likely origin), the Bitterroot off-site seed source, the Colville off-site seed source, and four plantations near Bonners Ferry (Deer Park, Brush Lake2, 3 & 4). The samples were processed at NFGEL to extract DNA (Project #70). DNA was sent to Dr. Craig Echt, Research Plant Molecular Geneticist, USDA Forest Service, North Central Experiment Station, Rhinelander, WI for analysis. A subset of the samples was screened at 15 chloroplast (cp) microsatellite (SSR) loci, and 2 loci were found to be polymorphic. These two loci were then genotyped on the full population set of 256 trees.

Results (provided by C. Echt)

One cpSSR locus had 2 alleles, the other 5 alleles. These 7 alleles produced 6 different chloroplast haplotypes among the 8 populations. Statistical tests (exact G-tests) were done to evaluate differences in haplotypic distributions between all possible pairs of populations, and probability (P) values were obtained to evaluate whether the observed differences were statistically significant (Table 1). Values < 0.05 indicate that two populations were different, while values > 0.05 suggest they were not different. The P values can also be used as a rough indication of how likely it is that two populations are genetically identical, but it must be kept in mind that the P values only hold for this single set of data, and cannot be extrapolated to other populations, or even be taken as literal interpretations for the study populations. More extensive sampling, or additional DNA marker data, may change the P values. The levels of cpSSR variation observed in ponderosa pine were lower than for any other pine populations examined by this or other laboratories.

	SPA	**MLO**	**BIT**	**COL**	**DPK**	**BL2**	**BL3**
MLO	.589						
BIT	.775	.296					
COL	.188	.168	.058				
DPK	1.00	.353	.286	.212			
BL2	.613	.092	.057	.198	.831		
BL3	.750	.833	1.00	.360	.373	.092	
BL4	.258	.383	.279	.065	.075	.012	.414

Table 1. P values for exact G-tests for the null hypothesis that there is no haplotypic differentiation .

Interpretation (provided by C.Echt)

The data are consistent with the hypothesis that the SPA originated from the adjacent native stand, although this interpretation has only moderate statistical support (P = 59%), and is confounded by the higher P values seen between SPA and BIT, DPK, BL2 and BL3. Additional DNA or isozyme marker genotyping of these and other native and off-site stands is warranted if a more definitive conclusion is needed concerning the origin of the SPA. It appears highly likely, however, that the Bitterroot source was the origin of the Brush Lake 3 plantation, and that the Brush Lake 2 and 4 plantations came from different seed sources.

The very low level of observed ponderosa pine cpSSR diversity may be explained by 1) a very small founder population having given rise to present day ponderosa pine populations in this region of the country, 2) planting practices based on seed stocks having very few pollen parents, or 3) a combination of both. Marker genotyping of additional known native populations is needed to determine whether the levels of diversity seen in the MLO stand and SPA are typical.

Future Work

An isozyme project will be carried out in FY99 to characterize the genetic diversity in the SPA and determine if the genetic base has been narrowed. The data will also be used to determine if the ponderosa pine SPA is a plantation of non-local seed source or a naturally regenerated stand by comparing the SPA to additional off-site sources. If funds allow, the DNA work will be pursued by screening nuclear SSR diversity. For the isozyme study, 31 ponderosa pine stands will be sampled from

(1) the Avery SPA
(2) the 150-yr-old stand down the ridge from the SPA (MLO) (if seeds are available)
(3) one Bitterroot NF origin
(4) three Colville NF origin
(5) one Wenatchee NF origin
(6) one Black Hills NF origin (Buskala SPA)
(7) 20 IPNFs origin (represents native material)
(8) five R1 Tree Improvement bulk (provenance) lots

Results will be combined with cpSSR diversity data and a manuscript prepared by C.Echt for publication.

(5) CLONAL IDENTIFICATION OF DOUGLAS-FIR

We used isozyme markers to determine if two Douglas-fir individuals were ramets of the same clone (Dean Davis, USDA Forest Service, Region 5: Project #71). Vegetative bud tissue was used to genotype the individuals at 26 loci. These individuals differed at 11 loci indicating they are not ramets of the same clone. The client may pursue identifying correct clonal parentage at a future date.

(6) GENETIC DIVERSITY IN CALIFORNIA SUGAR PINE

The goal of this work (Project #63) it to assess genetic variation in sugar pine (*Pinus lambertiana*) located throughout Region 5. Genotype data was generated for 286 sugar pine trees at 18 isozyme loci. This data will be added to previous sugar pine isozyme data generated at NFGEL for the USDA Forest Service, R5 Sugar Pine Blister Rust Resistance (SPBR) Program. The combined data set will be analyzed by the SPBR

Program Manager. In the course of genotyping the sugar pine trees, eight rust-resistant seedlots were found to be contaminated (containing seed from more than one tree).

(7) GENETIC DIVERSITY IN BLISTER-RUST RESISTANT SUGAR PINE SEED CROPS

This is a cooperative study between the California Department of Forestry and Fire Protection (CDF) (project initiator: Laurie Lippitt), USDA Forest Service-Region 5, and NFGEL (Projects #65 and #73). The project goal is to characterize the genetics of select Mt. Home sugar pine trees to address three objectives.

Objective 1. Compare the genetic diversity among Mt. Home clusters. Would genetic diversity levels decrease if seed from a subset of Mt. Home trees were used to produce seedlings? How much diversity is lost when number of trees are restricted?

Objective 2. Compare the Mt. Home group of sugar pine with sugar pine from all of seed zone 534. Is genetic diversity lost if only trees from Mt. Home are used? Do resistant seed from outside of Mt. Home need to be added to broaden the genetic base?

Objective 3. Characterize between year genetic variation in the progeny of Mt. Home sugar pine trees. Is there significant differences between years? Are many individuals contributing to the progeny or fewer? Are genetic differences dependent on the cluster in which the trees reside?

Objective 1 and 2 were addressed by genotyping 101 sugar pine parent trees (50 resistant to blister rust, 51 susceptible) at 25 isozyme loci. We found high levels of genetic diversity in the Mt. Home parents compared to other sugar pine. Clusters do not appear to be differentiated but instead contain comparable amounts of diversity (F_{ST}= 6.6%). There is a marked decrease of genetic diversity in the resistant parents compared to susceptible individuals in all estimators calculated.

Objective 3 was addressed by analyzing 1452 embryos from select sugar pine individuals. Embryos were genotyped at 15 isozyme loci. For statistical purposes, seedlots were chosen to provide certain numbers of trees/cluster, years/tree, and years/cluster. The overall goal was to genotype 100 embryos per year per cluster.

Final data is currently in analysis. Comparisons between Mt. Home material and seed zone 534 are being made. Embryo data analysis is underway including a mating system analysis (grouped by cluster and year), analysis of heterozygosity, and MANOVA of allelic frequencies.

(8) IDENTIYING LONGLEAF PINE X SLASH PINE HYBRIDS

Longleaf pine (*Pinus palustris*) and slash pine (*P. elliottii*) have been known to hybridize in zones of introgression. A stand on the Osceola National Forest, Florida, contains a mix of longleaf, slash, and suspected hybrids. To assure proper management of the stand and correct seed collection, managers wanted to develop laboratory markers that could distinguish the species and their hybrid (project contact: Tommy Spencer; Project #60).

The labwork began using DNA methodologies in an effort to sample more genetic variation than is available using isozymes. Variation in the nuclear ribosomal DNA internal transcribed spacer (ITS) was going to be used to distinguish taxa. Genomic DNA was extracted at NFGEL from thirty individuals (ten

trees each of longleaf, slash, and the putative hybrid) using needle tissue. DNA yields ranged from 50 to 369 micrograms per individual. NFGEL staff amplified the ~3kb ITS DNA region from the 30 individuals via the polymerase chain reaction (PCR).

Because of understaffing at NFGEL and a need to assess additional DNA technologies capable of hybrid ID, we entered into a collaborative arrangement with Drs. Tom Kubisiak and Ron Schmidtling, USDA Forest Service, Southern Research Station (SRS). Genomic DNA and the amplified DNA fragment for all thirty individuals was sent to the SRS where the analysis was continued. The ITS fragments were digested with eight restriction enzymes, looking for diagnostic banding patterns. Unfortunately, resulting band patterns were unable to distinguish the taxa. A RAPD analysis was then used to look for species specific markers. To date, RAPD data show that one of the longleaf is really slash pine, and that all of the putative hybrids are actually either longleaf or slash. Work is continuing on this project. If time and money allow, SSR primers will also be used to look for species specific alleles.


Elymus glaucus
Isozyme Group 1


Elymus glaucus
Isozyme Group 2

STAFF ACTIVITIES

Meetings, Shortcourses, and Workshops

Presentations

1998. V.Hipkins. NFGEL: Linking science and management. FR-PSW Research Station, Albany CA (April 21), NFS-Regional Office, Atlanta GA (May 5), FR-SRS, Saucier MS (May 7), FR-PNW, Portland OR (July 31).

1998. V.Hipkins. NFGEL: The role of laboratory genetics in the National Forest System. US Fish and Wildlife Forensics Laboratory, Ashland, OR, May 19.

1998. V.Hipkins. Lessons from conifer genetics: possible applications to walnut questions. *Juglans* Crop Germplasm Committee Meeting, Sacramento, CA, January 30.

1997. V.Hipkins. What managers need to know about genetic diversity. Region 5 Botany Workshop, USDA Forest Service, Morro Bay, CA, October 10-13.

1997. V.Hipkins. What do they do at NFGEL? Region 5 Botany Workshop, USDA Forest Service, Morro Bay, CA, October 10-13.

1997. V.Hipkins. Genetic structure of California native grasses. Society for Ecological Restoration, San Luis Obispo, October 2-4.

Attended

1998. Western Forests Range and Seed Council (R. Meyer).

Internal Activities

Member of the Eldorado National Forest Incident Purchasing Team (S.Carroll).

Member of the Eldorado National Forest Safety Committee (R.Meyer).

Union Representative - Eldorado National Forest (R.Meyer).

Participated in design, construction, and staffing of NFGEL booth at the County Harvest Fair (R.Meyer).

Participated in construction and staffing of Forest Service booth at the CA State Fair (R.Meyer).

Trained PSW technicians in the areas of DNA extraction and RAPD analysis (P.Skaggs).

Maintained and distributed the Genetic Resource Program Personnel Directory containing phone numbers, and mailing & IBM addresses of national NFS genetics employees (P.Guge).

Professional Activities

Schmidtling, R.C. and V.Hipkins. 1998. Genetic diversity in longleaf pine (*Pinus palustris*): influence of historical and prehistorical events. Canadian Journal of Forest Research 28(8):1135-1145.

Peer reviewer for Canadian Journal of Forest Research, Genetics, Current Genetics, Canadian Journal of Botany (V.Hipkins).

Received training and developed protocols in the Isoelectric Focusing technique at Isolab, Akron, OH (S.Carroll).

Visited the facility and viewed the operation of the US Fish and Wildlife Service Forensics Laboratory, Ashland OR (S.Carroll and V.Hipkins).

Visited the FR-PSW laboratory facility in Albany CA (V.Hipkins, R.Meyer, P.Guge, S.Carroll).

Hosted

NFGEL shared information and served as a training ground by hosting graduate students, Research branch scientists, university faculty, and other federal and state government agency employees. Tours of the facility and operation were provided to groups from the North American Forestry Commission - Insect and Disease Study Group, and from the People's Republic of China - Henan Province. Tours were also given to Forest Service employees representing four units of the Research branch, four Regions of the National Forest System, university faculty, and members of the public.

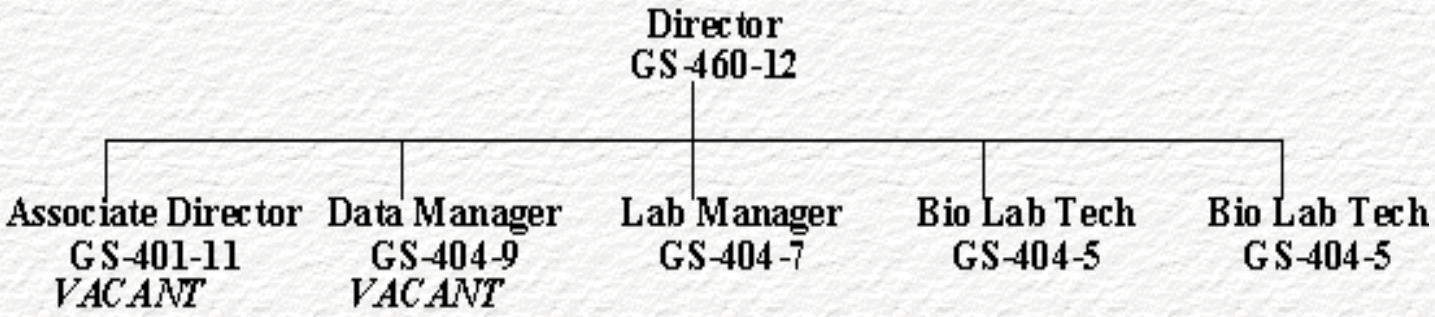
Collaboration and Cooperation

NFGEL has developed partnerships with FS Research Stations, American River College Internship Program in California, and State & Private Forestry/Oregon Department of Forestry. The Laboratory is collaborating on projects with Dr. Craig Echt, Forest Science Lab (NCRS), Rhinelander, WI and Dr. Tom Kubiask, Southern Research Station, Saucier MS. NFGEL continues to cooperate with Bureau of Land Management, US Fish and Wildlife Service, California Department of Forestry, Texas A&M University, and Oregon State University.

Cooperation with the Pacific Southwest Research Station (PSW) has been outstanding since the inception of NFGEL. While NFGEL's protein lab is located at the Placerville Nursery, Camino, CA, the DNA work is carried out in a cooperative arrangement with Dr. David Neale at the PSW Institute of Forest Genetics in Placerville, CA. PSW has shared facilities, equipment, personnel and supplies. NFGEL has reciprocated by providing supplies and equipment to PSW.

LAB MANAGEMENT

NFGEL Organizational Chart



NFGEL FY98 BUDGET

Expenditures (thousands of $)		Allocation (thousands of $)	
Salary (permanent positions)	149.9	NFFV	290.0
Salary (temporary positions)	29.5	soft money	22.7
Overhead to Eldorado NF	67.3		
Chemicals/Supplies	26.4		
Equipment	25.7		
Travel/Training	5.1		
Awards	4.4		
Fees	1.5		
Books	0.9		
Computer	0.6		
Repair	0.5		
Photos/Slides	0.4		
Postage	0.4		
TOTAL	312.6		312.7

NFGEL Projects (FY1998)

Project#	Collaborator	Species	Objective	Sample Type	Sample Size	Submission Dates	Preparation Dates	Electrophoresis Dates	Marker System	#Loci
65	Laurie Lippitt CA Dept. of Forestry	*Pinus lambertiana* (sugar pine)	Assess diversity among non-resistant and rust resistant tree clusters	Seed (megs)	97 indiv.	6/12/97	7/24 - 12/29/97	10/2-11/5/1997; 12/30/97	Isozymes (starch gel)	26
69	Nancy Grulke FR-PSW	*Pinus jeffreyi / ponderosa* (Jeffrey /ponderosa pines)	Species identification	Vegetative Buds	32 indiv.	10/24 - 11/19/1997	12/2/97	12/11, 12/17, 12/23/1997	Isozymes (starch gel)	18
68	Vicky Erickson NFS-R6	*Populus tremuloides* (quaking aspen)	Clonal ID/Conservation management	Mature Leaves	528 indiv.	8/27 - 9/5/1997	7/23 - 9/15/97	1/13 - 3/5/1998	Isozymes (starch gel)	18
64	Ricky Harrod, Carol Aubry, NFS-R6	*Hackelia* spp.	(1) Characterize genetic variation (2) Clarify taxonomic relationships	Leaves	259 indiv.	5/7 - 6/3/1997	5/15 - 6/3/97	3/10 - 4/9/1998	Isozymes (starch gel)	15
63	Safiya Samman NFS-R5	*Pinus lambertiana* (sugar pine)	Assess genetic variation thoughout R5 sources	Seed (megs)	258 indiv.	4/23/97	4/27 - 7/11/97	3/28 - 7/7/1998	Isozymes (starch gel)	18
71	Dean Davis NFS-R5	*Pseudotsuga menziesii* (Douglas-fir)	Ramet identification	Vegetative Buds	2 indiv.	3/5/98	3/11/98	3/12/98	Isozymes (starch gel)	
73	Laurie Lippitt CA Dept. of Forestry	*Pinus lambertiana* (sugar pine)	Relatedness of rust resistant vs. non-resistant seedlots	Seed (embryos)	1452 embryos	6/12/97	5/6 - 8/5/98	7/14 - 11/13/1998	Isozymes (starch gel)	16
60	Tommy Spencer NFS-R8 Tom Kubiask FR-SRS	*Pinus elliotii x P. palustris* (slash /longleaf pines)	Hybrid identification	Needles	31 Indiv.	5/29/97	5/29/97 - 6/27/97	7/1/97 - present	DNA: ITS/RAPD	--
70	Mary Frances Mahalovich NFS-R1 Craig Echt FR-NCRS	*Pinus ponderosa* (ponderosa pine)	Source identification/diversity of SPA	Vegetative Buds	256 indiv.	2/5 - 2/26/98	3/9 - 4/20/98	5/98 - present	DNA: microsatellites	2
Devel.	Allen Murray AR State Forestry	*Quercus falcata* var. *pagode folia* (cherry bark oak) *Quercus falcata* (southern red oak)	Varietal identification	Acorns	2 bulk seedlots	3/9/98	4/5/1998, 9/16 - 10/2/1998, 4/22 - 10/9/98	4/5/1998, 9/18 - 10/5/1996	Starch gel, IEF, DNA (RAPD)	20+
Devel.	Gerry Rehfeldt FR-RMRS	*Picea engelmanni* (Englemann spruce) *Picea glauca* (white spruce)	Hybrid identification	Needles	108 seedlings	6/5/97	6/12 - 6/27/97	6/17 - 8/10/1998	DNA (RAPD)	4

Last Updated on 2/22/99
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Workload by Region or Agency

(1) Isozymes

STARCH GELS

PROJECT#	REGION or AGENCY	SPECIES	#GELS	#DAYS	#WEEKS
65	CA Dept Forestry/NFS-R5	sugar pine	72	13	5
69	NFS-R5/FR-PSW	ponderosa/Jeffrey pine	9	3	2
68	NFS-R6	aspen	117	16	9
63	NFS-R5	sugar pine tests	13	2	0.5
78	NFS-R6	*Perideridia* tests	12	2	0.5
72	NFS-R6	lupine test	3	1	0.5
64	NFS-R6	*Hackelia*	47	11	5
71	NFS-R5	Douglas-fir	2	1	0.5
--	NFS-R8	northern red oak tests	17	2	1
75	NFS-R6	cottonwood	9	1	0.5
63	NFS-R5	sugar pine	156	16	10
76	NFS-R6	*Sisyrinchium* test	1	1	0.5
73	CA Dept Forestry/NFS-R5	sugar pine	170	32	18
		Total	628	101	53

ISOELECTRIC FOCUSING (IEF) GELS

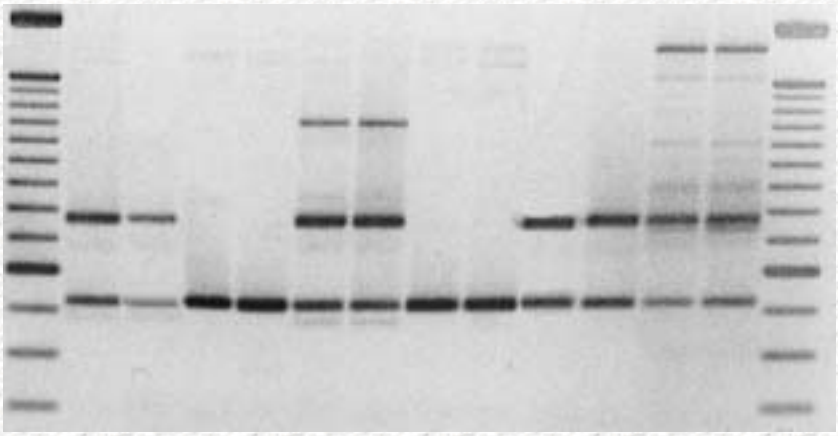
PROJECT#	REGION or AGENCY	SPECIES	#GELS	#DAYS	#WEEKS
--	AR State Forestry	cherry bark & so. red oak	10	3	1

Totals by Region/Agency: Isozymes

REGION or AGENCY	#GELS	#RUN DAYS	#WEEKS(*)
NFS-R5	180	22	13
NFS-R6	189	32	16
NFS-R8	17	2	1
AR State Forestry	10	3	1
CA Dept of Forestry/NFS-R5	242	45	23

(*) 54 weeks total because of overlapping run dates.

(2) DNA Activities



PROJECT#	REGION or AGENCY	SPECIES	#GELS	ACTIVITY
60	NFS-R8/FR-SRS	longleaf & slash pines	--	DNA extraction from 31 individuals
60	NFS-R8/FR-SRS	longleaf & slash pines	16	PCR of ITS region
70	NFS-R1/FR-NCRS	**ponderosa pine**	8	FastPrep DNA extraction of buds, development
70	NFS-R1/FR-NCRS	ponderosa pine	11	FastPrep DNA extraction of buds, 256 indiv
--	AR State Forestry	southern red & cherrybark oak	12	FastPrep DNA extraction of acorns, develop.
--	FR-RMRS/NFS-R1	Englemann & white spruce	24	Development of RAPD markers for hybrid ID

FY99 PLANNED PROJECTS

Project#	Collaborator	Species	Objective	Sample Type	Marker System	Planned Completion Date
73	Laurie Lippett, CA Dept Forestry	sugar pine (*Pinus lambertiana*)	Relatedness of rust resistant vs. non-resistant seedlots	embryos	isozymes (starch gel)	12/99 (8 weeks)
66	Floyd Bridgwater, FR-SRS	loblolly pine (*P. taeda*)	Estimate % contaminants in controlled cross treatments	meg/embryo pairs	isozymes (starch gel)	March 99 (12 weeks)
81	Dave Alicea, NFS-R5	sugar pine (*Pinus lambertiana*)	seedlot ID	megagametophytes	isozymes (starch gel)	February 99 (1 day)
82	Mary Frances Mahalovich, NFS-R1	ponderosa pine (*Pinus ponderosa*)	seed source ID, diversity in SPA	megagametophytes	isozymes (starch gel)	April 99 (6 weeks)
76	Dave Doede, NFS-R6	*Sisyrinchium* spp.	clonal ID, grazing effects	leaves	isozymes (starch gel)	May 99 (4 weeks)
75	Paul Berrang, NFS-R6	eastern cottonwood (*Populus*)	native vs non-native ID	mature leaves	isozymes (starch gel)	June 99 (8 weeks)
80	Ron Schmidtling, FR-SRS	slash pine (*Pinus elliottii*)	rangewide diversity	vegetative buds	isozymes (starch gel)	July 99 (6 weeks)
79	Joanna Clines, NFS-R5	*Collomia rawsoniana*	clonal ID, species diversity	leaves	isozymes (starch gel)	September '99
78	Paul Berrang, NFS-R6	*Perideridia* spp.	taxonomy/diversity	seedlings	isozymes (starch gel)	October '99
55	Janet Bair, USFWF	*Rorippa*	clonal ID/species diversity	leaves	isozymes (starch gel)	FY00
72	Dave Doede, NFS-R6	lupine	taxonomy	seed	isozymes (starch gel)	FY00
74	Chris Frisbee, NFS-R4	*Lewisia keloggii*	taxonomy	leaves	isozymes (starch gel)	FY00
77	Dave Doede, NFS-R6	*Sisyrinchium* spp.	hybrid ID	leaves	isozymes (IEF)	summer 99
70	Mary Frances Mahalovich, NFS-R1	ponderosa pine (*Pinus ponderosa*)	seed source ID, diversity in SPA	vegetative buds	DNA	ongoing

60	**Tommy Spencer, NFS-R8**	**slash pine (*Pinus elliottii*), longleaf pine (*P. palustris*)**	**hybrid ID**	**needles**	**DNA**	**ongoing**

Last Updated on 2/24/99
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NFGEL Projects (1988-1998)

Project#	Species	Region	Submission Date	Sample Size	Marker System	#Loci	Objective
Genetic Diversity/Structure							
73	*Pinus lambertiana* (sugar pine)	CDF,R5	1997	1452 embryos	Isozymes	16	Relatedness of rust resistant vs. non-resistant seedlots
68	*Populus tremuloides* (quaking aspen)	R6	1997	528 indiv.	Isozymes	18	Conservation management
65	*Pinus lambertiana* (sugar pine)	CDF	1997	97 indiv.	Isozymes	26	Diversity among non-resistant and rust resistant tree clusters
63	*Pinus lambertiana* (sugar pine)	R5	1997	258 indiv.	Isozymes	18	Assess genetic variation thoughout R5 sources
61	*Abies fraseri* (Fraser Fir)	FR-SRS	1997	36 indiv. 6 sources	Isozymes	18	Screen for polymorphic loci
59	*Bromus carinatus*	R5	1997	341 indiv. 13 pops.	Isozymes	13	Genetic structure within and between province and population
58	*Pinus palustris* (longleaf pine)	FR-SRS	1996	618 indiv. 20 sources	Isozymes	24	Baseline genetic diversity
55	*Rorippa subumbellata*	USFWS	1995-96	31 indiv. 2 sites	Isozymes	19	(1) Assess genetic variability in pops along shores of LakeTahoe (2) ID unique pops
52	*Pinus echinata* (shortleaf pine)	FR-SRS	1996	2 crosses 100 seeds each	Isozymes	21	Segregation distortion among clonal crosses in two environments
50	*Pinus flexilis* (limber pine)	R1	1995-96	594 indiv. 5 pops.	Isozymes	23	(1) Genetic diversity in isolated stands (2) Determine origin & location of founding trees

47	*Pinus palustris* (longleaf pine)	FR-SRS	1994-96	618 indiv. 20 sources	Isozymes	24	Baseline genetic diversity
46	*Pinus ponderosa* (ponderosa pine)	R5	1995	215 indiv.	Isozymes	29	Breeding zone development
45	*Elymus glaucus*	R6	1995-96	2746 indiv. 117 pops.	Isozymes	26	Genetic structure among and within population
44	*Purshia tridentata* (bitterbrush)	R5	1995	404 indiv.	Isozymes	16	Assess effects of silvicultural treatments on genetic diversity/structure
43	*Festuca idahoensis* (Idaho fescue)	R5	1995	401 indiv.	Isozymes	11	Assess effects of silvicultural treatments on genetic diversity/structure
42	*Pinus ponderosa* (ponderosa pine)	R5	1995	207 indiv.	Isozymes	26	Assess effects of silvicultural treatments on genetic diversity/structure
40	*Pinus echinata* (shortleaf pine)	R5	1994-95	9 clones 24 seedlots	Isozymes	22	Segregation distortion among clonal crosses in two environments
39	*Elymus glaucus*	R5	1994	598 indiv. 18 pops	Isozymes	30	Genetic structure within and between province and population
30	*Pinus ponderosa* (ponderosa pine)	R5	1993	370 indivs.	Isozymes	28	Breeding zone determination
29	*Pinus clausa* (sand pine)	R8	1993	49 clones	Isozymes	34	Clone identification & diversity
27	*Pseudotsuga menziesii* (Douglas-fir)	R6	1993-94	150 indivs.	Isozymes	20	Assess managed vs. native stand diversity
23	*Taxus brevifolia* (Pacific yew)	R5,6,1,10	1990-1991	54 pops.	Isozymes	11	Interspecific diversity

20	*Pinus lambertiana* (sugar pine)	R5	1991	563 indivs.	Isozymes	18	Genotyping rust resistant trees
16	*Pinus lambertiana* (sugar pine)	R6	1990	254 indivs.	Isozymes	13	Regional isozyme variation study
15	*Pseudotsuga menziesii* (Douglas-fir)	R6	1988-91	94 embryos	Isozymes	13	Determine levels of inbreeding
6	*Pinus lambertiana* (sugar pine)	R6	1989		Isozymes		
5	*Pinus lambertiana* (sugar pine)	R5	1988-89	378 indivs.	Isozymes	15	
Identification							
71	*Pseudotsuga menziesii* (Douglas-fir)	R5	1998	2	Isozymes	26	Ramet identification
70	*Pinus ponderosa* (ponderosa pine)	R1	1998	256 indiv.	DNA	--	Seed source identification
69	*Pinus jeffreyi / ponderosa* (Jeffrey /ponderosa pines)	FR-PSW	1997	32 indiv.	Isozymes	18	Species identification
66	*Pinus taeda* (loblolly pine)	FR-SRS	1997	61 seedlots	Isozymes	In progress	Estimate % contaminants in controlled cross treatments
60	*Pinus elliotii x P. palustris* (slash /longleaf pines)	R8/FR-SRS	1997	30 Indiv.	DNA	'CAP' w/ ITS region	Identify hybrid individuals
57	*Pinus virginiana* (Virginia pine)	FR-SRS	1996	3 individuals	Isozymes	23	Clonal identification of ramets
51	*Pinus taeda* (loblolly pine)	FR-SRS	1995	3 seedlots	Isozymes	17	Male parent identification
49	*Pinus lambertiana* (sugar pine)	R5	1995	6 seedlots	Isozymes	26	Clonal identification

48	*Pinus lambertiana* (sugar pine)	R5	1995	2 seedlots	Isozymes	26	Seedlot identification
41	*Pinus lambertiana* (sugar pine)	R5	1995	3	Isozymes	24	Ramet identification
38	*Pseudotsuga menziesii* (Douglas-fir)	R6	1994	373 ramets 5 seedlots	Isozymes	18	Determine seedlot contamination
37	*Pinus lambertiana* (sugar pine)	R5	1994	6 indivs.	Isozymes	29	SPBR seedlot identification
35	*Pinus lambertiana* (sugar pine)	R5	1993	6 indivs.	Isozymes	24	Ramet identification
33	*Pinus elliotii* (slash pine)	FR-PSW	1994	12 indivs.	Isozymes	17	Parental identification
32	*Pseudotsuga menziesii* (Douglas-fir)	R6	1994	2 indivs.	Isozymes	20	Clonal identification
31	*Pinus ponderosa* (ponderosa pine)	R5	1993	32 indivs.	Isozymes	24	Graft identification
28	*Pseudotsuga menziesii* (Douglas-fir)	R5	1993	4 indivs.	Isozymes	19	Clonal identification
26	*Pinus lambertiana* (sugar pine)	R5	1992	3 indivs.	Isozymes	11	Seedlot identification
24	Washoe & ponderosa pines	R5	1993	15 indivs.	DNA	RFLP & PCR	Species identification
22	*Pinus banksiana* (Jack pine)	R9	1991-92	11 indivs.	Isozymes	24	Determine seed origin
21	*Pinus taeda* (loblolly pine)	R8	1991-94	360 clones 523 ramets	Isozymes	21	Clonal identification
19	*Pinus palustris* (longleaf pine)	R8	1991	12 indivs.	Isozyme & DNA	12	Ramet identification
18	*Pseudotsuga menziesii* (Douglas-fir)	R6	1990	22 ramets	Isozyme	14	

17	*Pseudotsuga menziesii* (Douglas-fir)	R6	1990-91	35 clones	Isozyme	23	Ramet identification
14	*Larix occidentalis* (western larch)	R6	1990	4 seedlots	Isozyme	7	Seedlot identification
12	*Pinus taeda* (loblolly pine)	R8	1990	3 seedlots	Isozyme	12	Clonal identification
11	*Pinus taeda* (loblolly pine)	R8	1989	4 indivs.	Isozyme	12	Clonal identification
10	*Pinus lambertiana* (sugar pine)	R6	1989		Isozyme		
9	*Pinus monticola* (western white pine)	R1	1989	67 seedlots	Isozyme	16	
8	*Pinus taeda* (loblolly pine)	R8	1989	26 seedlots	Isozyme	9	Identify contamination in a controlled cross
7	*Pseudotsuga menziesii* (Douglas-fir)	R6	1989	63 seedlots	Isozyme	21	Identification of controlled crosses
4	*Pinus lambertiana* (sugar pine)	R5	1988-89	4 indivs.	Isozyme	15	Seedlot identification
3	*Pseudotsuga menziesii* (Douglas-fir)	R6	1989	32 indivs.	Isozyme		
2	*Pseudotsuga menziesii* (Douglas-fir)	BLM	1989		Isozyme	16	Estimation of orchard contamination
1	*Pseudotsuga menziesii* (Douglas-fir)	R6	1989	64 indivs.	Isozyme	13	Distinguish clones from rootstock
Taxonomy							
64	*Hackelia* spp.	R6	1997	259 indiv.	Isozymes	20+	(1) Characterize genetic variation (2) Clarify taxonomic relationships

56	***Saxifraga* spp.**	**R4 & R5**	**1995-96**	**260 indiv. 6 pops.**	**Isozymes**	**14**	**(1) Characterize genetic variation (2) Clarify taxonomic relationships**
54	***Frasera* spp.**	**R6**	**1996**	**235 indiv. 10 pops**	**Isozymes**	**13**	**(1) Characterize genetic variation (2) Clarify taxonomic relationships**
36	***Lewisia* spp.**	**R5**	**1994**	**20 indivs.**	**Isozymes**	**29**	**(1) Characterize genetic variation (2) Clarify taxonomic relationships**
Other							
67	***Populus trichocarpa* (black cottonwood)**	**R6**	**1997**	**61 indiv.**	**DNA**	**--**	**Extract genomic DNA**
62	***Pinus elliotii* (slash pine)**	**FR-PSW**	**1997**	**96 progeny 2 parents**	**Isozymes**	**27 (6 variable)**	**Provide segregating loci for genetic mapping**
53	**southern pines**	**FR-SRS**	**1996**	**4 indiv.**	**Isozymes**	**1 (IDH)**	**Marker compatability**

Dropped Projects: 13, 25, & 34

Last Updated on 2/22/99
By P.Skaggs
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PROJECT SUBMISSION

Projects may be proposed by any Forest Service employee or member of a cooperating agency. Project ideas should be discussed with the NFGEL Director prior to submission. Following preliminary approval, a brief proposal should be submitted summarizing background information, project objectives, and management implications. Projects are prioritized annually by a Steering Committee made up of national geneticists. Final proposals, including study objectives, sampling design, and analysis are developed through close cooperation between NFGEL and our clients. Questions regarding proposal format should be directed to NFGEL.

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