

Application of Genetic Analyses to Native Plant Populations¹

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The National Forest Genetic Electrophoresis Laboratory (NFGEL) was created in 1988 as a national facility to assist resource managers in the USDA-Forest Service (FS). State-of-the-art research methods and technology are utilized to help managers answer questions related to genetic diversity and conservation of native plant populations, as well as monitoring tree improvement issues.

NFGEL Mission

An appropriate discussion of NFGEL begins with our mission, which forms the foundation of our existence. Simply put, the NFGEL mission states, “To provide plant resource managers with the best genetic information possible”. Underlying NFGEL’s mission are three very important principles:

- 1) using Quality Assurance concepts to generate results and interpretations,
- 2) using the most Current and Appropriate Methods, and
- 3) producing results in Cooperation with Clients.

1) Quality Assurance

In today’s society, with increasing controversies surrounding natural resource management, it is crucial that critical eyes keep watch over the kind of data that are being used for policy decisions. The information that is generated needs to be “quality science”, which may, or may not, be equivocal to science per se. Good science is founded in the concepts of quality control and quality assurance. At NFGEL, the commitment to producing quality data comes with a price tag. It requires additional time and effort to ensure our results are repeatable and consistent over time.

2) Current and appropriate methods

NFGEL methods remain state-of-the-art through close cooperation with Pacific Southwest Forest and Range Experiment Station (PSW) and others in the research community. At the same time, we work closely with clients in defining the proposed project objectives and expected outcomes to ensure that our laboratory techniques are appropriate. Situations arise where other genetic analyses are more suitable (i.e. common garden studies). If so, we can recommend resources where you might accomplish the work. We do not hesitate recommending the most appropriate genetic tool available, even if it does not generate a project for NFGEL.

3) Cooperation with Clients

NFGEL strongly advocates an integrated approach to projects where interpretations and recommendations are a result of active participation by clients. The genetic component of a study is only one piece to the puzzle. Through cooperation, specialists provide the remainder of the story concerning biology, ecology, and politics surrounding a specific species and/or situation.

Hopefully, the resulting management decision reflects this clear intent of gaining a broad understanding of the issue.

Who We Are

NFGEL is administered by the Eldorado National Forest, in Region 5 of the Forest Service. Isozyme lab work is conducted at our lab at the Placerville Nursery in Camino, CA. DNA work is conducted through a cooperative arrangement with Dr. David Neale at the PSW Institute of Forest Genetics, Placerville, CA.

Current personnel include a Director, Assistant Director, three Biological Lab Technicians, and a part-time Computer Programmer. Services provided to clients include assistance in proposal formulation, sampling design and collection, conducting lab analyses, and generating results and genetic interpretations for reports and peer-reviewed publications.

Historically, funding has been provided through the FS Washington Office, Timber Management staff within the National Forest System (NFS) branch. More recently, projects have been funded through the FS Botany program, and other benefitting functions. In addition, funded projects have been completed for other agencies, such as the U.S. Fish and Wildlife Service and the Bureau of Land Management.

FS Genetic Resource Program Strategic Plan

In 1992, the FS geneticists convened to develop a strategic plan for the national genetics program. The five imperatives that were developed include:

- I. Genetic conservation
- II. Ecosystem management
- III. Tree improvement
- IV. Education
- V. Partnerships

As a result of this plan, NFGEL received a mandate to expand our scope of work. This expansion has included the addition of native plants, with particular emphasis on Threatened and Endangered species analyses. A tremendous amount of development work has been completed at NFGEL, since 1992, including studies on a broad range of plants, many with more challenging and complex genomes (polyploids) than the conifers we had concentrated on prior to 1992. The species studied to-date include those listed in Table 1:

Table 1. Different plants Studied by NFGEL.

Conifers

ponderosa pine

sugar pine

jack pine

jeffrey pine

longleaf pine

loblolly pine

sand pine

shortleaf pine

slash pine

E.white pine

limber pine

W.white pine

lodgepole pine

Douglas- fir

Fraser fir

Pacific yew

Other plants:

Grasses:

Elymus glaucus

Fescue idahoensis

Bromus carinatus

Plants:

Lewisia spp.

Hackelia spp.

Frasera spp.

Rorippa subumbellata

Saxifraga spp.

Purshia tridentata

What We Do

Currently, the questions posed to NFGEL fall in three main areas:

- 1) Tree improvement,
- 2) Conservation issues (threatened and endangered species), and
- 3) Native plants (restoration/revegetation).

Tree improvement studies focus on analyses generating information on conifer genetic patterns. Genotypes from individual clones are assayed to answer a multitude of concerns with superior trees, including seed identification, clonal mislabeling, effectiveness of controlled crosses, and overall effectiveness of seed orchards. Other studies assess geographic patterns of genetic diversity and management effects on genetic diversity occurring in natural stands.

In the Threatened and Endangered species arena, baseline information is needed, since the genetics of many plants have not been studied in the past. There is a need for information such as: How much diversity exists in this species? How genetically different are these populations? Do these populations constitute different species or subspecies? Has the genetic variation in a small, isolated population been reduced due to disturbance?

With the current focus on using natives for restoration/revegetation purposes, there is a need to develop seed transfer guidelines, similar to conifers. The most common questions deal with how far native grass seed can be transferred and how far we can go to collect seed/materials for restoring a site. Also, should species specific seed zones be delineated or can much more general zones be developed? If so, how? Another question relating directly to nurseries is whether genetic integrity of a collection has been maintained in the production of seed increases. For most of these questions, our laboratory methods are the most appropriate technique. However, for seed transfer guidelines, common garden studies are more appropriate due to the adaptive significance of the results.

Levels of Genetic Diversity

The level of genetic diversity that is studied in genetic analyses depends on the question at hand. Genetic differences can be estimated within individuals, among individuals, among populations, among species and other taxonomic levels, such as subspecies and varieties. At the individual level, several genes are examined to determine how many differences exist. This information on genetic differences builds over individuals and allows comparisons to be made relative to other individuals, other populations, other sites, other species and so forth. After collecting data from gels in the lab, various estimates are calculated to quantify levels of genetic diversity and amount of differentiation that occurs among groups. Examples for isozymes include: P (% polymorphic loci), A (# alleles/locus) and H_o , H_e (observed and expected proportions of heterozygous loci). This information can then be used to develop phenograms to describe similarities or genetic distances at the particular level of interest. Many questions related to ecosystem management can be answered through the use of genetic analyses. For example, determining how different or similar plant populations are relative to each other can be critical in determining conservation strategies.

Types of genetic analyses

Various tools have been developed in plant research based on earlier work in human genetics. Currently, some commonly accepted techniques include the following:

- Isozymes
- DNA
- RFLPs
- RAPDs
- Sequencing
- Common Garden studies

NFGEL conducts analyses looking at isozymes and DNA.

Comparison of Methods

It is essential that clear study objectives are determined to ensure that the appropriate genetic tool is utilized. To clarify how this determination is made, a brief comparison is presented here for three primary methods (isozymes, DNA, and common garden studies).

Isozyme methods have inherent advantages due to the large amount of published data which

exist to provide relative comparisons within and between species. Isozymes also provide multi-gene data within individuals. In addition, large numbers of individuals can be surveyed in relatively short amounts of time at a reasonable cost. A disadvantage with isozymes is the limited number of genes to survey which is approximately 30 loci. This is a narrow sampling of the genome using genes primarily involved in plant respiration and having no apparent correlation with adaptive variation (i.e. growth).

With DNA, each specific procedure has its own advantages and limitations. Overall, DNA results reflect a direct measure of genetic differences while providing an unlimited number of genes to assay. DNA work generally requires less plant tissue than isozymes, but is expensive both in chemicals and labor. The evidence is inconclusive whether DNA results will show any relationship with adaptive traits, but researchers continue to study this aspect.

Data from DNA and isozymes can be used together, utilizing the less costly isozymes for the bulk of the study and adding DNA to provide additional differences. The addition of DNA data can be beneficial when uniqueness of individuals cannot be derived with isozymes alone. The data are somewhat interchangeable when investigating questions regarding genetic variation and the distribution of genetic patterns both within and among populations.

Common garden studies are sometimes discounted for the more appealing state-of-the-art molecular analyses. But, in much of the needed native plant work, common garden experiments are still a preferred option. Common garden experiments are best applied in situations where managers are concerned with survival and adaptation of plant materials on a restoration site. Morphological traits measured in these studies demonstrate adaptiveness of individuals through their growth and survival in a spectrum of environments. By eliminating the environmental variance, the remaining genetic influence can be evaluated. It would be most beneficial to spend the time and energy to conduct common garden evaluations, particularly in polyploid grasses where genetic interpretation is harder to obtain. The drawbacks in common gardens occur in the relatively slow time-frame and expense required, although, in comparison with conifers, this time-frame should be greatly reduced with native grasses.

How Do We Look at Genes?

Materials for DNA and isozyme analyses are collected and sampled similarly, although strategies and sampling intensities may differ, depending on the particular method and genetic level of measurement. Normally, fresh materials are shipped to NFGEL as seed, vegetative tissue, or wood. Collections for DNA analyses are more flexible than isozymes since DNA is present in all materials. Isozymes require specific collection windows when enzyme activity is optimal. Isozyme analysis usually requires germinated seed to stimulate enzyme activity before sample preparations are completed. In some cases, amplification of DNA is necessary to increase the amounts available to work with. Various methods are used to isolate and examine sections of DNA depending on which type of DNA is of interest (examples include ribosomal DNA, mitochondrial DNA, and chloroplast DNA). DNA and protein samples are placed on a gel medium and connected to power sources to separate protein/DNA based on mobility differences. After several hours, solutions are applied to make genetic differences visible on the gels. In the case of isozymes we are viewing products

of genes (proteins) whereas DNA methods provide glimpses of the actual DNA. Patterns on gels are “scored” to generate data for several genes for each individual sampled. Once these data have been genetically interpreted and analyzed, we work with clients, integrating our genetic information with their localized knowledge of the question at-hand.

Examples of NFGEL Projects

In 1989, Hurricane Hugo swept through the Southeastern U.S. and decimated the FS Francis Marion Seed Orchard in South Carolina. Clonal identities were lost for the longleaf pine blocks with few superior genotypes remaining. Seed orchard managers were in need of any additional selected materials that could be identified from the remnant trees. NFGEL’s task was to determine how many different clones remained in the salvaged individuals. Isozyme analysis was used to assess uniqueness between individuals with additional differences provided by DNA analyses. The final results showed that isozymes provided sufficient information at 6 gene loci to determine that 6 of the 12 individuals were unique, while 1 group of 4 and 1 group of 2 appeared to be genetically the same. With additional DNA information using RFLP analysis, uniqueness was increased to 9 individuals with the last group of 3 individuals probably belonging to the same clone.

An example of a Threatened and Endangered species project is the following study of two closely-related species of *Lewisia*. FS botanists in Region 5 disagreed with the recent treatment of these species in the *Jepson Manual*. *L. serrata* was taxonomically submerged under *L. cantelovii* to create one species. In addition, botanists were concerned with increasing collection pressure due to the desire to use *Lewisia* in urban landscaping. On the national forests, both are perennial species occurring on rocky cliffs above drainages.

Following isozyme analysis, NFGEL’s conclusion agreed with FS botanists that distinct patterns occur in *L. serrata* and *L. cantelovii* with no gene flow between the two taxa. Our recommendation based solely on genetic interpretation is for *L. serrata* to remain a separate taxon.

Current NFGEL Projects:

- Black’s Mountain Experimental Forest - FS, Region 5, & PSW
 - Establish baseline levels of genetic variation for species in these different life forms (ponderosa pine, bitterbrush, and Idaho fescue) to evaluate effects from silvicultural treatments

- Aspen - FS, Region 6
 - Estimate levels and patterns of genetic diversity to assist in development of conservation strategies for restoration of aspen in riparian areas

- *Bromus carinatus* and *Elymus glaucus* - FS, Region 5 & 6
 - Estimate levels and patterns of genetic diversity to obtain a cursory look at seed transfer guidelines

- Loblolly pine controlled crosses - FS, Southern Forest Experiment Station
 - Evaluate percent of contaminants in seed produced from controlled pollinations based on various bag installation and removal dates

- *Hackelia venusta* - FS, Region 6 & USFWS
 - Clarify taxonomic status of a blue-flowered species and a white-flowered species
 - Determine if gene introgression has occurred between *Hackelia venusta* into another *Hackelia* spp.

- Sugar pine - California Dept. Of Forestry (CDF)
 - Compare genetic diversity levels of rust resistant trees and seed crops with the nearby seed zone at Mountain Home State Forest in California

- Limber pine - FS, Region 1
 - Characterize the genetic diversity in an isolated stand in sw North Dakota
 - Determine whether the stand originated from material brought in by Native Americans

Challenges

As needs for native plants increase, resource managers need facilities to grow materials and have the confidence that materials used in site revegetation will be adapted and survive. The genetic integrity of plants collected for restoration needs to be maintained throughout sample collection, production, seed increases and outplanting. Any genetic shifts in plant materials from origin to restoration site should be monitored. With the current technologies available, these are some areas that NFGEL can help monitor.

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