GENETIC STUDIES IN NATIVE PLANTS

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Abstract

The genetic variation contained within a species is paramount for its survival and future evolution. Species exhibit a large range in their levels and patterns of genetic variation. This range in population structure is basic to the use and conservation of genetic diversity in plants. In order to understand, conserve, and manage plant populations, it is necessary to measure the levels of genetic variation within a species. We have at our disposal a variety of estimation tools. These tools provide information about plant identity, taxonomy, hybridization, parentage and mating systems, and levels and structure of genetic diversity. Genetic information can be used to guide restoration and revegetation projects, conservation concerns, and seed transfer movement. Our role at NFGEL is to conduct laboratory genetic tests and provide information to land managers so that they may better utilize and manage plant species.

Keywords

allozyme, molecular marker, restoration, conservation

Introduction

Genetic factors play a significant role in ecosystem health, species survival, and future population persistence and evolution. Therefore, they should play a role in conservation planning. Land managers need to consider genetic issues such as variation and diversity, population structure, hybridization, mating systems, parentage, identification, and taxonomy. Our role at the USDA Forest Service National Forest Genetic Electrophoresis Laboratory (NFGEL) is to provide genetic information to managers as an aid for their resource decisions.

Much of NFGEL's recent work reflects land manager's increasing interest in using native plants. Native plants are preferred not only because they are part of our natural heritage, but also because they perform important functions in native ecosystems and promote healthy environments. However, challenges face the land manager considering native plants. Genetic information increases our understanding of native species and provides clearer management direction.

Genetic Considerations in Managing Native Plants

The genetic challenges of using natives can be divided into two categories, adaptation and genetic integrity. Adaptation is the ability of the plant to germinate, grow, and reproduce on the site. Although adaptation depends on genetics, it is not easily measured with genetic markers. The genetic test best suited for measuring adaptation is the common garden study. Genetic integrity involves a range of issues including identifying appropriate plant species or subspecies; disrupting local gene pools; measuring, restoring, and maintaining original levels of genetic diversity; intra- and interspecific hybridization and introgression ("genetic contamination"); maintaining original genetic structure; and maintaining sufficient genetic diversity to allow future adaptation and evolution. Aspects of genetic integrity can be estimated by measuring genetic variation and structure in the laboratory using genetic markers.

NFGEL is often asked to assist with three kinds of projects: restoration projects, seed transfer zones, and agronomic efforts (seed increases) (Table 1).

Restoring native ecosystems obviously requires adaptation — the plants must grow. Less obvious genetic issues are equally important. Genetic goals include restoring the original range of genetic diversity and maintaining sufficient genetic diversity to permit future adaptation and evolution. Measuring genetic diversity allow us to maintain variation and avoid depleting our ecosystems of this resource. Restoration projects designed without considering genetic components risk producing genetic monocultures in our native species. Genetic markers also help in such restoration goals as correctly identifying plant material and capturing maximum genetic diversity. Markers may also allow estimation of the risk of disrupting local gene pools.

Native plants are used not only for restoring natural ecosystems, but also for erosion control, forage, shelterbelts, and other projects. Defining acceptable seed transfer zones improves the chance the projects will succeed and minimizes their negative impacts. Managers need to know how far seed can be moved and whether it should be moved between habitats or regions. Concerns include both adaptation (whether the plant will grow) and genetic integrity. Genetic integrity concerns include losing rare alleles, contaminating local

Table 1. Activities where genetic information can aid resource decisions.

MANAGEMENT ACTIVITY	GENETIC COMPONENT	WHY
restoration/conservation	genetic diversity	maintain variation and ecosystem health
	genetic structure	maintain variation and ecosystem health
	taxonomy/identification	confirm plant id confirm genotypes
	phylogeny	understand relationship to other taxa determine conservation value of species
seed transfer	adaptability	ensure material will survive, grow, and reproduce
	genetic integrity	ensure species survival and future evolution
seed increases and other agronomic manipulation	taxonomy/identification	identify species determine purity and stability determine authenticity of genotypes
	genetic diversity/structure	maintain variation and ecosystem health

gene pools, and conserving adequate genetic variation. Only if the species' genetic structure and breeding system are known can genetic risks of seed transfer be estimated.

Establishing an adequate supply of seed (or vegetative propagules) may require establishing a cultivated seed source. However, is the seed returned the same species and lineage as that planted? Are desired levels of genetic variability maintained? Are a few genotypes increasing at the expense of others? Is the material genetically pure, or is there introgression from other accessions grown at the same facility? Authenticity and variability of genotypes can be verified using genetic markers in the laboratory. Any method used to address plant identification questions should be repeatable (both within and between labs), objectively scored, and able to discriminate between the genotypes and varieties of concern.

Methods to Obtain Genetic Data

With the advance of molecular genetic techniques, there are many ways to measure genetic variation and thus contribute to the management of native plants. Basically, techniques can be divided into Laboratory or Field methods. Each technique has its own advantages, disadvantages, and uses (Table 2).

Laboratory methods include techniques that resolve proteins (isozyme analysis, isoelectric focusing), or DNA (RFLPs, RAPDs, PCR, microsatellite analysis, DNA sequencing). Protein level techniques primarily look at genetic changes in coding regions that have altered amino acid sequences, while DNA level techniques look at changes to the DNA itself. In general, lab techniques are relatively quick and indicate actual levels of genetic diversity. However, the relationship between protein or DNA markers and adaptive traits is usually unclear. Compared to DNA methodologies, isozyme analyses are more efficient and cost effective but provide less resolution (measure less of

Table 2. Application of various methods used to measure genetic variation.

TECHNIQUE	COMMON USES
Laboratory	
protein:	
starch gel electrophoresis (isozymes)	genetic diversity and structure assessment identification mating system assessment and parentage phylogeny/taxonomy (most effective when applied to the study of congeneric species or species in closely related genera)
isoelectric focusing	distinguish and identify plant genera, species, subspecies, varieties, hybrids patent protection genetic purity in cultivation
DNA:	
RFLP	genetic diversity and structure assessment taxonomy/phylogeny forensics/identification
RAPD	genetic diversity and structure assessment identification
DNA sequencing	phylogeny/systematics
Field	
common garden test	population structure (esp. along environmental gradients) seed transfer guidelines adaptation

the existing genetic variation).

Field methods often utilize common garden studies to measure morphological and physiological traits (e.g., survival, height, seed weight) of direct interest to adaptive fitness. Material from diverse sources are grown under uniform conditions to determine the degree of environmental vs genetic control of a measured trait, and thus the adaptedness of the plants to a given environment. Unfortunately, common garden tests can be expensive and slow to yield results.

An informed choice must be made when choosing a technique to measure genetic variation. Available tissue, cost, time, labor, and, of utmost importance, management objective must be considered, and the appropriate measurement tool chosen. Before any measurement technique is employed, library searches and consultations with experts (university faculty, research scientists, field botanists) should be performed. Some management questions could be resolved inexpensively if a morphological examination of plant material preceded laboratory or field study (Table 3).

Examples of Genetic Analyses at NFGEL

Elymus glaucus Isozyme Variation: Implications for Restoration

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.

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, and even populations that are a short distance from each other are generally genetically distinct. However, populations from high altitudes are genetically differentiated from those in low elevations.

To maximize genetic diversity, collect from as many populations as practical. It is just as important to collect from populations growing closely together as those growing far apart (because the geographically close populations are as genetically different from each other as are populations separated by a great distance). This study also suggests that isozymes are not measuring adaptive traits. Common garden tests are needed to determine how far seed can be moved and still thrive. Until then, using local seed or seed from multiple sources is preferable to using seed from any single non-local source.

Isozyme Pattern Analysis of *Frasera fastigiata* and *F. umpquaensis*: Taxonomic Implications

Frasera umpquaensis is a R-6 Forest Service sensitive species. The bulk of

Table 3. Methods for answering management questions about native plants. 1-3 equals the order in which the method should be applied in the initial study. Often, answering a question fully will require, for example, a morphological examination followed by an isozyme study followed by a second morphological examination that is focused on characteristics that might distinguish genetic groups identified with isozymes. The order is based on the amount of information the method is likely to yield, the application of that information to the question, and cost.

TYPE OF STUDY	LIBRARY/ CONSULTATION	MORPHOLOGY	LABORATORY (PROTEIN OR DNA)	COMMON GARDEN
taxonomy	1	2	3	4
adaptation	1		(3)	2
genetic integrity	1	3	2	
phylogeny	1	3	2	
parentage		2	1	
identification of cultivars, increased seed		2	1	

the population lies along the Rouge-Umpqua divide with much smaller disjunct populations occurring on Eugene and Medford BLM administered land and on the Willamette, Siskiyou and Shasta-Trinity National Forests. *F. fastigiata's* primary range is within Idaho but also spills over to the Blue Mountains of northeast Oregon. *F. umpquaensis* was recently submerged under *F. fastigiata*. However, there has not been unanimous support for this and botanists in Oregon remain skeptical of the reduction. If, in fact, *F. umpquaensis* deserves no taxonomic recognition then there would probably be no need to manage the taxon as a sensitive species. A genetic study was initiated between NFGEL and Forest Service Botanist Richard Helliwell (Umpqua NF) to characterize isozyme patterns from several populations of both *Frasera umpquaensis* and *F. fastigiata*, and infer taxonomic distinctions between *Frasera* species.

One hundred seventy eight (178) individuals of *F. umpquaensis* and 75 individuals of *F. fastigiata* were analyzed. Genetic markers were used to calculate diversity measures within populations and to understand the relationships among the populations. Based on the way the variation is distributed within and among the taxa, our data suggests that *F. umpquaensis* deserves separate taxonomic recognition, and should not be submerged under *F. fastigiata*. The number of unique bands in each taxa and the degree of separation between the taxa indicates that *F. umpquaensis* and *F. fastigiata* are not exchanging genes and are evolutionarily diverged. The Shasta-Trinity population also appears to contain some unique variation and may be worthy of special consideration within the *F. umpquaensis* complex.

The National Forest Genetic Electrophoresis Laboratory

NFGEL is a national Forest Service facility funded mainly by appropriated dollars and administered through the Eldorado National Forest. Services are provided to managers within the Forest Service, other government agencies, and non-government organizations for assessing and monitoring genetic diversity. Current techniques include starch gel electrophoresis (isozymes), isoelectric focusing, PCR-based technologies such as RAPDs, microsatellite analysis, and RFLPs. We work closely with research institutions to incorporate the latest technological advancements into our program.

History and Organization

NFGEL was established in 1988 as part of the National Forest System of the USDA Forest Service. We are located at the Placerville Nursery in Camino, California. Our protein work is conducted in Camino, and our DNA analyses are carried out in a cooperative arrangement with Dr. David Neale at the PSW Institute of Forest Genetics in Placerville. At the inception of our lab, we focused exclusively on tree improvement issues. It has been a decade of change and varied opportunities as we have expanded our scope of activities to address genetic conservation and management of all plant species.

NFGEL is staffed by a Director, Associate Director, Lab Manager, two Biological Lab Technicians, and Computer Programmer. The NFGEL staff provides varied services to clients. We function as a clearinghouse of genetic information as well as providing genetic lab services. Laboratory services include developing project proposals, formulating sampling design and collection strategies, conducting electrophoresis of protein and DNA markers, analyzing and interpreting data, reporting results, and drawing management implications.

Workload

To date, we have studied 20 different gymnosperms (all but one of which belongs to the family Pinaceae), 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 NFGEL studies 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.

Conclusion

Genetic analyses have immediate practical application to the resource manager. Plant identification, measurement of genetic variation in plant species and populations, determination of adaptedness, and maintenance of genetic integrity are all possible with the use of genetic estimation tools.

Genetic tools offer objective options for the assessment of diversity and can contribute to appropriate management strategies.

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