US Forest Service, Washington Office,
Forest Management

The National Forest Genetics Lab (NFGEL)

FY16 Annual Report

October 1, 2015 – September 30, 2016

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Message from the Director

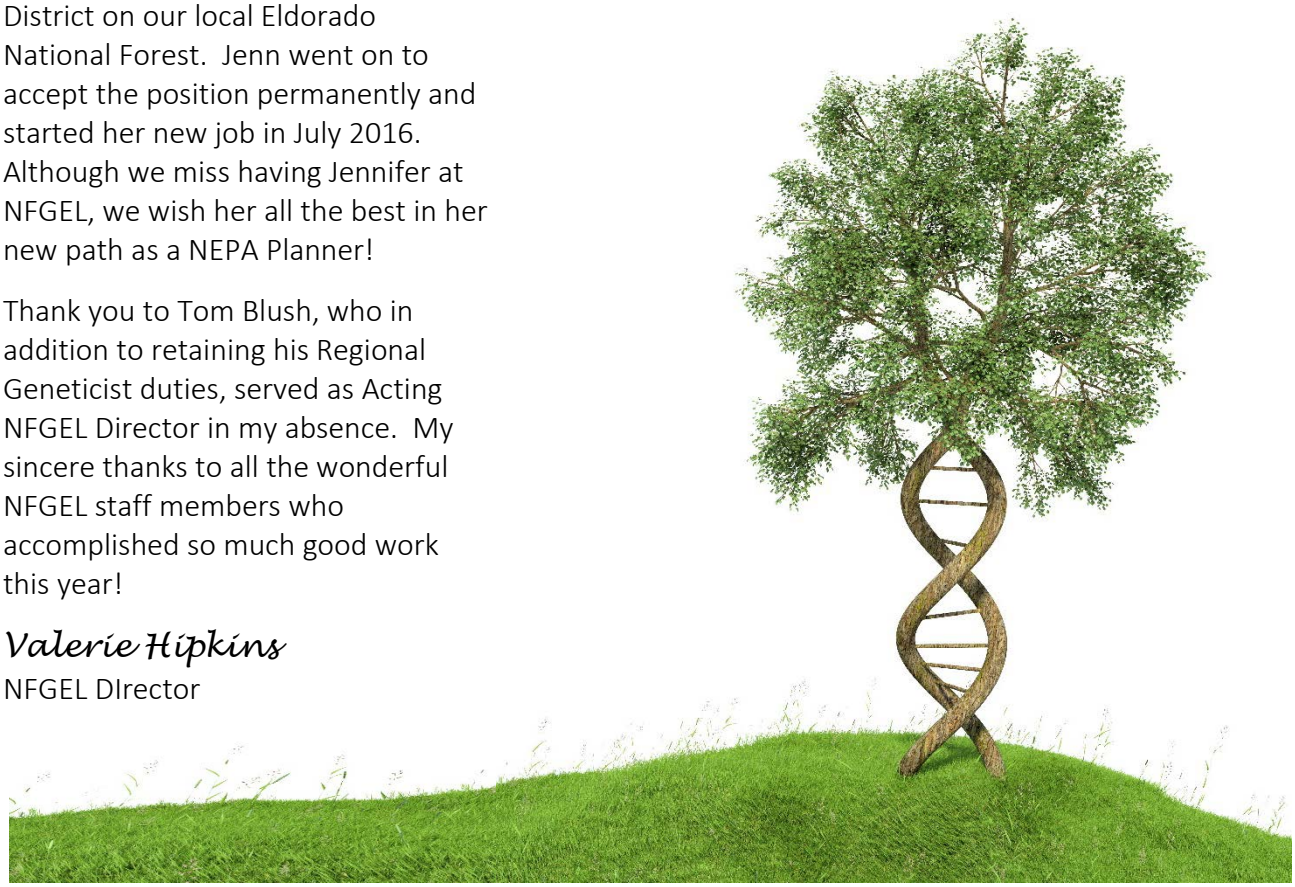
This report covers laboratory activities and accomplishments during Fiscal Year 2016 (October 1, 2015 through September 30, 2016).

Fiscal Year 2016 proved to be a challenging staffing year for NFGEL. Although we operated with an extremely reduced number of employees, I'm pleased to report that we were still able to complete 11 projects. Our work continued to guide restoration and conservation efforts, identified mislabeled reforestation material and unwanted hybrids between native and exotic species, and determined if tested plants were species and/or varieties that warranted special protection. All lab accomplishments were achieved this year by a total of 16 employees that accounted for only 2.5 full-time equivalents (FTEs).

Reduced lab staffing levels were mostly due to detail opportunities taken by NFGEL employees. I was on a 4-month detail as the Acting National Reforestation and Nurseries Program Manager in our Washington Office Forest Management staff, Washington D.C. This was a wonderful opportunity to work at a National level on nursery and reforestation issues that affect the establishment of healthy and resilient forests. I want to thank my supervisors and Forest Management staff members for offering me the chance to work in this area and being such gracious, supportive and inclusive colleagues. Also on detail this year was NFGEL geneticist, Jennifer DeWoody. Jennifer took a 6-month detail as a NEPA Planner at the Placerville Ranger District on our local Eldorado National Forest. Jenn went on to accept the position permanently and started her new job in July 2016. Although we miss having Jennifer at NFGEL, we wish her all the best in her new path as a NEPA Planner!

Thank you to Tom Blush, who in addition to retaining his Regional Geneticist duties, served as Acting NFGEL Director in my absence. My sincere thanks to all the wonderful NFGEL staff members who accomplished so much good work this year!

Valerie Hipkins
NFGEL DIrector



NFGEL Projects

NFGEL develops reliable, effective, low-cost, and time-efficient technologies for characterizing genetic variation in all plant species to aid in adaptive management and planning efforts on forest and rangelands throughout the Nation. Projects are prioritized using a set of seven criteria. Once proposals have been accepted, projects are subject to a ranking within the laboratory-scheduling queue dependent on various factors.

NFGEL Project Prioritization Criteria

- Strategic Alignment
- Value to Customer
- Availability of Existing Technology
- Availability of Sample Material
- Importance to Risk Mitigation
- Leverage Potential
- Project Costs

NFGEL Project Scheduling Factors

- Date of total sample receipt
- Total project sample size
- Availability of markers
- Availability of operating and appropriate laboratory equipment
- Availability of laboratory staff (professional and technician)
- Purchase and availability of materials and supplies
- Completion of a signed Contract, Agreement, or funds transfer document
- Compatibility with other projects in the lab (species, size, laboratory protocols)

NFGEL projects were processed to meet a variety of management objectives. Project results were used to guide restoration and conservation projects, and assist in silviculture and tree improvement activities. Eleven project summaries are included in this Annual Report.

PROJECT #	PARTNER	PROJECT TITLE
268	USFS R6	Oregon white oak restoration: genetic diversity and geographic differentiation in the Cascade Range
298	USFS R6	Isozyme analysis of atypical *Packera cana* in southern Washington and Northern Oregon
299	NGO	Genetic studies of *Chorizanthe parryi* var. *fernandina* (S. Watson) San Fernando Valley Spineflower
310	USFS R1-R10	Technology transfer of allozyme markers for species identification in *Botrychium* (moonworts)
317	Tree Improvement co-op	Ramet identification and parental verification in western hemlock (*Tsuga heterophylla*) orchards
319	USFS R6 / USFWS	Genetic variation in *Artemisia campestris*: do distinct genetic varieties exist within the species?
321	Private Company	Genetic fingerprinting: ramet identification and parental verification in loblolly pine (*Pinus taeda*) clones
323	Private Company	Verification of clonal genotypes in Douglas-fir (*Pseudotsuga menziesii*)
324	Private Company	Clonal and parental identification in Douglas-fir (*Pseudotsuga menziesii*) seed orchards
325	USFS R9	Distinguishing pure Butternut (*Juglans cinerea*) from Butternut hybrids
330	NGO	Analysis of DNA content variation in San Diego thornmint (*Acanthomintha ilicifolia* (A. Gray) A. Gray)

Oregon white oak restoration: genetic diversity and geographic differentiation in the Cascade Range.

NFGEL Project 268. Project cooperator: Andy Bower, USFS – Region 6.

Oregon white oak foliage and acorns. Donald Owen, California Department of Forestry and Fire Protection. Bugwood.org

A restoration strategy is being developed for Oregon white oak (*Quercus garryana*) on the east side of the US Forest Service Mt. Hood and Gifford Pinchot National Forests and the Columbia River Gorge National Scenic Area. Gene conservation from these eastside stands is an area of interest but acorns cannot be stored for more than a few years at best. Oak stands on the west side of the Cascade mountains could be a potential source for reforestation in the event of catastrophic damage to eastside oak stands, but it is unknown whether there are genetic differences between stands on opposite sides of the Cascade mountains. Oregon white oak is fairly widespread in areas west of the Cascade mountains in Oregon and Washington, but has a limited distribution east of the mountains. Knowledge of the level of genetic differentiation between east- and westside stands will be important in determining if acorns from the westside may be appropriate for use in restoration treatments on the eastside.

The objective of this project is to determine the level of genetic diversity in eastside Oregon white oak as compared with west-side stands, and determine if these stands are genetically differentiated. In order to more robustly address this objective, this project was conducted in collaboration with Dr. Travis Marsico at Arkansas State University. Dr. Marsico has already conducted a genetic diversity study of Oregon white oak using microsatellites [*Travis D. Marsico, Jessica J. Hellmann, and Jeanne Romero-Severson. 2009.*

Patterns of seed dispersal and pollen flow in Quercus garryana *(Fagaceae) following post-glacial climatic changes. Journal of Biogeography 36, 929–941*]. The majority of the samples used in that study were located from west of the Cascades. In this NFGEL Project, we generated data from samples collected from the east side of the Cascades and measured variation using the same loci as those used in Dr. Marsico's Journal of Biogeography manuscript.

NFGEL generated data at 12 SSR loci (7 nuclear, 5 chloroplast) for 564 trees from 19 stands. All DNA samples used by Dr. Marsico in his study were shipped to NFGEL and a subset were genotyped at NFGEL to assure compatibility between the datasets. Eleven of the 12 loci were compatible between the two labs, with one locus unusable due to data incompatibility. Data were sent to partners for analysis on April 13, 2016. Travis Marsico and Andy Bower will take the lead on analysis and drafting a manuscript for publication. The combined dataset includes genotypes of 875 trees located from 40 populations separated into two 'regions'. Preliminary results indicate that gene flow is high and that there may be no special ecotype conserved east of the Cascades.

Loci used in the final genetic analysis of Oregon white oak.

0C19: Added 1bp to NFGEL score to match Marsico data.
0G12: Matches Marsico data.
0M05: Matches Marsico data.
1G13: Matches Marsico data.
1M17: Matches Marsico data.
ZAG36: Added 1bp to NFGEL score to match Marsico data.
ZAG9: Matches EXCEPT for Marsico 252 and 253 alleles. NFGEL scored these as 253. To make our datasets compatible, Marsico alleles 252 will need to be changed to 253 (so no 252 allele would be in the datasets).
UDT1: Matches Marsico data.
UDT3: Added 1bp to NFGEL score to match Marsico data.
UDT4: Matches Marsico data.
UCD4: Subtracted 1bp from NFGEL score to match Marsico data.
UCD5: NOT COMPATIBLE – DROP LOCUS

Isozyme analysis of atypical *Packera cana* in southern Washington and Northern Oregon.

NFGEL Project 298. Project cooperator: Mark Darrach, USFS – Region 6.

Packera cana, *(woolly groundsel).* ©2005 Steve Matson. calphotos.berkeley.edu.

The genus *Packera* (Asteraceae) is composed of ca. 60 species and is of New World origin. Originally described as a segregate of *Senecio*, the genus was recognized based on its unique base chromosome number (x=22 or 23) and morphological distinctiveness when compared to the broad taxonomic group. Phylogenetic relationships within *Packera* and among related genera (in the tribe Senecioneae) are complex, partly due to hybridization, both as a driver of historic speciation and as an ongoing process in sympatric populations, and partly due to incomplete lineage sorting among closely related species.

Within *Packera*, hybridization appears to be the driver of complex taxonomic relationships and inconsistent signals from chloroplast (maternally inherited) and nuclear (bi-parentally inherited) genetic data used in phylogenetic studies. Hybridization leads to the combining of distinct gene pools in nuclear markers, allowing the hybrid (or hybrid-derived species) to be identified as intermediate (or closely related) to the two parental taxa. For chloroplast markers, however, only the maternal line is observed, meaning hybrids may not be identified. Thus, for studies of putative hybrid species or complex taxonomy, a combination of nuclear and plastid loci, or solely nuclear markers if the study is limited, are preferred.

Packera cana (Hooker) W.A. Weber & A. Love is a wide ranging species found throughout the mountainous western North America and into the High Plains (Flora of North America, efloras.org, accessed Sept. 22, 2015). Unlike other members of the genus, *P. cana* is reported to be morphologically uniform, with the exception of high elevation specimens. However, atypical populations of *Packera* most closely resembling *P. cana* have recently been identified in the Umatilla National Forest, and in the region of southern Washington and northern Oregon. Morphological and ecological assessment by Mark Darrach and Dr. Debra Trock (California Academy of Sciences) has confirmed distinct morphology and habitat preferences of the atypical populations compared to typical *P. cana*. The question now is whether the distinct populations represent a novel taxon, and if so, at what level they differ from *P. cana*.

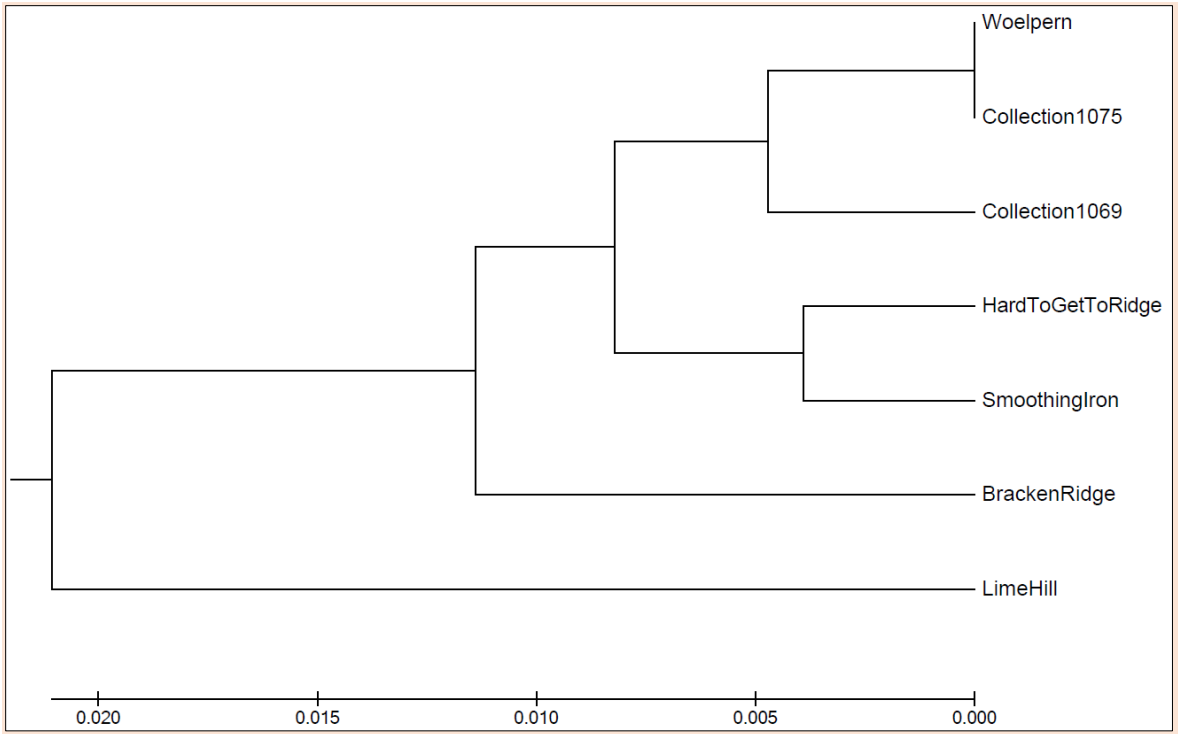
NFGEL conducted a molecular genetic study using bi-parentally inherited, putatively neutral markers (isozymes) of seven collections of *Packera* spp. to address two objectives:

1. Is the newly discovered group of populations worthy of separate taxonomic status, and, if so, what level of recognition is appropriate?

   Analyses of nine isozyme loci revealed moderate levels of genetic variation (3.5 mean alleles per locus over all; 1.6 effective alleles per locus per population, accounting for varying sample sizes). Populations of *Packera* were significantly differentiated overall (F_{ST} = 0.069, P<0.0001). When populations were grouped as "*P. cana*" or a putative "*P. sp. nov.*" the differences between groups was not significantly different from the overall measure (F_{RT} = 0.073), indicating the genetic structure did not support the hypothesis of distinct taxa.

   If the two morphologies represented distinct taxa then the population phylogeny based on the genetic distance between populations should reveal two clades clustering typical and atypical populations. The UPGMA population phenogram built from pairwise estimates of Nei's (1978) genetic distance placed the atypical *Packera* populations within the typical *P. cana* populations, inconsistent with the morphology representing distinct species.

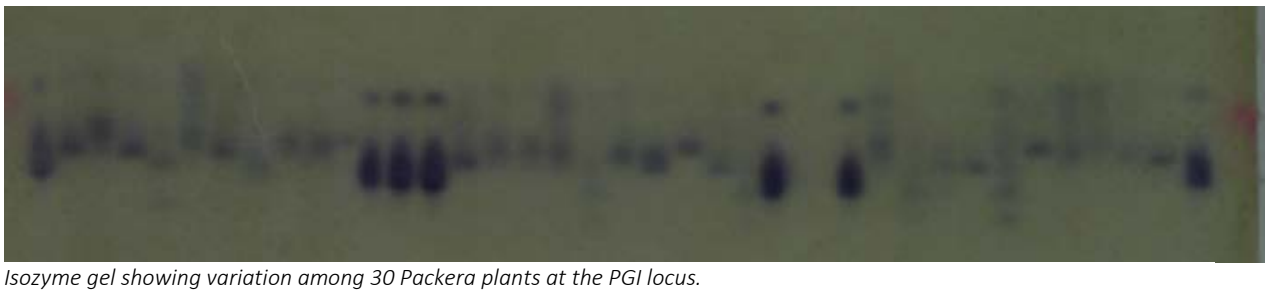
   Population assignment tests based on Bayesian likelihood methods did not identify differences between morphological groups. Typical and atypical populations showed similar patterns of population assignment.



Population phenogram built from Nei's (1978) genetic distance between Packera populations, estimated from variation at 9 isozyme loci. Four populations were identified as P. cana a priori: Collection 1069, Collection 1075, Lime Hill, and Woelpern. The remaining populations (Bracken Ridge, Hard-to-get-to-ridge, and Smoothing Iron) displayed atypical morphology. If the two morphotypes represented distinct species, populations displaying the same morphology would be expected to cluster together.

2. Can these newly recognized populations be effectively compared in a molecular genetic setting alongside more typical Packera cana?

   Atypical populations of *Packera* displayed putative triploid banding patterns more frequently, which may be consistent with divergence through some hybridization or polyploidy event. Examination of ploidy variation was conducted in NFGEL Project #331 (results presented in this FY16 Annual Report) after the acquisition of a new ploidy instrument.



Isozyme gel showing variation among 30 Packera plants at the PGI locus.

Genetic studies of *Chorizanthe parryi* var. *fernandina* (S. Watson) San Fernando Valley Spineflower

NFGEL Project 299. Project cooperator: Deborah Rogers, Center for Natural Lands Management.

Chorizanthe parryi (San Fernando Valley Spineflower). ©2015 Anuja Parikh and Nathan Gale

Chorizanthe parryi var. *fernandina* (S. Watson) (San Fernando Valley Spineflower, SFVSF) is listed as endangered by the state of California and is a federal candidate for the same status. SFVSF is an herbaceous annual found within coastal sage communities at elevations below 350 m. After being considered extinct for a time, SFVSF has been found in two locations—the Ahmanson Ranch in Ventura County and Newhall Ranch in Los Angeles County — where it occurs primarily in dry, sandy places within coastal sage in dense patches of several hundred plants. Although both ranches support large populations of spineflower, fewer than 20 acres of habitat at the Ahmanson Ranch, and no more than 25 acres at Newhall Ranch are known to support his species. Therefore, a total of not more than 45 acres of occupied habitat are known at present.

Ongoing mitigation projects have initiated studies of SFVSF ecology, phenology, and reproductive biology, and incorporated findings into in conservation efforts. Observations of SFVSF indicate the plant follows the canonical seasonal reproductive pattern for annuals in a Mediterranean climate, germinating after sufficient winter rain, bolting, flowering, and setting seed prior to summer heat, usually between April and May/June.

Surveys of floral visitors identified at least ten taxa (morphospecies) carrying pollen of SFVSF, including ants, beetles, flies, and bees. The observed population did not appear to be pollen-limited, and the main pollinator was likely an introduced ant. Pollinator-exclusion experiments indicated SFVSF to be self-

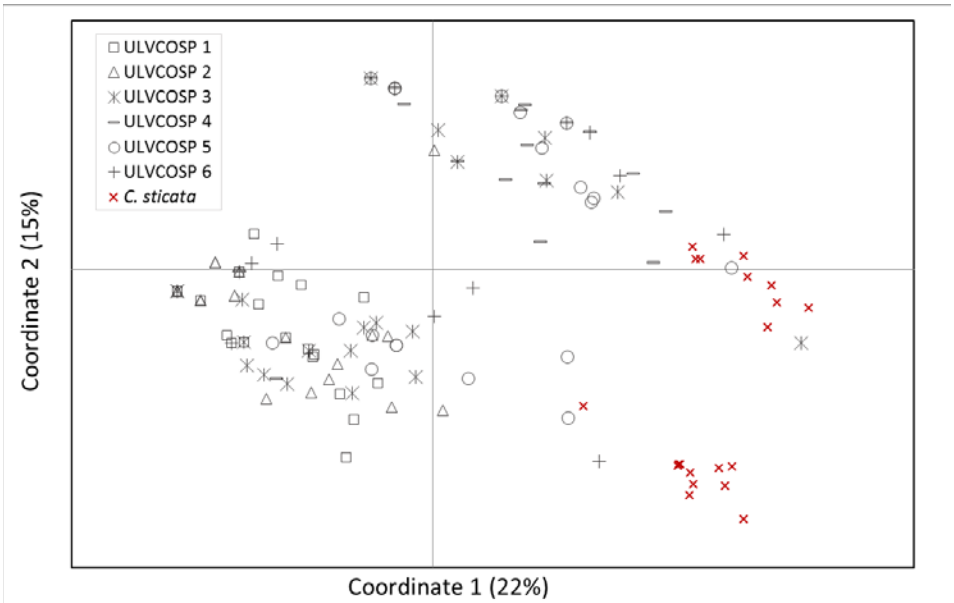
compatible to a certain extent (around 22% seed set). The presence of ants increased both the percentage seed set and the viability of the resulting seed, indicating they are likely an effective and important pollinator. Germination rates in the open-pollinated treatments were higher than those in the pollinator exclusion tests. The viability tests may be consistent with some level of inbreeding depression in self-pollinated seed.

Individual SFVSF plants may be moderate to prolific seed producers (up to 300 involucres per plant). Plants remain intact following senescence, with seeds remaining within the involucres until some physical disturbance breaks apart the structures. This mechanism has been proposed to be a safe-site adaptation; or as a mechanism for dispersal via small mammals, either through seed caching or via attachment to fur. Either process would likely limit the gene flow between patches of SFVSF, and certainly reduce the likelihood of seed dispersal between distinct populations, thus limiting gene flow and influencing the genetic structure of the species.

Conservation interests for SFVSF include a better understanding of the amount and structure of genetic diversity within the remaining populations, estimating the degree of inbreeding in the extant populations, and determining whether there is evidence of current or imminent inbreeding depression. These interests have been represented as the following two research objectives:

1. Describe (selectively neutral) genetic diversity and genetic structure of the extant populations, using a congeneric sympatric species to provide some context for interpretation.
2. Using the same methods used to describe genetic diversity, provide diversity and inbreeding coefficients from samples from all extant populations that have been produced under controlled crossing conditions and represent a) selfed and b) outcrossed plants. Measure several phenotypic attributes of these selfed and outcrossed plants over one growing season as a first estimate of phenotypic expression of inbreeding depression.

A four-part study plan was developed to address the objectives using samples of established plants (leaves) and germplasm (seed) collected from the two SFVSF populations. First, microsatellite primers from previous published studies would be screened to develop an SSR panel to assess genetic variation in each population (from leaf samples). This data would be used to describe the genetic structure of the extant populations (Objective 1). Second, seed from a subset of the collection sites would be grown under greenhouse conditions and the microsatellite panel would be used to genotype each plant to be used in the greenhouse experiment. Third, controlled crosses would be conducted to test self-fertilization, crosses within a population, and crosses between populations. Fourth, the F1 seed generated by the controlled crosses would be grown under greenhouse conditions and assessed for microsatellite variation (to confirm parentage) and phenotype associated with fitness (to measure inbreeding effects, Objective 2).



Principal coordinate analysis of data from five microsatellite loci reveals possible genetic structure in the ULVCOSP collections of SFVSF and a congener, C. sticata.

A number of protocols and methods were tested to assess the genetic structure of San Fernando Valley spineflower. Several laboratory protocols produced genetic marker data, or show the potential of providing sequence data with further testing. The initial results indicate SFVSF harbors low levels of genetic variation within and significant genetic structure among subpopulations. The protocols detailed here should expedite the collection of the remaining molecular data from the remaining samples.

Attempts to germinate, emasculate, and cross-pollinate SFVSF plants in a greenhouse setting identified a number of challenges to studying this small herb. While the direct-sow method appears to be more successful for germinating SFVSF, plants established at a very low rate, indicating additional tests are needed to find an efficient protocol. Despite specialized equipment, the methods to emasculate developing flowers were time-intensive and potentially damaging to the remaining flower organs. However, a large amount of seed from both populations is available for future work, and many of the seed lots have been cleaned and are ready for germination. NFGEL has made available to other laboratories both DNA samples and tissue resources for further study. We also proposed some next steps based on the results of the preliminary analyses and protocol tests.

Three outcomes of the experiments reported here provide guidance for future work to assess the genetic structure of and potential effects of inbreeding on SFVS. First, the germination tests indicated that controlled conditions rather than a greenhouse are indicated for progressing with the controlled-crosses. While low germination rates are common for wild-collected seed, the low percentage of seedlings that became established indicates that conditions were not optimal for SFVS. Together, low germination and establishment rates meant that a large number of seed would need to be sown to reach the targeted number of flowering plants from each population for the controlled crosses. Once established, the variable temperature and humidity measured in the greenhouse indicates the environment may more variable than desired for measures of F1 growth as a proxy for inbreeding depression.

Second, the while the greenhouse tests indicated that emasculation of the small flowers of SVFS is possible, the fertility of the remaining ovaries and style was not verified. It is possible that the emasculation procedure may damage the developing ovary or style, reducing the likelihood of

viable seed being produced from controlled crosses. The germination and greenhouse tests indicate that further attempts to conduct controlled crosses and assess plant growth will require a collaborator with specialized experience working with this type of annual or further investment in developing protocols for cultivation, as well as more controlled growing facilities.

In the laboratory analyses, the low success rate of amplifying the microsatellite primers described for a related genus (*Eriogonum*) in SFVS resulted in a small number of promising loci for molecular tests. In addition, the possibility of null alleles at these loci reduces the amount of information provided by each locus and biases the allele frequencies and thus statistical analyses, ultimately reducing the power of the microsatellite data for individual identification and paternity confirmation tests as specified in the study design.

Given the outcomes of the molecular analyses, additional markers will be required to assess the level of fixation (potential inbreeding) and genetic structure in the leaf collections and/or for individual identification and paternity checks in the greenhouse experiments.

If the molecular analyses are continued, the five microsatellite markers may be sufficient to assess population structure in the leaf samples, particularly if protocols are further refined to amplify the two loci with variable amplification. These data could test for isolation or gene flow between the Newhall Ranch and ULVCOSP populations, but would likely be insufficient to quantify inbreeding.

If the greenhouse studies are refined and completed, the controlled crossing experiments would provide the most direct estimate of the effects of inbreeding, and no further molecular data may be necessary. The examination of greenhouse material using microsatellites was for verification purposes only.

However, given the difficulty encountered in the greenhouse experiments and the high level of specialized skill required to conduct the controlled crosses, a molecular approach to assess inbreeding may be feasible. One possibility is to assess a number of seed per mother plant (at least 10, more robustly 20, depending on the available markers) to estimate the number of pollen parents and thus effective outcrossing rates in natural populations. This molecular approach would require a greater number of loci (at least the equivalent of 15 microsatellite loci) that are designed for SFVS or display near perfect amplification (no null alleles). Alternatively, novel high-throughput sequencing (HTS) technologies may provide an option to assess nucleotide variation across the genome, given sufficient HTS experience and skill in bioinformatics.

Technology transfer of allozyme markers for species identification in *Botrychium* (moonworts)

NFGEL Project 310. Project cooperator: Larry Stritch, USFS – Region 1-Region 10.

For several years, Forest Service Botanists across the continental US and Alaska have contracted with Donald Farrar (Prof. Emeritus, Iowa State University) to positively identify samples of *Botrychium* (moonworts) using allozyme markers. As most species of *Botrychium* are rare and several are of TES concern, accurate identification of specimens is critical to management efforts. Dr. Farrar is retired and planning to decommission his allozyme lab, potentially leaving National Forest System botanists without the benefit of molecular genetic information, as identifying some species based on morphological features is difficult, particularly if specimens are young or degrading. In 2014 the USFS and Dr. Farrar began an effort to transfer the isozyme methodology and interpretation used to identify *Botrychium* to NFGEL to support management efforts. The prep method, gel and stain recipes, and gel loading protocols used by Dr. Farrar were all transferred to NFGEL in fiscal year 2014 and are recorded in Project 305.

Botrychium simplex. Anne Russell, US Forest Service.

While the laboratory methods were successfully adapted by NFGEL, the resolution of the banding patterns and their interpretation to identify *Botrychium* to the species level were not achieved in NFGEL Project 305. In fiscal year 2015, additional tests were conducted to refine the electrophoresis and interpret the allozyme banding patterns using Dr. Farrar's methods and data. The 2015 work was conducted as NFGEL Project 310, and this report details the methods and findings of the additional tests.

Specifically, the objective of Project 310 was to improve isozyme electrophoresis methods to replicate banding patterns closely enough to apply D. Farrar's species identification methods. If additional testing failed to improve data quality, the lab would reassess the potential for NFGEL to conduct this work.

NFGEL analyses confirmed that the written protocols were sufficient to replicate the same quality of gels observed in NFGEL Project 305. Furthermore, similar migration and activity was revealed when using different lots of starch, with slightly crisper bands in the older (Farrar) lot. However, the variation in gel resolution was not sufficient to change the banding interpretation. Thus, the source of starch does not explain the differences in migration and resolution observed between NFGEL and D. Farrar photos.

Additional analyses compared stain recipes for three isoenzymes and revealed differences in enzyme activity. Neither the NFGEL nor the Farrar recipe for TPI produced bands sufficient to score the samples. For both ACO and DIA, the Farrar recipe produced greater activity than the NFGEL recipe, although for both recipes, the DIA bands were faint and fuzzy and likely insufficiently resolved to identify species (a function of the electrophoresis not staining). The NFGEL recipe for 6pgd produced darker bands than the Farrar recipe, although the Farrar recipe was sufficient to score the gels.

However, overall resolution of the starch gels was insufficient to score the alleles according to D. Farrar's method or accurately identify species. Of the 11 stain-buffer combinations tested, five had sufficient activity (dark enough bands) and migration (relative the D. Farrar's gels) to measure alleles and attempt to identify the species (S7-AAT, S7-PGI, S9-PGM, S11-SKD, S11-IDH); three had sufficient activity but did not migrate with sufficient resolution to distinguish alleles according the D. Farrar's methods (S9-6pgd, S9-MDH, S9-PGI), and three were not sufficiently active to distinguish alleles in all tests (S7-TPI, S11-ACO, S11-DIA). Thus, the majority of loci examined were not fully resolved as would be required to identify samples of *Botrychium* based on isozyme banding patterns.

Further, given the specialized knowledge and skills required to interpret the results, it is not clear that NFGEL could address the needs of the Regional and Forest Botany staff using isozyme data alone. Dr. Farrar is an expert in *Botrychium* morphology as well as isozyme interpretation, and no staff of NFGEL will be trained to identify species morphologically. Thus, it would be imperative for the botany field staff to narrow down the potential species identification based on habitat and location information and providing this information to NFGEL. Even then, fully understanding and practicing the catalog of isozyme patterns will require more staffing time than available given the overall priorities of the NFGEL workflow.

Based on the inconclusive results of the 2015 isozyme testing and changes in lab staffing, NFGEL will conduct no further tests to adapt D. Farrar's methods for species identification of *Botrychium* samples. Although this means the Lab will not be able to assist forest-level botanists with species identification, the records and information gained through Projects 305 and 310 will provided a foundation for any future studies of *Botrychium* species. Currently at least one university lab is developing markers based on DNA sequence data for species identification (M. Stensvold, pers. comm.). Depending on the findings, it may be of interest for NFGEL to test those markers for use in the lab as the transfer of DNA techniques is often more successful than isozyme interpretations. Even if a robust marker system is transferred to NFGEL, the Forest Service will need to develop protocols for field identification, sample submission, voucher maintenance, and data stewardship in order to use molecular data for species identification on a national level.

Although no further laboratory work will be conducted, NFGEL will maintain the samples previously submitted and prepared for analysis. Each sample of *Botrychium* sent to NFGEL as part of Project 305 was prepped for isozyme analysis and then pressed for morphological verification or vouchering purposes. The isozyme preps remain in frozen storage at NFGEL. In May 2016, the pressed specimens were shipped to D. Farrar for his use and cataloging.

Ramet identification and parental verification in western hemlock (*Tsuga heterophylla*) orchards

NFGEL Project 317. Project cooperator: Keith Jayawickrama, Northwest Tree Improvement Cooperative.

The use of molecular markers for orchard management remains an essential tool to verify and confirm the genetic identity of selected material. We used five microsatellite (SSR) loci to genotype a total of 1,043 western hemlock trees with the objectives of:

1. Confirming clonal identity of submitted ramets.
2. Confirming parentage of submitted progeny (forward selections).

Western hemlock. iStock.com

The variability in the SSR loci was high among the clonal genotypes. The number of alleles per locus was 27.6 (7.9), effective number of alleles per locus equaled 15.0 (4.5), expected heterozygosity was 0.925 (0.025), and the fixation index equaled 0.159 (heterozygote deficiency). The SSR data were able to resolve 238 unique genotypes among the 1,043 individual samples used for ramet identification.

The Probability of Identity (the average probability that two unrelated individuals, drawn from the same population, will by chance have the same multilocus genotype) is 6.9×10^{-11}, indicating ample discriminatory power of the data for distinguishing unique genetic individuals. These SSRs had a mean error rate of 5.8%.

Among parental trees, fifteen other genotypes were detected that were the result of mislabeling or some other source of mis-identity. Fourteen of these genotypes were unique and matched no other genotype detected in the study. The fifteenth genotype did not match other ramets of that labeled clone, but instead matched the genotype of an alternate clone. Among the progeny ramets, there were three trees whose genotypes did not match the genotypes of other ramets of the same clone number. Only 1.7% of the ramets submitted for analysis (18/1043) contained mislabeling (mislabeling of the ramet, overgrown rootstock, etc.).

A total of 448 ramets from 100 forward selections were submitted to confirm parentage. Genotype data at five SSR loci confirm that the parentage is not correct in 19 of the 100 forward selections.

Genetic variation in *Artemisia campestris*: do distinct genetic varieties exist within the species?

NFGEL Project 319. Project cooperators: Mark Skinner and Andy Bower, US Forest Service – Region 6, and Ted Thomas, US Fish and Wildlife Service – Pacific Region

Artemesia campestris. R.W. Smith, www.wildflower.org

The pattern of variation in certain herbaceous *Artemisia campestris* along the Columbia River is complex and does not lend itself to simple taxonomic classification. In the Flora of the Pacific Northwest, *Artemisia borealis* is treated as a subspecies of *A. campestris*, and *A. campestris* is described as "A highly polymorphic species, divisible into a number of rather diverse but apparently wholly confluent subordinate taxa". The taxonomic history of *Artemisia campestris* and *A. borealis* is more like a braided stream than the expected tree and taxonomists disagree about how to treat the taxa.

To add to the confusion, both *A. borealis* and *A. campestris* are variable. Two forms of *A. borealis* and/or *A. campestris* occur along the Columbia River in Washington and, historically, in Oregon. One form, "*wormskioldii*," is typically biennial and is short, with hairier leaves and stems, slightly broader leaves, and more compact inflorescences with bigger flowers.

It blooms in April and May with occasional stragglers blooming into later summer. The other form, "*scouleriana*," is usually perennial and is much taller, though heights overlap, with less hairy leaves and stems, narrower leaves, and long, open inflorescences with smaller flowers. It blooms in August and September. The *wormskioldii* plants resemble arctic/alpine *A. borealis*. The *scouleriana* plants resemble widespread low elevation *A. campestris.* Intermediates occur, and seeds collected from *wormskioldii* plants occasionally produce an offspring with the *scouleriana* phenotype. The *wormskioldii* form lives on sand dunes and similar habitats along the Columbia. This habitat has been mostly destroyed as a consequence of dam-building, and two *wormskioldii* populations remain, at Beverly and at Miller Island. The *scouleriana* form is widespread in the Pacific Northwest. It is fairly common at several sites along the Columbia River, including Beverly and Miller Island.

The *wormskioldii* form is a listed rare plant; critically imperiled globally, endangered in Washington, and a candidate species with the USFWS. It is heavily managed and new populations have been established. However, the mixed populations at Beverly and Miller Island, and the existence of morphologically

intermediate plants raise the question of whether *wormskioldii* is a real taxon or a set of extreme phenotypes in variable *scouleriana* populations. If *wormskioldii* is a not a real taxon, then it could be delisted and resources used to preserve it could be re-directed to manage other rare plants.

This isozyme study was initiated to assess the genetic variation within and among *wormskioldii* and *scouleriana* occurrences to determine if these forms are genetically distinct. The evolutionary relationships of both of these forms were also compared to *A. borealis* samples. For purposes of this report, the three putative taxa tested are identified as *A. borealis, A. campestris* var. *scouleriana,* and *A. campestris* var. *wormskioldii*.

This study was initiated to test whether *wormskioldii* populations and *scouleriana* populations of *Artemisia campestris* should be treated as distinct taxonomic entities. The isozyme diversity analyzed indicates that there are moderate levels of genetic variation and significant differentiation among the ten populations of *Artemisia* sampled. The structure of the genetic diversity supports the taxonomic distinction of the *A. borealis* samples from the *A. campestris* var. *wormskioldii* and var. *scouleriana* samples. However, these markers do not support the separate taxonomic status of the two putative varieties within *A. campestris*. The genetic differentiation was not consistent with taxonomic divergence between the two *A. campestris* varieties and therefore does not support the idea that *wormskioldii* and *scouleriana* are distinct taxa.

Significant differentiation was observed among locations of sampled populations. The two populations growing at a given location were more similar to each other than were populations identified as the same variety. In other words, if the varietal status is valid, we would expect all populations of a variety to be more genetically similar to each other than they are to another variety or taxon. This was not the case and instead genetic clustering occurred by location suggesting that geography explains a greater proportion of the genetic variability than does the *a priori* varietal designations. What we found is that plants growing in the same place are more similar to each other than to more distant populations. The *scouleriana* and *wormskioldii* samples at Beverly have a genetic identity of 0.992. Those growing on Miller Island have a genetic identity of 0.982. However, the genetic identity of *wormskioldii* populations growing on Beverly and Miller Island is only 0.944 and the genetic identity of *scouleriana* populations at these two sites is only 0.951. In general, comparisons of the other *scouleriana* populations with the Beverly or Miller Island populations of that putative taxon are intermediate. More important than absolute numbers is the expected pattern of variation; we would expect members of a single subspecies to be more similar to each other than to members of a different subspecies.

Morphological traits that can also function as genetic markers indicate that the two varieties are different, perhaps with different ancestry, even though intermediate plants exist. The morphological and phenological differences between the *wormskioldii* and *scouleriana* plants along the Columbia are often clear, and they ally *wormskioldii* with *borealis,* not *scouleriana*. These differences are exhibited in plants growing together at the same sites, so they are not just environmentally induced. In other words, these traits are good genetic markers, like the isozymes studied here. Morphology and phenology indicate that *wormskioldii* and *scouleriana* are different entities, although the extent in which the *wormskioldii* phenotype is heritable should be determined through common garden experiments.

However, isozyme variation was not patterned in a way expected if *wormskioldii* and *scouleriana* are different taxonomic groups, and was not able to genetically separate *wormskioldii* from *scouleriana*. The

genetic identity of 0.992 between *wormskioldii* and *scouleriana* at Beverly is very high and indicates great gene flow between the two forms. Looking at isozymes alone, we would call these the same taxon. However, it should be stressed that the course resolution provided by these genetic markers reveal broad-scale patterns of differentiation and may not be sufficient to provide more fine-scale resolution. Further studies using alternative DNA-based nuclear or organelle markers may reveal additional information about the varietal relationships.

What could explain this discrepancy between isozyme markers and morphology/phenology markers? Three scenarios seem likely. Perhaps *wormskioldii* and *scouleriana* split relatively recently from a common ancestor, and morphology and phenology are changing under selection, but the more neutral enzymes studied here are not. Alternatively, perhaps *wormskioldii* were once different, but interbreeding with *scouleriana* is slowly reducing the neutral genetic differences. Finally, it is possible that the environmental conditions on sandy or gravelly sites at the edge of the river select for the *wormskioldii* phenotype and early flowering, but are neutral about the basic metabolic enzymes studied here. Therefore, isozymes may be indicative of strong gene flow, but morphology and phenology are reflective of strong selection pressures.

The Puget Sound *scouleriana* are definitely different from *wormskioldii,* but that may be an indicator of peculiarities of the Puget Sound plants, rather than the relationships among Columbia River *scouleriana* and *wormskioldii*. Hitchcock et al. (1955, p. 60) wrote: "A few of the plants from our range, particularly about Puget Sound, are apparently referable to the otherwise more eastern ssp. *caudata* . . . . The ssp. *caudata* seems fairly well distinguished from ssp. *borealis* in c. and c. U.S., but westerward it becomes difficult to distinguish from var. *scouleriana* of ssp. *borealis*." It would be interesting to know about the genotypes of plants upstream of Beverly or away from the main stem of the Columbia, which presumably would not be ssp. *caudata*.

Morphology strongly suggests that *wormskioldii* is the same as *borealis.* However, *wormskioldii* differs from the *borealis* samples in DNA content per cell and in isozyme variation. It is possible, though, that even if *wormskioldii* is related to *borealis,* it is not closely related to the *borealis* populations of the Olympic Peninsula. There is no likely recent path of plant migration between Beverly and the Olympic Mts. However, *borealis* from the Rocky Mountains could easily have washed down the Columbia River during normal floods or the Bretz floods some 15,000 years ago, and established population on the shores. It is interesting to speculate that the hairiness, short stature, and early maturity of alpine *borealis* gave such migrants an advantage on sandy or gravelly banks that went dry early in the season.

Although isozyme variation provided no evidence of two distinct genetic varieties within *A. campestris*, the significant allele frequency differentiation among populations indicated that genetic structure exists among the sampled populations. Morphological, ecological, and genetic divergence between samples is ultimately required for taxonomic divisions. The genetic variation detected among populations studied here may be insufficient to restrict or promote taxonomic changes.

Genetic fingerprinting: ramet identification and parental verification in loblolly pine (*Pinus taeda*) clones

NFGEL Project 321. Project cooperator – Private Company

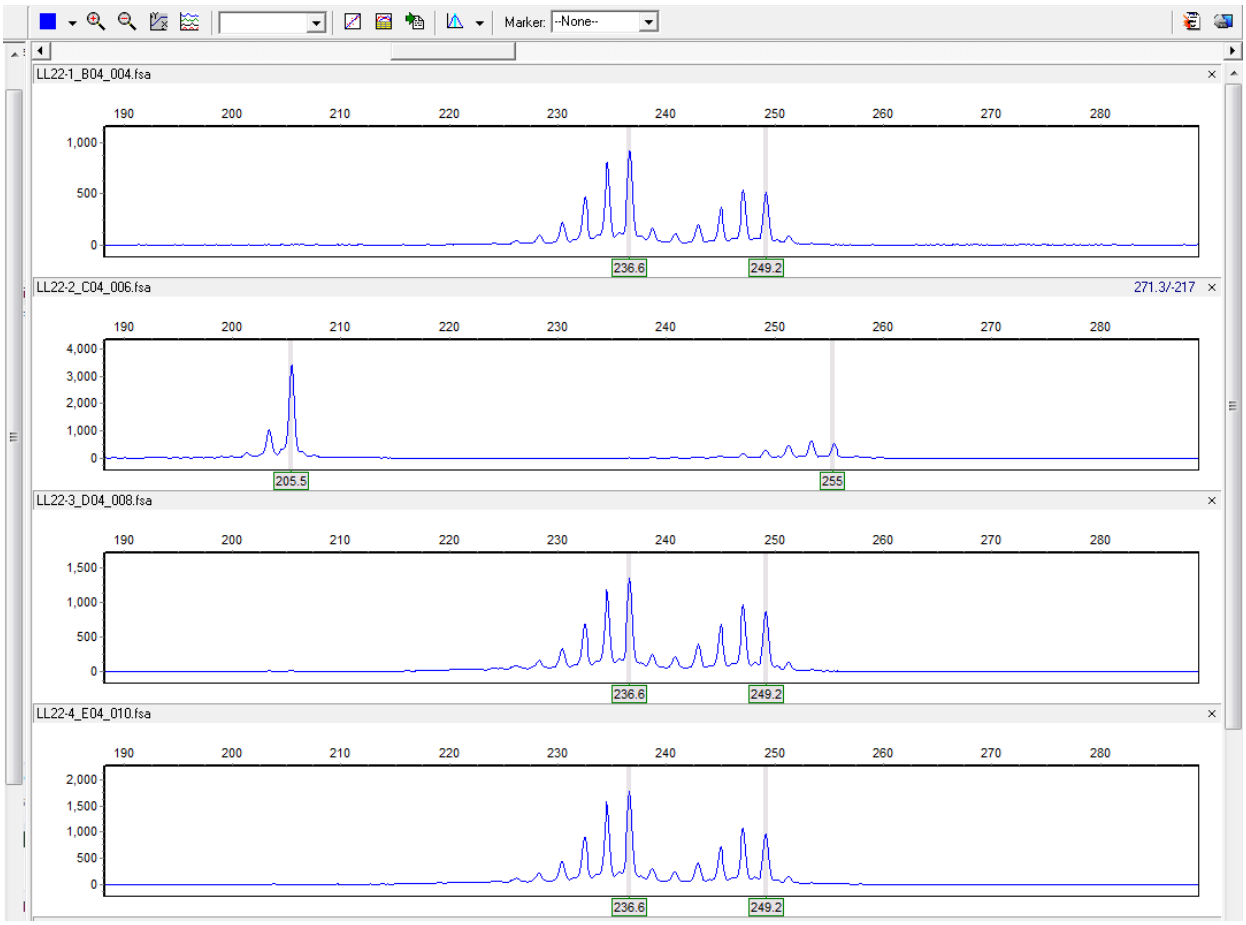
NFGEL employed molecular markers to verify and confirm the genetic identity of selected material in loblolly pine breeding programs. We used six microsatellite (SSR) loci to genotype a total of 1,132 loblolly pine trees with the objectives of:

1. Confirming clonal identity of submitted ramets.
2. Confirming parentage of submitted families.

This set of SSRs are appropriately variable to support orchard and breeding program management. The Probability of Identity (PI) (the average probability that two unrelated individuals, drawn from the same population, will by chance have the same multilocus genotype) determined among the trees using these SSR loci is 2.7×10^{-8}, indicating ample discriminatory power of the data for distinguishing unique genetic individuals. These SSRs had a mean error rate of 0.3%.

A total of 3.3% of the ramets submitted for analysis (35/1070) contained mislabeling. Of the 35 mislabeled trees, 6 had unique genotypes that matched no other genotype in this study, 4 trees matched a clone different from its designated label, 2 mislabeled trees matched each other, but matched no other genotype, and 23 mislabeled ramets labeled as being clonal actually matched the genotype of an alternate clone.

Three to 30 progeny from 6 families were submitted to confirm parentage. Genotype data at six SSR loci confirm that the parentage is correct in one of the six families. Five of the families contained various levels of parentage mislabeling (or mis-identity).



An example of SSR data from one locus for four trees labeled as being ramets of a single clone. In this example, three ramets have matching genotypes (the first, third, and fourth trees) while the second tree from the top has a different genotype, and is therefore not a ramet of the same clone as the other samples.

Verification of clonal genotypes in Douglas-fir (*Pseudotsuga menziesii*)

NFGEL Project 323. Project cooperator – Private Company

As for our genotyping work in support of pine and hemlock orchard management, we also provided molecular testing for Douglas-fir (*Pseudotsuga menziesii*) breeding programs. The objective of this project is to genotype Douglas-fir trees from a pacific-northwest tree improvement program to verify their clonal genotypes and parentage.

A total of 756 Douglas-fir trees were genotyped at six microsatellite loci. These markers provided a Probability of Identity of 1.1×10^{-12}, indicating sufficient discriminatory power of the data for distinguishing unique genetic individuals. The mean number of alleles per locus was 26.7 (6.4), effective number of alleles per locus equaled 13.9 (3.7), expected heterozygosity was 0.922 (0.021), and the fixation index equaled 0.258 (heterozygote deficiency).

A total of 26 ramets did not match the expected clonal genotype and resulted in a 3.4% 'mislabeling' rate (26/756). Genetic data also did not support the male parental identity for one submitted family of Douglas-fir.

Douglas-fir. iStock.com

Clonal and parental identification in Douglas-fir (*Pseudotsuga menziesii*) seed orchards

NFGEL Project 324. Project cooperator – Private Company

In our efforts to support tree improvement programs, we continued our work to fingerprint individual Douglas-fir trees from additional orchards using six microsatellite loci.

In this project we genotyped a total of 426 trees. Data was combined with genotypes obtained in prior NFGEL projects. Variability in the SSR loci was moderate to high among the clonal genotypes. After combining the Douglas-fir clonal genotypes from NFGEL Project 307 with the scores from NFGEL Project 324, the number of alleles per locus was 24.3 (6.7), effective number of alleles per locus equaled 13.1 (4.3), expected heterozygosity was 0.910 (0.046), and the fixation index equaled 0.292 (heterozygote deficiency). The Probability of Identity (PI) was2.9×10^{-12}. There markers had a mean error rate of 0.91%.

Only 3 out of 295 parent ramets (1.0%) analyzed contained mislabeling (mislabeling of the ramet, overgrown rootstock, etc.). Only nine progeny out of 123 total progeny analyzed appeared to have mis-identified maternal &/or paternal parents.

Distinguishing pure Butternut (*Juglans cinerea*) from Butternut hybrids

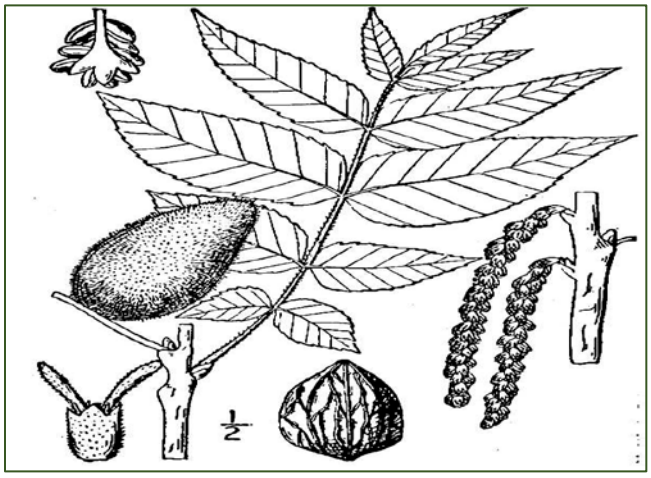
NFGEL Project 325. Project cooperator: Scott Rogers, US Forest Service – Region 9

Butternut (Juglans cinerea) foliage. Bill Cook, Michigan State University, Bugwood.org.

NFGEL completed three prior butternut identification projects using molecular markers:

- NFGEL Pj#198: Identifying Butternut (*Juglans cinerea*) in the Northeast U.S. using Molecular Markers, December 2008 (revised).
- NFGEL Pj#286: Identification of Butternut (*Juglans cinerea*) and Japanese Walnut (*J. ailantifolia*) Hybrids (Region 8), August 2014.
- NFGEL Pj#306: Distinguishing Butternut (*Juglans cinerea*) from Japanese Walnut (*J. ailantifolia*) and their Hybrids (Region 9), February 2015.

This project (NFGEL Project #325) used the same three molecular markers as were used in Pj#286 and #306 (markers *trnT-F*, ITS, and 22-5) to assess the species and hybrid identity of submitted putative butternut samples. Specifically, the project objective was to distinguish pure, or native, butternut (*Juglans cinerea* L.) from the exotic Japanese walnut (*Juglans ailantifolia*) species or hybrids.



Butternut.

Twenty-one samples were submitted for analysis. Their genetic data match that of known *J. cinerea* (butternut) and no indication of hybridity was observed in the genetic data.

Analysis of DNA content variation in San Diego thornmint (*Acanthomintha ilicifolia* (A. Gray) A. Gray)

NFGEL Project 330. Project cooperator: Deborah Rogers, Center for Natural Lands Management.

San Diego thornmint. Sarah Godfrey.

This study was designed to test for putative ploidy variation in San Diego thornmint (*Acanthomintha ilicifolia* (A. Gray) A. Gray) (Lamiaceae) first detected in a previous NFGEL study (Project 279). The previous study analyzed isozyme variation in ca. 20 populations of San Diego thornmint in conjunction with a common garden study conducted by another group. Possibly the most interesting aspect of the isozyme study was the identification of three putative ploidy levels based on banding patterns, primarily at PGI-2: diploid, tetraploid, and hexaploid patterns. Deborah Rogers presented these findings to a meeting of land managers and conservation groups and several showed interest in and concern about the potential for managers to unknowingly mix ploidy levels during conservation and restoration activities.

Interpreting ploidy from isozyme patterns does not verify cytotype variation as the banding patterns may either underestimate the ploidy level (a diploid pattern may be produced in a balanced tetraploid) or overestimate variation (a duplicated locus will appear polyploid in a diploid individual). Flow cytometry data compares the relative DNA content of nuclei, and is one way to verify ploidy differences among populations.

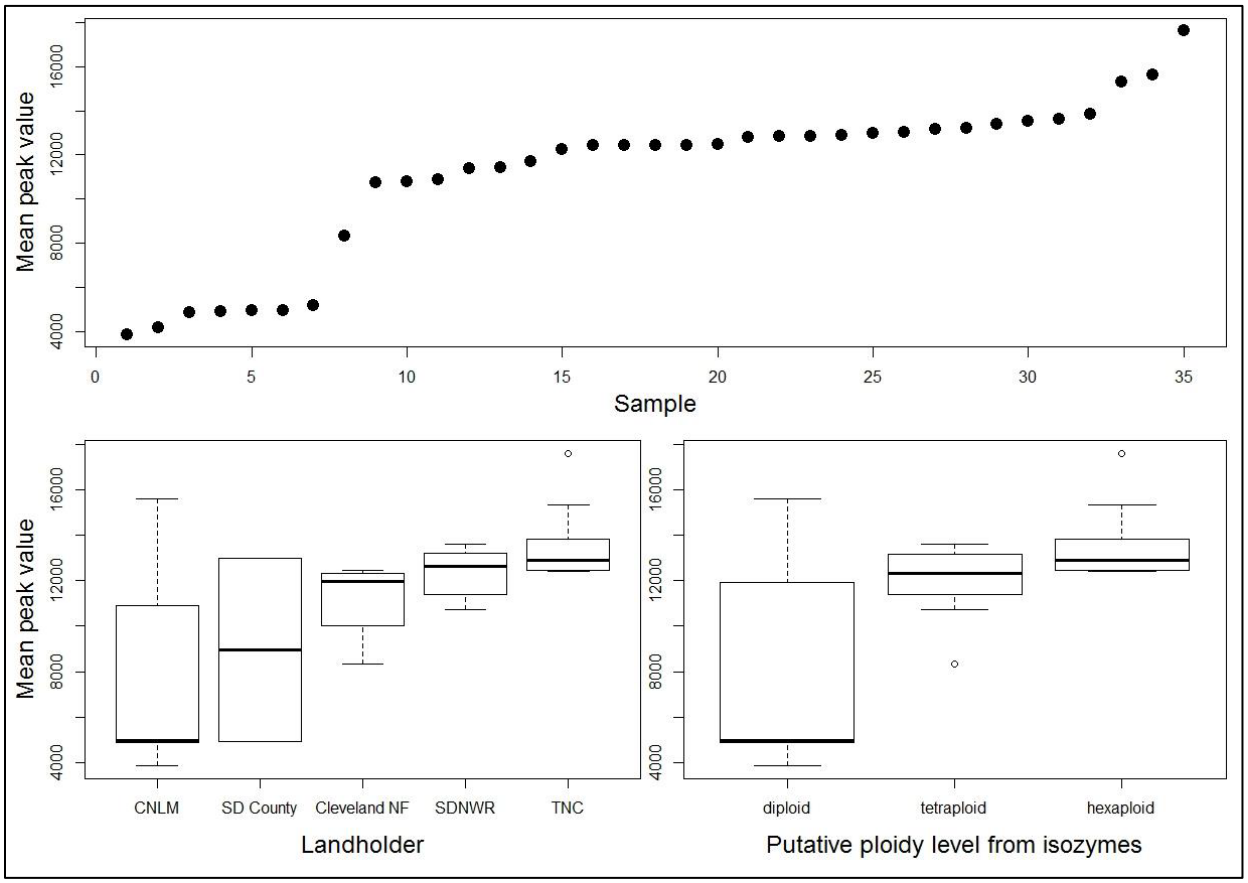
The objective of this project was to use flow cytometry (FCM) to determine if field-collected samples of San Diego thornmint display variation in DNA content. Specifically, the DNA content (interpreted from the FCM data) was compared among populations displaying the putative diploid, tetraploid, and hexaploid isozyme patterns to determine if banding patterns corresponded to cytotype (ploidy) variation among populations of thornmint.

A total of 55 flow cytometer analyses were conducted on 35 samples. Three peak patterns were observed in the flow cytometry histograms. The mean peak values (DNA content) varied over all samples, over collection sites, and over putative ploidy classes based on the isozyme data. All three genome sizes were observed in the putative diploid populations, while the small genome content pattern was not observed in the putative tetraploid or putative hexaploid populations. Only three samples displayed the large genome content pattern, and these were collected from one putative diploid population and the putative hexaploid population. Thus, the putative diploid populations were more complex than predicted, while none of the putative tetraploid or hexaploid samples were classified as a small genome.

This pattern indicates isozymes likely underestimated ploidy level (DNA content) variation in San Diego thornmint.

While the range of mean peak values was not always consistent with the putative ploidy level from isozyme data, it was consistent with cytotype variation in San Diego thornmint. The underlying ploidy levels or genetic mechanisms of this variation cannot be determined from these data alone, although the mean peak values do provide a quantitative measure of relative DNA content. The most biologically feasible cytotypic model, but far from the simplest model, may be where the medium genome content corresponds to hexaploid individuals, the small genome content corresponding to diploids, and the large genome content corresponding to octoploids.

The main objective of this project was to confirm whether variation in ploidy inferred from isozyme banding patterns corresponds to differences in DNA content, a more direct measure of cytotype variation. The flow cytometry data gathered for five collections of San Diego thornmint indicate that plants vary in DNA content within and among populations, and the variation did not strictly follow the predicted geographic patterns. Three genome contents were identified in the flow cytometry patterns, which may reveal complex cytotype variation in this endangered plant. As such, conservation and management strategies would benefit from considering possible ecological (fitness) or demographic (fertility) consequences of managing populations of differing cytotypes.



DNA content varies among thornmint samples.

NFGEL Requests for Assistance

In addition to accepting and processing Projects, NFGEL also fulfills requests for assistance. A Project is characterized by (1) the presence of a project plan with defined management objectives and sampling design, (2) the receipt of plant material needed to meet objectives, (3) a strategy for the laboratory methodology needed to meet objectives, and (4) the design for the interpretation and reporting of results. Significant work done by NFGEL Staff outside the Project definition is captured by the use of "Requests for Assistance (RAF)" numbers.

16-RAF016: DNA isolation from poplar leaf tissue

Partner: Héloïse Bastiaanse, US Forest Service Pacific Southwest Research Station

NFGEL extracted DNA from approximately 700 poplar leaf samples and delivered the quantified DNA to our partner. DNA isolation was conducted using Qiagen DNeasy 96-well format kits, employing a proteinase-K step and tissue homogenization under liquid nitrogen using the Qiagen Mixer Mill with tungsten beads. DNA concentrations were determined using pico green. The average sample DNA concentration was 92ng/ul, with an average DNA yield of 16ug per sample. Poplar is of research interest for understanding the genes and mechanisms that regulate wood formation.

Poplar trees. iStock.com

Staffing and Organization

NFGEL Staff

During FY 2016, NFGEL was staffed with 1.7 permanent FTEs (three employees), and multiple staff on temporary tours (13 employees). Temporary employees accounted for 0.8 FTEs for the reporting year, or 2.5 total FTEs at the lab.

EMPLOYEE	POSITION	TOUR (% FTE for year)	DATES
Valerie Hipkins	Director	Permanent (67%)	10/1/15 – 6/3/16
Jennifer DeWoody	Geneticist	Permanent (31%)	10/1/15 – 10/16/15 4/18/16 – 7/22/16
Randy Meyer	Lab Biotech	Permanent (72%)	10/1/15 – 9/30/16
Jian Alsarraj	Lab Biotech	Temp-NTE (40%)	10/1/15 – 9/30/16
Amanda Cutler	Lab Biotech	Temp-NTE (38%)	10/1/15 – 2/19/16
Anne Russell	Lab Biotech	Temp-NTE (0.01%)	3/11/16 – 3/14/16 4/22/16 – 4/25/16
James Boom	Lab Biotech	Contract (1%)	4/6/16 – 5/10/16
Aubrey Carr	Lab Biotech	Contract – High School student (07%)	10/1/15 – 11/17/15
Anika Morkowski	Lab Biotech	Contract – High School student (07%)	10/1/15 – 11/17/15
Lubna Abdulsattar	Lab Biotech	Volunteer (1%)	2/22/16 – 5/2/16
Yehya Alsarraj	Lab Biotech	Volunteer – High School student (0.03%)	7/16 – 8/16
Mark Wolford	Intern	Student Conservation Assoc (0.08%)	5/4/16 – 8/16
Jorge Sanchez	Intern	Student Conservation Assoc (0.08%)	5/4/16 – 8/16
Keishla Perez	Intern	Student Conservation Assoc (0.08%)	6/6/16 –9/16
Noelani Parker	Intern	Student Conservation Assoc (0.08%)	6/13/16 –9/16
Maricela Abarca	Intern	Student Conservation Assoc (0.08%)	6/13/16 –9/16

NFGEL Steering Committee

NFGEL is guided by a Steering Committee made up of Agency professionals with an interest in the genetic assessment of our nation's resources. Steering Committee members:

1. oversee and ensure the accomplishments of the agreed upon work of NFGEL,
2. assist in setting national priorities for NFGEL workload, and
3. assist in securing necessary resources to accomplish the program of work.

MEMBER	POSITION	LOCATION
John Crockett, Chair	Deputy Director - Forest Management	Washington Office, Washington DC
Tom Blush	Regional Geneticist	Region 5, Placerville CA
Barbara Crane	Regional Geneticist	Region 8, Atlanta GA
Keith Woeste	Acting National Program Leader, Genetics and Global Change Research - Forest Management Sciences	Northern Research Station, West Lafayette, IN
Gary Man	Forest Health Specialist, State and Private Forestry	Washington Office, Washington DC
David Pivorunas	Assistant Threatened, Endangered, and Sensitive (TES) Species Program Leader; Watershed, Fish, Wildlife, Air, and Rare Plants	Washington Office, Washington DC
Vacant	National Botanist - Rangelands Management & Vegetation Ecology	Washington Office, Washington DC
David Gwaze	National Silviculturist and Genetics Lead	Washington Office, Washington DC

Staff Activities

Publications

Shinneman, DJ, RE Means, KM Potter, and VD Hipkins. 2016. Exploring climate niches of ponderosa pine (*Pinus ponderosa* Douglas ex Lawson) haplotypes in the western United States: implications for evolutionary history and conservation. PLoS ONE 11(3): e0151811. doi:10.1371/journal.pone.0151811.

Best Practice Guide for Forensic Timber Identification. 2016. United Nations Office on Drugs and Crime (UNODC). PDF guide. (Hipkins)

Presentations

Choosing molecular markers for *Botrychium* taxonomy. Ninth Montana Plant Conservation Conference. Missoula, Montana. 9 February, 2016. (Hipkins)

Molecular genetic markers to achieve productive and resilient forests. Forest Genetics for Productivity Conference. Rotorua, New Zealand. 14-18 March, 2016. (Hipkins)

Latitudinal cline in genetic structure identified from a range-wide collection of Pacific madrone (*Arbutus menziesii* Pursh). The Future of Pacific Madrone Meeting. Puyallup, Washington. 19-20 April, 2016. (Hipkins)

Attendance

Second expert group meeting on forensic analysis in support of law enforcement operations related to forest crime. United Nations. Vienna, Austria. 5-9 October 2015. (Hipkins)

Reforestation, Nurseries, and Genetic Resources Board of Directors meeting. Washington, DC. 9-10 November, 2015. (Hipkins)

Redwood Genetics Workshop. San Francisco, California. January 2016. (Hipkins)

Workshop held at the Smithsonian to develop a portable DNA barcoding device that can be used to combat timber theft. Washington, DC. 26 August, 2016. (Hipkins)

Gene Conservation of Tree Species Workshop – Banking on the Future. Chicago, Illinois. 16-20 May, 2016. (Hipkins)

Professional Activities

Detail: NEPA Planner, Placerville Ranger District, Eldorado National Forest (DeWoody). (10/19/15 –April 18, 2016)

Detail: Acting National Reforestation and Nurseries Program Manager, Forest Management, Washington Office (Hipkins). (6/6/16 – 10/14/16)

Collaborated on a Partnership for Enhanced Engagement in Research (PEER) proposal (administered by the U.S. National Academy of Sciences (NAS)). Resource enhancement and ecorestoration of the African Alpine Bamboo (*Yushania alpina*) in the highlands of East Africa; Ms Judith Nantongo (PI), Uganda's National Forestry Research and Resources Institute (NaFORRI), Kampala, Uganda. (Hipkins)

Evaluated the promotion potential of Faculty Research Assistant, Oregon State University, Department of Forest Ecosystems & Society. (Hipkins)

Renewed 'Approved Laboratory Permit' with California Department of Food and Agriculture. November 2015. (Hipkins)

Reviewed manuscripts for Tree Genetics and Genomes, American Journal of Botany, and New Forests. (Hipkins)

Team Participation

Member of USDA Forest Service National Safety Committee (January 2001 - Current). (Meyer)
Member of the PSW Placerville Safety Committee (January 2013 – Current). (Meyer)
Member of the PSW-RS Community Enhancement and Diversity (civil rights) Team. (Meyer)
Member of the US Forest Service Integrated Vegetation Management, Forest Management, Washington Office: Career Development and Mentoring Team (Hipkins)
Member of the US Forest Service Integrated Vegetation Management, Forest Management, Washington Office: Communication Team (Meyer)
Co-Lead of organizing committee for "Gene Conservation of Forest Trees – Banking on the Future". Workshop held May 2016, Chicago, Illinois. (Hipkins)

Technology-Transfer

Shared protocols for DNA extraction from pine needles with Colorado State University professor (Hipkins)

Union Activities

Union President – Pacific Southwest Research Station (PSW-RS), and Chief Steward Eldorado National Forest (ENF) – Region 5. (Meyer)
NFFE Forest Service Council, Safety. (Meyer)
NFFE Forest Service Council, Safety Committee Chair (2006 – Current). (Meyer)
NFFE Forest Service Council, Union Representative on USDA Strengthening Services Administrative Solutions (SSAS) Homeland Security Workgroup. (Meyer)
NFFE Forest Service Council, Union Representative, Work Group, Comprehensive Safety Policy Review. (Meyer)
NFFE Forest Service Council, Union Representative on Accident Investigations. (Meyer)
Sierra Fire LTBMU Fatality, Team Member.
NFFE, Union Representative, Federal Advisory Council on Occupational Safety and Health (FACOSH) on Field Federal Safety and Health Council (FFSHC) Improvements. (Meyer)
NFFE Forest Service Council, Union Representative, Coordinated Response Protocol (CRP). (Meyer)
NFFE Forest Service Council, Union Representative, Emergency Medical Services (EMS). (Meyer)
NFFE Forest Service Council, Union Representative, eMedical Data Base. (Meyer)
NFFE Forest Service Council, Union Representative, eCheck-In Check-Out. (Meyer)
NFFE Council Vice President – Region 5, Union Representative, Region 5 Self Contained Breathing Apparatus (SCBA) Working Group (Meyer)
NFFE Forest Service Council, Union Representative, eSafety – OWCP Project working with Kirk Powel (Meyer)
NFFE Council Vice President – Region 6, Union Representative, Supervisor Training and Team Education Program (STTEP) Pilot (Meyer)
NFFE Forest Service Council, Pre-Assignment Drug Testing Of Seasonal Employees Negotiation Team (Meyer)
NFFE Forest Service Council, Union Representative, Facilitated Learning Analysis Continuous Improvement Team (FLACIT) (Meyer)
NFFE Forest Service Council, Union Representative, USDA USFS Avian Flu Support Working with APHIS (Meyer)

NFFE Council Vice President – Region 5, Union Representative, Growth Respect Opportunity Workshop (GROW) PTSD Sub-Group (Meyer)

NFFE Forest Service Council, Union Representative, National T&D Safety and Occupational Health Steering Committee (Meyer)

NFFE Forest Service Council, Union Representative, USDA PDI Draft Leave Departmental Manual (4060-630-1) (Meyer)

Sir Peter Crane speaking at the banquet dinner held at the Morton Arboretum during the Gene Conservation of Tree Species Workshop, Chicago, IL. Richard Sniezko, US Forest Service.

Gene Conservation of Tree Species Workshop field trip, Chicago, IL. David Pivorunas, US Forest Service.

Tours

NFGEL continues to host a variety of visitors throughout the year. Tours of the facility and operation are provided that range from simple walk-through visits of the laboratory (usually 30 – 60 minute duration) to more extensive experiences where visitors get hands-on opportunities to extract DNA, work with liquid nitrogen, pipette liquids, dissect owl pellets, and explore other forest conservation and restoration efforts including soil stability, bark beetle biology, and forest tree disease pathology (1 – 6 hour duration).

The National Hispanic Environmental Council (NHEC) visited the US Forest Service National Forest Genetics Laboratory in November 2015 as part of their first annual "Western Environmental Science/Research Institute".

TOUR DATE	TOUR GROUP	NFGEL GUIDES
October 26, 2015	Boy Scout Troup #758 (6 boys + 4 chaperones)	Meyer, Hipkins
November 12-13, 2015	National Hispanic Environmental Council, Western Environmental Science/Research Institute. 22 students	Meyer, Cutler, Hipkins
January 13, 2016	Public. College freshman and adult	Hipkins
January 27, 2016	Tahoe Expedition Academy. 3rd graders	Hipkins
February 9, 2016	UCCE – Tree Fruit Research and Education	Meyer
February 4, 2016	Forest Management Staff (WO and FT Collins)	Hipkins
March 31, 2016	PSW Civil Rights Team	Hipkins
April 8, 2016	Option for Youth Public Charter School, Rancho Cordova	Hipkins
April 29, 2016	Public (retired scientists)	Meyer, Hipkins
May 10, 2016	Mira Loma High School, high school biology classes (50 students, 2 teachers)	Meyer, Boom, Hipkins
June 10, 2016	'Well-Seasoned Women' (16 members)	Meyer
September 16, 2016	Colfax Garden Club	Meyer
September 19, 2016	Rescue School, 5th graders (25 students, 3 adults)	Meyer

Budget

NFGEL receives an annual allocation from the Washington Office, National Forest System's Forest Management staff group. From FY09 – FY13, NFGEL received $480,000 each year. The FY14 and FY15 allocations were approximately $475,000 each year . The allocation increased in FY16 to $508,937. In addition to these funds, NFGEL expended $26,570 individual partner program dollars collected for non-NFVW projects in FY16. These dollars were used for additional salary, chemical, supply, equipment, repair needs, and travel.

FY16 NFGEL BUDGET

ALLOCATION

ITEM	AMOUNT
WO - Forest Management	$508,937
Private Companies (Douglas-fir, slash pine, loblolly pine)	$17,443
Artemisia (Pj 319)	$7,500
PSW Research Station (poplar)	$1,627
TOTAL	$535,507

EXPENDITURES

ITEM	AMOUNT
Salary - Permanent Employees	$263,350
Salary - Temporary Employees	$27,882
Salary - Contracts	$34,307
Site Utilities and Rents	$21,100
Chemicals and Supplies	$79,141
Equipment and Repair	$19,441
Computer and Office Supplies	$305
Postage	$69
Administrative Costs	$182
Vehicle	$3,506
Contract – USDA (Video Development)	$5,000
Contract – USFS (Gene Conservation)	$25,000
Donation –WCF Special Project	$41,250
Travel and Training	$14,870
TOTAL	$535,403

BALANCE

ITEM	AMOUNT
DIFFERENCE	**$104**

Mission and Purpose

The National Forest Genetics Laboratory (NFGEL) provides genetic testing and information for integrated solutions to on-the-ground problems faced by natural resource managers and policy makers. Solutions are provided for public agencies, non-government organizations, and private industries across the United States, often spanning geographical and organizational boundaries. NFGEL addresses conservation, restoration, and management of all plant species using molecular genetic techniques.

The purpose of NFGEL is to analyze molecular genetic markers (protein 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 genetic improvement program, and contributes information in the support of conservation and restoration programs, especially those involving native and TES (threatened, endangered, and sensitive) species. NFGEL serves the needs of the national forests and provides natural resource managers with the means for evaluating the genetic consequences of vegetation establishment actions. All Laboratory information can be found on-line.

Alignment to National Strategic Plans

NFGEL's work is consistent with the strategic direction outlined in the USDA Strategic Plan (2011 – 2015) and the Forest Service Strategic Plan (2015 – 2020). Our work aligns to the following Agency Strategic Plan measures:

> Goal 1: Sustain our Nation's Forests and Grasslands, with the objective to foster resilient, adaptive ecosystems to mitigate climate change.
>
> Goal 3: Apply Knowledge Globally, with the objectives to (a) advance knowledge, (b) transfer technology and applications, and (c) exchange natural resource expertise.
>
> Goal 4: Excel as a High-Performing Agency, with the objectives to (a) recruit a diverse workforce, (b) promote an inclusive culture, and (c) attract and retain top employees.

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