
THE NATIONAL FOREST GENETICS LABORATORY (NFGEL)

FY17 ANNUAL REPORT

USDA FOREST SERVICE
NFGEL
2480 CARSON ROAD
PLACERVILLE, CA 95667
[NFGEL Website](#)
530-622-1609



MESSAGE FROM THE DIRECTOR

October 6, 2017

NFGEL work supports native plant species management, determines genetically appropriate reforestation stock, and sustains ecosystems and species in response to environmental stressors such as insect and disease pressures and climate change.

During FY17 (October 1, 2016 – September 30, 2017) we completed fourteen project reports for six different Regions of the US Forest Service and five external partners representing Industry, Universities, and other Federal Agencies.

My thanks to the land management professionals who continue to value and utilize genetic information for the establishment and maintenance of healthy forests and grasslands!

Valerie Hipkins

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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. Fourteen project summaries are included in this Annual Report.

PROJECT #	PARTNER	PROJECT TITLE
294	USFS - Region 8	Population genetic evaluation of Table Mountain pine
295	USFS - Region 1	Isozyme and DNA content variation in wild and cultivar collections of <i>Festuca</i> from the northern Rocky Mountains
304	USFS - Region 6	Resolving taxonomic confusion around <i>Sidalcea setosa</i> and <i>Sidalcea oregana ssp. spicata</i>
316	Private Company	Identifying poplar escapes
328	Private Company	Parental identification of loblolly and slash pine progeny
329	USFS - Region 8	Genetic diversity in isolated populations of <i>Paxistima canbyi</i> (Canby's Mountain Lover)
332	Private Company	Genetic fingerprinting: loblolly pine (<i>Pinus taeda</i>) and slash pine (<i>P. elliotii</i>) clonal identification
333	USFS - Region 1	Howell's gumweed (<i>Grindelia howellii</i>) genetic diversity and conservation
335	USFS - Region 1-4	5-Needle pine species identification
336	Private Company	Clonal identity of questionable Douglas-fir ramets
338	USFS - Region 8	Levels of hybridization with loblolly pine in shortleaf and longleaf pine seedlots
340	BLM	Species identification in whitebark and limber pines
341	USFS - Region 1-4	5-Needle pine species identification: whitebark and limber pine species identity
342	UC-Davis	Varietal and clonal identification in tea

NFGEL PROJECT SUMMARIES

Population genetic evaluation of Table Mountain pine.

NFGEL Project 294. Project Cooperators: Barbara Crane, USFS – Region 8; Craig Echt and Sedley Josserand, USFS – Southern Research Station; Robert Jetton, CAMCORE; and Kevin Potter, NCSU.

Table Mountain pine (TMP) is a highly endangered conifer species in the Southern Appalachian mountains. There are less than 30,000 acres (which are not pure stands) of TMP left in the native forests. TMP is a serotinous species, but due to lack of prescribed fire and increased southern pine beetle infestations, it has become increasingly more rare. It has been identified (in *FORGRAS*, 2010) as one of the top ten critically endangered tree species in Region 8 of the US Forest Service.



In partnership with Camcore (North Carolina State Univ., Raleigh, NC), range wide cone collections were made from north Georgia to Pennsylvania. This was a 4-year collection project funded by both USFS R8's Regional Office Forest Management Unit and the Washington Office's Forest Health Protection Unit.

Knowledge about the genetic diversity of the species will enable managers of the US Forest Service to make more informed decisions, including recommendations for appropriate seed deployment, management of the populations that are the most imperiled or degraded, establishment a TMP conservation seed bank (for seed production), and support for increased prescribed burns in areas of sufficient genetic diversity to promote natural regeneration.

NFGEL received, isolated DNA, and genotyped 855 TMP trees at 7 microsatellite (SSR) loci.

Tissue Received: 2/19/14 – 10/8/2015

Sample Prep Dates: 2/24/2014 – 11/5/2015

DNA Extraction Dates: 3/7/2014 -- 1/6/2016

Sequences for 8 SSR loci were received from Echt/Josserand on 2/29/2016.

SSR Analysis Dates: 4/24/2016 -- 8/14/2017 (includes protocol testing and sample genotyping)

Final SSR data was sent to Project Cooperators for analysis on 8/15/2017. Objectives to be addressed in the analysis include:

- What are the levels of genetic diversity within and among the TMP collections?
- Are the populations genetically diverse enough to support specific seed zones?
- Is the species inbred and/or genetically bottlenecked due to its scarcity?
- Are any of the mother trees collected genetically related?

Isozyme and DNA content variation in wild and cultivar collections of *Festuca* from the northern Rocky Mountains.

NFGEL Project 295. Project Cooperator: USFS - Region 1, Mary Frances Mahalovich and Susan Rinehart.

Idaho fescue (*Festuca idahoensis* and *F. idahoensis* ssp. *idahoensis*) has recently been identified as one of the core species in the native plants program in the Northern Region. Sparse genetic information is available for this species and is usually inferred from associated *Festuca* spp. *F. idahoensis* has a chromosome number $2n=28$ and is thought to be autotetraploid.

The species has been the focus of an ongoing common garden experiment at CDA Nursery (Coeur D'Alene, ID), with the aim of informing restoration activities. Results indicate that 72% of the putative native collections of Idaho fescue strongly resemble the Winchester selected germplasm release (larger plant size and green, rather than the blue blade color indicative of Idaho fescue) in the common garden study.

A number of related species that occur near or in sympatry with Idaho fescue were included in the project in order to examine possible hybridization in natural collections: *F. occidentalis* (FEOC); *F. viridula* (FEVI); *F. campestris* (FECA); and *F. ovina* (FEOV). Seed availability limited the sheep fescue (FEOV) seed lots to one cultivar and two varietal releases. The 'Covar' cultivar, an aggressive competitor, highly drought tolerant and prolific seed producer, establishes an effective ground cover for erosion control, has its origins from Konya, Turkey. 'Bighorn' is a PVP (plant variety protection) turf grass release of unknown nativity and release date by Turf-Seed Inc., and 'MX-86' is a turf grass variety developed by Jacklin Seed Company in 1986 of unknown nativity. In addition, three NRCS releases, which are commonly used in restoration projects, are included to resolve the genetic source of the material and test for possible hybridization: 'Winchester' selected germplasm and two cultivars, 'Nezpurs', and 'Joseph'.



Festuca

This project was designed to develop or apply existing genetic markers to address three objectives:

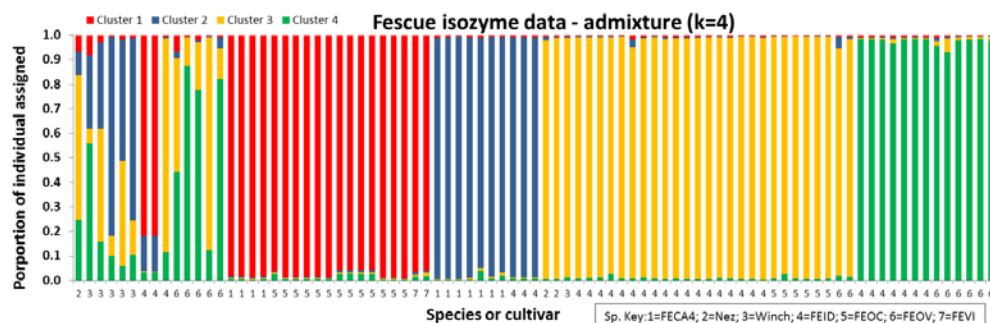
- Resolve the taxonomic identification of the "wild" seed (putatively *Festuca idahoensis*) collected from Region 1. Priority will be given to the seed lots collected under the "research" protocol rather than those collected in the less stringent "operational" manner.
 - a. Ideally, tests will positively identify each collection as one of 5 "like" *Festuca* native to Montana (FEID, FEOC, FEVI, FECA, FEOV).

- b. At a minimum, confirm whether the “wild” collections are *F. idahoensis* (meaning a positive identification with relation to the other species may not be possible).
- Determine if the “wild” seed collections include either of three NRCS releases (Winchester, Josef, or Nespurs), or hybrids between the releases and wild-type *F. idahoensis*.
 - a. Winchester selected germplasm may be another species (*F. viridula*)
 - b. Josef and Nespurs are thought to be derived from *F. idahoensis*
- Examine relative DNA content among *Festuca* species.

Methods used included various marker systems: the internal transcribed sequences of the nuclear ribosomal DNA loci (ITS) region, plastid and nuclear microsatellite loci, isozymes, and ploidy level determinations.

Broad results are:

- ITS sequence variation does not appear species-specific in these *Festuca* samples, and the pattern of species and seed lots splitting amongst clades is consistent with heterozygosity and potential paralogy among the sequenced loci.
- The SSR markers were able to separate some FEVI samples from other taxa, and identified the FEID Kenelty Mtn collection as being divergent. In all other cases, there was a general lack of discrimination among samples.
- Isozyme results indicate that some collections of FEID are highly differentiated and may represent either genetically distinct locations (Mica Mountain), potential areas where hybridization occurs, or evidence of misidentification during seed collection (Moose Camp and Kenelty Mountain). The lack of discrimination between FEID and both FEOC and FEOV means historic or ongoing admixture between these three species cannot be ruled out. The placement of the Winchester selected germplasm and Nezpus cultivar within the majority of the FEID collections in the some analyses is not consistent with the cultivars being highly differentiated, but the assignment of the Nezpus collection with the FEOV-‘Covar’ cultivar in both assignment tests is not consistent with a pure FEID origin of the cultivar. More powerful markers designed to discriminate between these target species would be required to fully resolve the objectives.
- Although it was acknowledged that DNA content determinations were somewhat arbitrary, these values seemed to fall out into five basic groupings or DNA content ‘classes’. The majority of samples (70%) had an intermediate DNA content, with 16% of samples having smaller DNA contents and 14% of samples with larger DNA contents. The Joseph and Nezpus cultivars contained the intermediate DNA content level (except for one seedling of Joseph that had the highest DNA content value), and the Winchester selected germplasm contained a larger DNA content value relative to the common intermediate level.

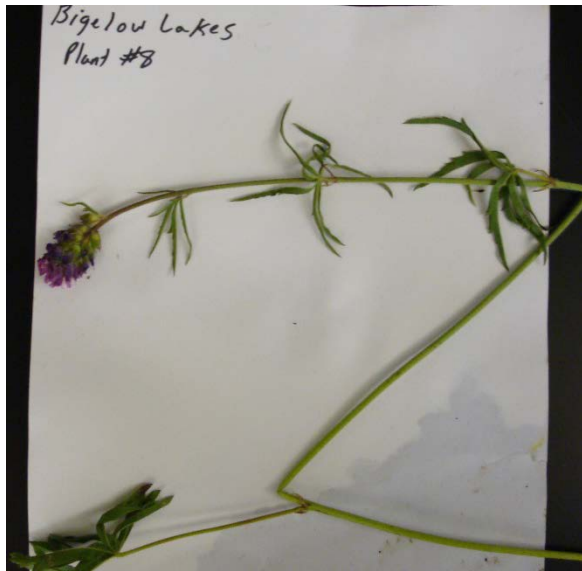


Resolving taxonomic confusion around *Sidalcea setosa* and *Sidalcea oregana* ssp. *spicata*.

NFGEL Project 304. Project Cooperator: Clint Emerson, USFS - Region 6; Barbara Wilson, Carex Working Group.

A small population of *Sidalcea* (checkermallow, checkerbloom) grows on Oak Flat near Agness, Curry County, Oregon. This population is geographically isolated from other similar plants. Specimens were collected from the Oak Flat population five times over a 12-yr period in 1941, 1943, 1947, 1949, and 1953. The population disappeared for many years but eventually it reappeared and an additional specimen was collected (*Stansell* 3308, specimen at the herbarium at Oregon State University). In 2014 the population had dwindled to four plants. The small size of this isolated population raised concern that it would disappear again, this time forever.

Loss of this plant would remove an important forb component from the area's oak savannah. It would end the more-or-less independent evolution of an isolated, peripheral population in a somewhat different environment from the main, more inland species range. Such a population could become so different it could become an independent taxon. One Oak Flat specimen, *Hitchcock* 1953 (deposited at the herbarium of the University of Washington) became the type specimen of *Sidalcea setosa* C.L. Hitchc. subsp. *querceta* C.L. Hitchcock, known only from Oak Flat. Taxonomy of southwest Oregon *Sidalcea* is, however, controversial.



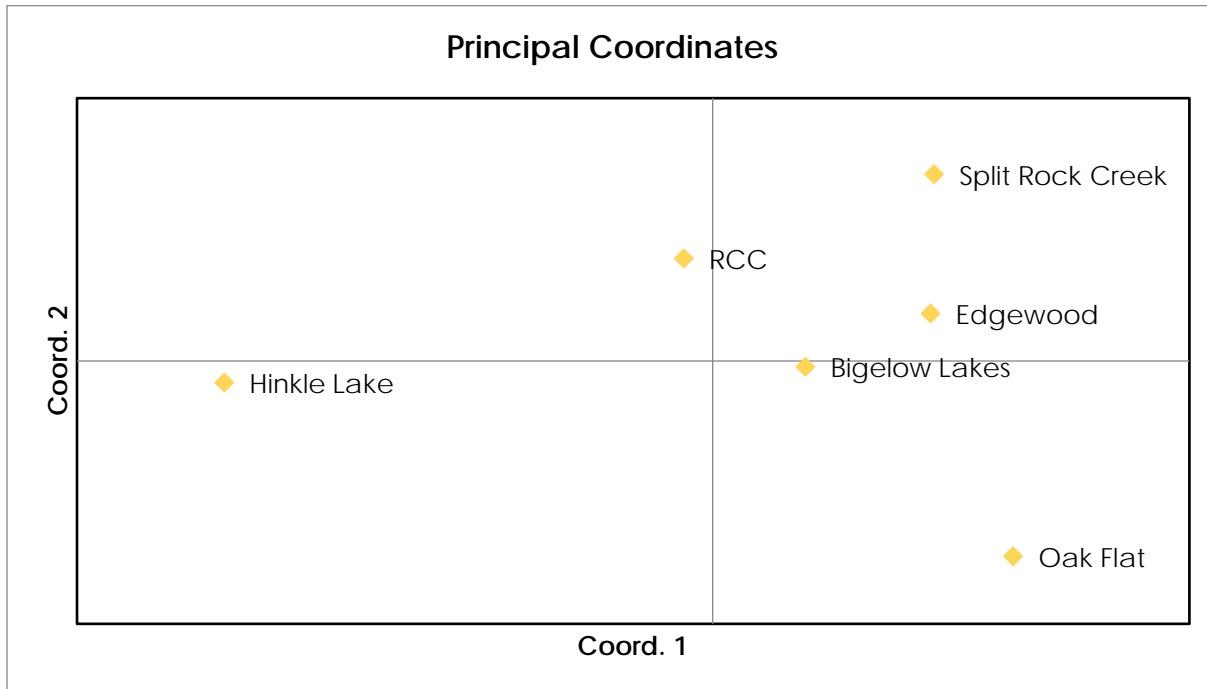
This study was initiated to determine, if possible, whether the isolated Oak Flat population of *Sidalcea* is different enough to be considered a distinct taxon. Genetic variation in and among samples was assessed at 14 isozyme loci.

Sidalcea taxonomy in southwest Oregon is controversial because the plants are highly variable morphologically. Traits that might be useful for identification, such as hairs on the stem and calyx, vary from individual to individual and sometimes within an individual. Flower size varies depending on sexuality of the flowers more than it does between species. Leaf shape varies from orbicular and crenate or lobed to palmately compound depending on their location on the

plant. Calyx size is used to distinguish taxa, but the calyx can continue to grow after flowering.

Serious taxonomists of the group have come to contradictory conclusions about the best classification system. To account for the variation, Hitchcock (1957) recognized both *Sidalcea oregana* and *S. setosa* in the region. He recognized five subspecies in *S. oregana*, and within *S. o. ssp. oregana* he recognized five varieties. Within *S. setosa*, he named *S. s. ssp. querceta* based on an Oak Flat specimen with many stellate hairs and shorter calyces than typical *S. setosa* (Hitchcock 1957, p. 55-56). Dimling (1991) surveyed variation in this group of *Sidalcea* and concluded that the plants were variable but that there were few obvious gaps in the variation that suggested boundaries of taxa. The observation that isozyme variation in this study occurred mainly within populations is consistent with Dimling's observations of variation within populations.

She agreed with Roush (1931) in treating *S. setosa* as particularly hairy individuals of *S. oregana* ssp. *spicata*. However, this did not end the controversy. At the present, the Oregon Flora Project (2016) recognizes only *S. o.* ssp. *oregana* and *S. o.* ssp. *spicata* (Jaster et al. 2016), while the Jepson Manual of the Plants of California (Hill 2016) accepts *S. setosa* as a rare taxon and recognizes Hitchcock's (1957) varieties in *S. o.* ssp. *oregana*.



Principal coordinates analysis (PCA) based on isozyme frequencies in sampled populations of *Sidalcea*.

This isozyme study shows that while the small Oak Flat population appears to be somewhat genetically different from the other *Sidalcea* populations sampled, it is not as distinct as the Hinkle Lake population. In addition to the isozyme results, the limited study of *Sidalcea* morphology reported here suggests that the Oak Flat samples available fit within the variation exhibited by other Oregon *S. oregana* ssp. *spicata* samples. The Oak Flat population falls comfortably within the broad *S. oregana* ssp. *spicata* taxon concept currently in use in Oregon. Plans to preserve this population should be based on its role in the heavily impacted oak savannah habitat of Oak Flat and its geographic isolation, but not on its taxonomy.

Reference

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- Oregon Flora Project. Oregon Plant Atlas, <http://www.oregonflora.org/atlas.php>, accessed 23 August 2016.
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Identifying poplar escapes.

NFGEL Project 316. Project Cooperator: Private Company.

We used nine microsatellite loci to determine if there are any non-*Populus trichocarpa* genetic material carried by any of the submitted samples. These samples were located in or near monitoring plots used to demonstrate that hybrid poplars are not proliferating beyond managed plantations.

Because candidate parent trees were not available to us for this study, a traditional paternity analysis was not conducted. Instead, we took a less specific, more generalized approach by looking at the genetic composition of the submitted samples and assessing their genetic similarity to other known poplar species previously genotyped at NFGEL. The results showed that the unknown poplar samples were most genetically similar to known *P. trichocarpa* clonal genotypes.

Poplar tree (iStock.com)



Parental identification of loblolly and slash pine progeny.

NFGEL Project 328. Project Cooperator: Private Company.



Microsatellite markers have been shown to be very useful for confirming parentage in many species. Here, we used six such markers to confirm the parentage of loblolly (*Pinus taeda*) and slash pine (*P. elliotii*) progeny. Putative parent tree genotypes were compared to the genotype of the individual progeny to confirm the cross. In some cases we did not have the genotype of one of the parents, however the parent genotype could be inferred from the progeny array.

Slash pine (iStock.com)

Genetic diversity in isolated populations of *Paxistima canbyi* (Canby's Mountain Lover).

NFGEL Project 329. Project Cooperator: USFS - Region 8, Barbara Crane, Duke Rankin; Chicago Botanic Gardens, Evelyn Williams and Abigail White.

Paxistima canbyi A Gray (Canby's Mountain Lover) is a native evergreen shrub restricted to the Cumberland Plateau (KY, TN, VA, WV) and central Appalachian region of the United States. This low-growing shrub primarily grows on the edge of rocky cliffs. Within its range, only 67 isolated populations of *P. canbyi* are currently known, and many of those populations are rapidly declining. As a result, this imperiled species is listed as threatened in Kentucky and endangered in Maryland, Ohio, Pennsylvania, and Tennessee. One of the major threats to *P. canbyi* is the Euonymous scale (*Unaspis euonymi*), which is native to Japan and China and is now well established in the United States and Canada. The scale causes permanent leaf damage and in some cases plant death. Generally found on *Euonymous* and *Pachysandra* species, the scale has expanded its reach to include populations of *P. canbyi*.

In light of this threat, restoration and conservation efforts are needed to facilitate recovery. *Paxistima canbyi* has been included in many conservation plans as a species of interest, but land managers currently do not know how to best manage the species. This is largely because the breeding system and levels of genetic diversity have not yet been investigated. *Paxistima* plants appear to be clonal, forming mats, and the number of distinct individuals in a population can be difficult to assess. If this species is primarily clonal, populations comprised of many individual plants may actually represent one or a few genotypes. In those cases, negative genetic factors may be present before census numbers suggest decline. For *P. canbyi*, many populations flower, but do not produce seed, which indicate that genetic factors are already negatively impacting this species' ability to reproduce sexually. Thus, determining the extent of clonal growth and levels of genetic diversity in populations of *P. canbyi* would help guide conservation efforts and inform future management.

Here, we provide information on the basic reproductive biology and genetic structure of *P. canbyi* populations using a conservation genetics approach. Specifically, we addressed two main objectives using neutral microsatellite DNA markers. The first was to determine the extent of clonal growth within West Virginia and Ohio populations of *P. canbyi*. Second, we measured levels of genetic diversity and differentiation in those populations. The results of this study will be used to aid the recovery of this species by informing future restoration and conservation efforts.

Leaf tissue samples from nine populations of *Paxistima canbyi* in West Virginia and Ohio were submitted for this project. We found that the populations of *P. canbyi* analyzed in this project rely heavily on clonal growth and exhibit low genotypic diversity. Of the 119 sampled individuals, our microsatellite analysis revealed only 35 unique multi-locus genotypes (MLGs). As a result, population census numbers overestimate the size of the breeding population, which has important implications for the restoration of this species. Clonal growth favors immediate fitness and ensures survival when there are barriers to sexual reproduction. However, clonality is unlikely to sustain *P. canbyi* populations long-term, as resistance to disturbance and environmental change is often lower in clonal organisms. This is particularly true given that this species is threatened by the Euonymous scale. Therefore, identifying populations with unique genotypes (i.e., genetic repositories) will be important. Greater sampling and additional microsatellite markers would provide even more information about how extensive clonal growth is across this species' range.



Clonal map of *P. canbyi* at one site. Each number on the map is the location of an individual plant. Each symbol represents a different clone.

Given the high levels of clonality detected in West Virginia and Ohio populations of *P. canbyi*,

the recovery plan for this rare evergreen shrub should ideally focus on facilitating sexual reproduction by increasing genotypic diversity, which would increase the number of compatible mates. Alternatively, maintaining genotypically diverse clonal populations is a viable option. Identifying populations with high genotypic diversity and then obtaining source material from those populations may be useful for introducing more diverse material to wild populations, as well as for supporting the *ex situ* conservation of this species. In light of how quickly this species is suffering from the Euonymous scale, producing new ramets for reintroduction, transplantation, breeding programs, and/or conservation could be an important next step.

In terms of managing these populations, reducing stressors such as scale infestation and deer herbivory may improve survival. Building enclosures to exclude deer and monitoring/removing the scales on a regular basis could be effective strategies.

Genetic fingerprinting: loblolly pine (*Pinus taeda*) and slash pine (*P. elliotii*) clonal identification.

NFGEL Project 332. Project Cooperator: Private Company.



Loblolly pine (iStock.com)

Branch tips from a total of 1,299 loblolly and slash pine trees were received by NFGEL for the purpose of determining clonal identity. Ramets were either from uniquely labeled clones, or one of 30 blind samples. Six microsatellite loci were used to assess DNA variability. Markers were developed by Dr. Craig Echt and Sedley Josserand, US Forest Service, Southern Institute of Forest Genetics, Saucier, MS.

All clones (over 100) have unique genotypes at these six SSR loci. 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) remains high among these individuals, indicating ample discriminatory power of the data for distinguishing unique genetic individuals (PI = 4.9×10^{-9} for all samples, 3.2×10^{-8} for the loblolly pine clones, and 4.5×10^{-7} for the slash pine clones). We detected clonal mislabeling at a level of 1.7%.

Howell's gumweed (*Grindelia howellii*) genetic diversity and conservation.

NFGEL Project 333. Project Cooperator: USFS - Region 1, Karen Stockman; Chicago Botanic Gardens, Evelyn Williams and Abigail White.

Grindelia howellii (Asteraceae; Howell's Gumweed) is a restricted species endemic to Montana and Idaho. There is debate over classification of the species; *G. howellii* has been grouped with *Grindelia paysonorum* and considered similar to *Grindelia nana*. However, *G. howellii* has distinctive glands on the stem and leaves, making it sticky to the touch. The most recent molecular phylogeny of the genus confirms its place in the "Pacific" Clade with other species in the region. However, this genetic study only tested one herbarium specimen (accession) for *G. howellii*, and other species with multiple accessions per species were polyphyletic. These results

point to a complicated relationship between taxonomy and evolutionary differences in the genus.



Grindelia squarrosa (iStock.com)

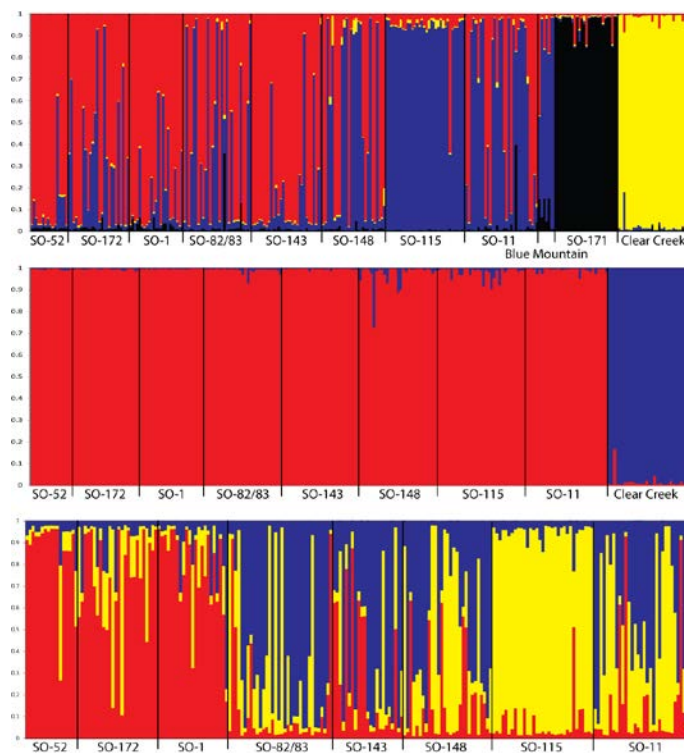
Beyond these taxonomic issues, *G. howellii* is considered a sensitive species in Montana by the US Forest Service. It is found in open habitats such as roadsides and open wetlands, and could be negatively impacted by road management or development. Also, given its restricted range, populations may have low genetic diversity and/or require protection in order to perpetuate the species. Despite local abundance, restricted endemics can be limited by dispersal distances and low genetic diversity. Their limited range and habitat preference can make them more susceptible to fragmentation and loss of genetic diversity. However, endemism and restricted range does not always indicate low genetic diversity or high inbreeding. In addition to occupying a small range, *G. howellii* may hybridize with other species in the genus, such as *Grindelia squarrosa*, which is widely distributed across the United States. *Grindelia squarrosa* has reportedly hybridized with at least one other species, *G. nana*. Hybrid populations may exist where species overlap in their ranges, and produce morphologically intermediate individuals.

Here, we used microsatellite loci and samples from *G. howellii* and *G. squarrosa* populations to examine genetic diversity and possible hybridization. The main questions to be answered in this study were: 1) Are the *G. howellii* populations' sizes, genetic diversity, and variability ideal to preserve genetic integrity? 2) How do the genetics of *G. howellii* interact with conservation efforts, restoration, and management for this species? 3) How closely related are *G. howellii* and *G. squarrosa*? 4) What is the relationship between geographic range size and levels of genetic diversity in *G. howellii* and *G. squarrosa*?

We used genetic data from nine populations of *Grindelia howellii*, one population of *Grindelia squarrosa*, and one putative hybrid population to examine genetic diversity and variability. The number of total individuals in the sampled populations varies greatly (30 – 8,800), but genetic diversity is not strikingly different across populations. If we accept the general northern, central, and eastern groupings of the Seely Lake (Montana) populations as ‘meta-populations’, the northern meta-population has lower genetic diversity than the central and eastern meta-populations. If the northern populations are large, the relatively low genetic diversity could be of concern.

Our results do not indicate any immediate threat to the sampled populations in terms of low genetic diversity, and indeed the Montana populations seem to be acting as a large meta-population, with few genetic differences between populations. In particular, SO-143 is found along a road that may be developed in the future. Looking at its genetic diversity and the STRUCTURE results, it is not distinctly different from SO-148 and SO-82/83. However, it does have a private allele found only in that population. If the population is destroyed, it may be worth transplanting individuals and/or collecting seeds to add to the SO-148 and SO-82/83 populations.

Species identification within *Grindelia* is hard due to the wide range of morphological variation within and among species, and previous genetic studies have found that ‘species’ aren’t always



unique genetic units. The putative hybrid population in this study, SO-171, is quite distinct from the other *G. howellii* Montana populations. However, if it is a hybrid, we’d expect it to share genetics with both the Blue Mountain population of *G. squarrosa* and Montana populations of *G. howellii*. However, Blue Mountain, though different from the Montana populations, isn’t strikingly so – as a different species, we’d expect it to be quite distinct. The SO-171 population could be a hybrid, or it could be that geographic distance from the other Montana populations has led to genetic differentiation. The Clear Creek population is also quite distinct. This could be because of geographic distance or because it is not *G. howellii*. At this point, we recommend collecting from more populations of *G. squarrosa* and putative hybrid populations.

STRUCTURE results for three genetic datasets from *Grindelia*.

Without more *G. squarrosa* populations, we can’t relate population genetic levels to range sizes. The single included putative *G. squarrosa* population doesn’t have markedly different levels of genetic diversity compared to the *G. howellii* populations.

5-Needle pine species identification.

NFGEL Project 335. Project Cooperator: USFS - Region 1-4, Mary Frances Mahalovich.



Interior whitebark pine plus trees in the shared services genetics program occasionally exhibit cone and pollen morphology that is intermediate to whitebark and limber pines: 1) cones are longer than most whitebark, 2) cones are closed like whitebark, but have some scales that are somewhat open like limber, and 3) pollen catkin color is a mixture of both carmine (whitebark) and yellow (limber) within the same catkin cluster. Species identity of four trees are in question that are located where both species are known to co-occur in Montana. While there is no confirmed hybridization between these species (i.e., reproductive barriers between members of the stone and white pines), these four trees would have the highest probability of being the first documented hybrids. Bingham et al. produced putative hybrids between in the 1970s; however, the seed was never germinated and before it could be confirmed, the seed lot was later

damaged/lost during a power outage in 2014 at FSL Moscow. It is documented that due to the maternal contribution in *Pinus* spp. reproduction, it is possible for seeds and cones to develop with an unfertilized embryo (i.e., but no hybridization has occurred). Project objectives include:

1. Species identification of four trees in the genetics program.
2. Rule out naturally occurring hybridization between whitebark and limber pines.

Genetic markers were determined through the work of Rachel Milliano, Pacific and Yukon Laboratory for Environmental Testing, Canada (rachel.milliano@canada.ca), and received through Mary Frances Mahalovich. Four markers were amplified based on sequence data that was shown by Milliano to differentiate WBP from LP as determined by representative sequences retrieved from GenBank and by direct sequencing of trees located in Canada. Two of the markers (ycf1 and trnG) amplify chloroplast DNA loci, and the IFG8612 markers amplify the same basic region of nuclear DNA. The genetic data indicated that in some cases, the mother trees were whitebark pine, in another cases the mother was limber pine, and some situations where seed came from apparently LP x WBP hybrid trees.

Clonal identity of questionable Douglas-fir ramets.

NFGEL Project 336. Project Cooperator: Private Company.

The objective of this project was to compare genotypes from questionable Douglas-fir ramets to those of known clones to determine the identity of the questionable material. Six SSR markers were used in the clonal identification of these Douglas-fir samples: 2G12, 2C3, 3B2, 4A7, 1C3, and 3G9. Eighteen trees were received labeled with 'confident' clonal identities. SSR data was able to uniquely identify all 18 clones. Twenty-eight trees were identified as having 'questionable' clonal identity. Samples were run multiple times in an attempt to fill in missing data and for error rate determination. Genotypic scores at the six SSR loci were used to compare 'questionable' samples to their corresponding 'confident' ramet. In the majority of cases, the questionable ramet did not match the genotype of the corresponding confident ramet.

Levels of hybridization with loblolly pine in shortleaf and longleaf pine seedlots.

NFGEL Project 338. Project Cooperator: USFS - Region 8, Barbara Crane and Craig Echt.



Shortleaf pine orchard and germinating seed (R8 - USFS)

Because of environmental changes in temperature, longleaf (LL) and shortleaf (SHL) pines are suspected to be more susceptible to hybridizing with loblolly pine. This has serious negative ramifications on some inherent adaptive traits and unique biological traits of each species.

In R8, both LL and SHL pines are targeted as priority restoration species, by the agency and by many partner organizations. Seed is scarce, expensive, and cone crops are every 5-7 years. The Forest Service owns & manages 70% of all longleaf & 95% of all shortleaf orchard resources across the entire south. Many state agencies' tree improvement programs have been terminated. They now depend on the FS for a supply of genetically improved seed. Some organizations who want to start or rebuild their orchards depend on FS seed orchards & progeny tests for scion material. We want to make sure that we are providing the correct clone and pure seed to our partners for their successful reforestation needs. Planting with correct material is critical for successful reforestation projects.

NFGEL used genetic markers developed by Craig Echt (USFS-SRS) on a project brought to us by Barbara Crane (R8 Regional Geneticist). We are checking R8's shortleaf and longleaf orchards to see if any tree is actually a hybrid with loblolly (and not a pure shortleaf and longleaf), and also checking shortleaf and longleaf orchard seedlots to see if any of the seed are loblolly hybrids. Prevailing thought (supported by Stewarts paper and the odd morphology observed in many trees/seedlings today) suggests that short and longleaf hybridization with loblolly is quite high and of major concern.

NFGEL checked about 200 seed per seedlot from 2 seedlots of SHL and 9 seedlots of LL. R8 selected these 11 seedlots to start with because these came from very high loblolly pollen flight years – so if hybridization was happening, the thought was we should be seeing it in these seedlots. A total of 2,095 seed were checked to see if they were loblolly hybrids. For SHL, one seedlot contained less than 1% hybrids, while the second seedlot was comprised of only 2.6% hybrid seed. The longleaf seedlots also did not contain significant hybridization with loblolly. The majority of seedlots tested had less than 1% hybrids (and no hybrids were detected in four of the lots). One Texas seedlot contained near 3% hybrids. The Region can chose to screen more

seedlots from various years to confirm these low hybridization rates; they could also screen seed from natural stands if they want to distinguish what's going on in natural stands vs orchards.

Percent loblolly pine hybridization in shortleaf pine and longleaf pine seedlots.

Seedlot	Species	# lob hybrid seed detected in seedlot	% lob hybrids in seedlot
AR-OZ 03-3-110-1-14-01	SHORTLEAF	1	0.52%
MS 23-1-110-1-14-01	SHORTLEAF	5	2.62%
S. AL. 01-2-121-1-14-01	LONGLEAF	0	0.00%
N. AL. 01-3-121-1-14-01	LONGLEAF	1	0.53%
FL 09-1-121-3-14-01	LONGLEAF	0	0.00%
FL 09-1-121-3-14-02	LONGLEAF	0	0.00%
LA 17-1-121-1-14-01	LONGLEAF	1	0.53%
MS 23-1-121-1-14-01	LONGLEAF	0	0.00%
TX 42-1-121-1-14-01	LONGLEAF	1	0.52%
TX 42-1-121-3-14-01	LONGLEAF	5	2.63%
TX 42-1-121-3-14-02	LONGLEAF	3	1.58%

Species identification in whitebark and limber pines.

NFGEL Project 340. Project Cooperator: Bureau of Land Management (BLM), Maureen Hartshorn and Joshua Jackson.



Limber pine (iStock.com)

BLM foresters in Rawlins, WY have identified some pine trees that are presenting strangely. The trees are found in what is thought to be a limber pine (*Pinus flexilis*) stand on Shirley Mountain, but they have purple male cones indicative of whitebark pine (*P. albicaulis*). Whitebark pine has

not been mapped previously on Shirley Mountain. The project objective is to determine if these trees are whitebark pine (WBP), limber pine (LP), or hybrids.

The same markers used in NFGEL Project 335 (summarized in this FY17 Annual Report), were used in this project: chloroplast markers *ycf1* and *trnG*, and the nuclear IFG8612 marker. Data indicate that the ten tested trees are limber pine (or another way of expressing this is that the data do not show evidence of any whitebark pine alleles in the ten sample trees using these for markers). *trnG* and *ycf1* are markers that are paternally inherited through the chloroplast and are haploid (only one fragment per tree is amplified). All trees genotyped contain a 325bp fragment at *trnG*, and a 287bp fragment at *ycf1*. These fragments have only been found in our limber pine controls. The IFG8612 marker is bi-parentally inherited through nuclear DNA. The fragment sizes observed in this marker (225bp and 406bp, respectively), are the same fragment sizes observed only in the limber pine controls. Therefore, according to the data at these markers, the ten tested trees are limber pine, not whitebark pine or a limber X whitebark pine hybrid.

5-Needle pine species identification: whitebark and limber pine species identity.

NFGEL Project 341. Project Cooperator: USFS - Region 1-4, Mary Frances Mahalovich.



Whitebark pine (iStock.com)

This project continued the work of NFGEL Project 335 (summarized in this FY17 Annual Report). The goal was to identify the species of two trees in the USFS genetics program and determine if either is a naturally occurring hybrid between whitebark pine (WBP) and limber pine (LP). Approximately 10 seed per tree were analyzed individually at the same suite of chloroplast and nuclear markers as in the prior project. The haploid megagametophyte (meg) and diploid embryo from each seed were genotyped individually at the four genetic markers. The haploid

meg carries one allele per locus from the maternal tree, while the diploid embryo contains up to two alleles per locus, one from the maternal parent tree and the second from the paternal pollen parent. Data suggest that one of the two trees is a whitebark pine, while the other tree is a limber pine x whitebark pine hybrid. The first tree appears to have been pollinated by whitebark pine, producing whitebark pine seed; the hybrid tree appears to have been pollinated by both limber pine and whitebark pine, producing a mix of seed with both some LP and some WBP pollen parents.

Varietal and clonal identification in tea.

NFGEL Project 342. Project Cooperator: **University of California - Davis, Jackie Gervay-Hague.**



Tea (iStock.com)

Remnant 'mother plants' (ortets) and other materials of *Camellia sinensis* (tea) exist from a 1967 experiment. Before these plants can be used for further research and extension, their genetic identity must be confirmed. The objective of this work is to identify distinct genotypes among tea plants submitted for genetic analysis. Specific project goals are to see if we can quickly screen SSR markers from the literature to find a small subset of loci capable of distinguishing genotypes.

DNA was isolated from submitted samples and genotyped using nine SSR markers. The SSR data was able to resolve 21 unique genotypes among the 31 individual samples. Some putative clonal ramets matched as was expected; however some putative clonal ramets did not have matching genotypes. Genetic data was able to resolve apparent identity naming errors among the samples.

STAFFING AND ORGANIZATION

NFGEL STAFF

During FY 2017, NFGEL was staffed with 1.7 permanent FTEs (two employees), and multiple staff on temporary tours (10 employees). Temporary employees accounted for 1.1 FTEs for the reporting year, or 2.8 total FTEs at the lab.

EMPLOYEE	POSITION	TOUR (% FTE for year)	DATES
<i>Valerie Hipkins</i>	Director	Permanent (100%)	10/1/16 – 9/30/17
<i>Randy Meyer</i>	Lab Biotech	Permanent (73%)	10/1/16 – 9/30/17
<i>Jian Alsarraj</i>	Lab Biotech	Temp-NTE (37%)	10/1/16 – 8/9/17
<i>Margaret Wisniewski</i>	Lab Biotech	Contract - MobilizeGreen (25%)	3/11/17 – 9/30/17
<i>Yehya Alsarraj</i>	Lab Biotech	Contract (6%)	12/27/16 – 1/9/17 7/3/17 – 8/9/17
<i>James Boom</i>	Lab Biotech	Contract (4%)	1/10/17 – 2/3/17
<i>Kasey Cope</i>	Lab Biotech	Contract (4%)	3/11/17 – 9/30/17
<i>Forrest Stull</i>	Lab Biotech	Contract (11%)	5/22/17 – 7/25/17
<i>Ella Schultz</i>	Lab Biotech	Contract (11%)	6/12/17 – 8/3/17
<i>Slaney Stringer</i>	Lab Biotech	Contract (7%)	3/11/17 – 9/30/17
<i>Grayson Egbert</i>	Lab Biotech	Contract (2%)	3/11/17 – 4/27/17
<i>TD Forestry</i>	Lab Biotech	Contract (1%)	3/8/17 – 4/5/17

NFGEL STEERING COMMITTEE

NFGEL is located within the US Forest Service National Forest System. We are a detached Washington Office unit belonging to the Forest Management, Rangeland Management & Vegetation Ecology staff group. 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
<i>J. Eric Davis Jr., Chair</i>	Assistant Director - Forest Management, Rangelands Management, and Vegetation Ecology	Washington Office, Washington DC
<i>Tom Blush</i>	Regional Geneticist	Region 5, Placerville CA
<i>Barbara Crane</i>	Regional Geneticist	Region 8, Atlanta GA
<i>Keith Woeste</i>	Molecular Geneticist and Hardwood Breeding	Northern Research Station, West Lafayette, IN
<i>Gary Man</i>	Forest Health Specialist, State and Private Forestry	Washington Office, Washington DC
<i>David Pivorunas</i>	Threatened, Endangered, and Sensitive (TES) Species Program Leader; Watershed, Fish, Wildlife, Air, and Rare Plants	Washington Office, Washington DC
<i>William Carromero</i>	National Botanist - Forest Management, Rangelands Management, and Vegetation Ecology	Washington Office, Washington DC
<i>David Gwaze</i>	National Silviculturist and Genetics Lead - Forest Management, Rangelands Management, and Vegetation Ecology	Washington Office, Washington DC

STAFF ACTIVITIES

PUBLICATIONS

- Potter, KM, RM Jetton, A Bower, DF Jacobs, G Man, VD Hipkins, and M. Westwood. 2017. Banking on the future: progress, challenges and opportunities for the genetic conservation of forest trees. *New Forests* 48(2): 153-180.
- Bower, A and VD Hipkins. 2017. Genetic diversity and population structure in the rare, endemic baker cypress (*Hesperocyparis bakeri*). *Madroño* 64(2):71-82.
- Willyard, A, DS Gernandt, K Potter, V Hipkins, P Marquardt, M Mahalovich, SK Langer, FW Telewski, B Cooper, C Douglas, K Finch, HH Karemera, J Lefler, P Lea, and A Wofford. 2017. *Pinus ponderosa*: a checkered past obscured four species. *American Journal of Botany* 104(1):161-181.

PRESENTATIONS

- Managing an endangered southern California herb in the context of climate change: the contribution of genetic information to management strategy. Natural Areas Conference, Davis, California. 18 October 2016. (DeWoody)
- Integrating molecular genetics into seed management programs. National Native Seed Conference. Washington DC. 13-16 February 2017. (Hipkins)
- Current state of genetic technologies. Tools to Combat Illegal Timber Theft Workshop. Seattle, WA 28 February – 1 March 2017. (Hipkins)
- Integrating molecular genetics into land management projects. Collaborative Forest Landscape Restoration Program (CLRP) national conference call. 27 July 2017. (Hipkins)
- Use of genotyping technologies to identify tree variability. Application of High Throughput Genotyping Technologies for Forest Tree Species Identification and Timber Tracking Meeting. Madrid, Spain. 13-15 September 2017. (Hipkins)

ATTENDANCE

- Discussion on Gene Conservation Efforts with USFS and American Public Gardens Association. Arcadia, CA. 23-24 January 2017. (Hipkins)
- Use of mass-spectroscopy for wood identification. US Fish and Wildlife Forensics Laboratory. Ashland, OR. 15-16 March 2017. (Hipkins)
- Congressional Briefing Training. Washington DC. 12-16 June, 2017. (Hipkins)

PROFESSIONAL ACTIVITIES

- Renewed 'Approved Laboratory Permit' with California Department of Food and Agriculture. September 2017. (Hipkins)
- Reviewed manuscripts for Tree Genetics and Genomes and New Forests. (Hipkins)
- Technical Coordinator for the GTR Proceedings of the Gene Conservation of Tree Species—Banking on the Future Workshop, Chicago II. (Hipkins)

TEAM PARTICIPATION

Member of USDA Forest Service National Safety Committee (January 2001 - Current). (Meyer)
Member of the PSW Placerville Safety Committee (January 2001 – Current). (Meyer)
Member of the PSW-RS Community Enhancement and Diversity (civil rights) Team. (Meyer)
Member of the US Forest Service Leadership Forum (Hipkins)
Member of the Center of Excellence in Forensic Science at the University of Washington.
(Hipkins)

TECHNOLOGY-TRANSFER

Shared protocols with international and national partners for tissue desiccation and storage processes, starch gel electrophoresis, and marker choice for conifer species (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, Work Group, Comprehensive Safety Policy Review. (Meyer)
NFFE Forest Service Council, Union Representative on Accident Investigations. (Meyer)
• Preacher Fire FLA Team, Team Member.
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 Forest Service Council, Union Representative, Facilitated Learning Analysis Continuous Improvement Team (FLACIT) (Meyer)
NFFE Forest Service Council, Union Representative, National T&D Safety and Occupational Health Steering Committee (Meyer)

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). NFGEL staff also hosts groups on site by being the point of contact for conference room reservations, and providing room and site access while assuring that site and participant safety and security issues are addressed.

TOUR DATE	TOUR GROUP	NFGEL GUIDES
<i>October 24, 2016</i>	Rescue Middle School. 5 th grade class #2 (25 students, teacher, parents)	Meyer
<i>October 25, 2016</i>	Rescue Middle School. 5 th grade class #3 (24 students, teacher, parents)	Meyer
<i>October 27, 2016</i>	Placerville Nursery Clients Meeting. USFS employee participants.	Hipkins
<i>November 8, 2016</i>	Client tour	Hipkins
<i>February 9, 2017</i>	USFS R5 ER Specialists (10 members)	Meyer
<i>April 26, 2017</i>	Camino Charter School. 6 th – 8 th grade classes (40 students)	Hipkins, Alsarraj, Curless
<i>April 27, 2017</i>	El Dorado High School. 9 th grade Natural Resource class (30 students)	Hipkins, Egbert, Stringer, Curless
<i>May 16, 2017</i>	Client tour	Hipkins
<i>June 12, 2017</i>	USFS Washington Office staff tour	Meyer
<i>June 21, 2017</i>	Client tour	Hipkins

EVENT DATE	EVENT GROUP	NFGEL HOST
<i>December 16, 2016</i>	Eldorado NF Placerville RD Meeting	Meyer
<i>January 29-31, 2016</i>	USFS PSWRS Video Shoot – Tree Mortality	Meyer
<i>February 22, 2017</i>	Eldorado NF LEOs and Dispatch	Meyer
<i>May 11, 2017</i>	Eldorado NF LEOs FPO Training	Meyer
<i>June 15, 2017</i>	Eldorado NF LEOs FPO Training	Meyer
<i>July 22, 2017</i>	Eldorado National Forest Interpretive Association	Meyer

BUDGET

NFGEL receives an annual allocation from the Washington Office, National Forest System's Forest Management, Rangeland Management & Vegetation Ecology staff group. From FY09 – FY13, NFGEL received \$480,000 each year. The FY14 and FY15 allocations were approximately \$475,000 each year, and the FY16 and FY17 budgets were roughly \$500,000. In addition to these funds, NFGEL expended \$43,550 individual partner program dollars collected for non-NFVW projects in FY17. These dollars were used for additional salary, chemical, supply, equipment, repair needs, and travel.

FY17 ALLOCATION

ITEM	AMOUNT
WO - Forest Management	\$508,786
Private Companies (slash pine, loblolly pine, Douglas fir)	\$30,610
Alaska Yellow Cedar (Pj 334)	\$9,000
International Programs	\$3,940
TOTAL	\$552,336

FY17 EXPENDITURES

ITEM	AMOUNT
Salary - Permanent Employees	213,076
Salary - Temporary Employees	10,763
Salary - Contracts	167,223
Site Utilities and Rents	21,100
Chemicals and Supplies	84,048
Equipment and Repair	36,398
Computer and Office Supplies	233
Postage	422
Administrative Costs	1,010
Vehicle	3,434
Travel and Training	14,241
TOTAL	551,947

BALANCE

	AMOUNT
DIFFERENCE	+\$389

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.

