

**Evaluation of Montane Forest Genetic Resources in the Lake Tahoe Basin:
Implications for Conservation, Management, and Adaptive Responses of *Pinus monticola*
to Environmental Change**

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This proposal addresses **Theme 5, Climate Change (5a - Managing for climate change) and Theme 4, Watershed Restoration (4b and c - wildlife and habitat restoration and invasive species)** in the published RFP.

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Abstract. Forest trees species are primary terrestrial ecosystem components and conservation of their genetic resources warrants special attention. Evaluation of adaptive genetic diversity of forest resources in the Lake Tahoe Basin will allow us to detect the sensitivity and potential vulnerability of populations of *Pinus monticola* (western white pine) to environmental change (introduced organisms, climatic warming, and climate-driven outbreaks of native insects). Identifying patterns of adaptive variation at the landscape-level will constitute a valuable tool to design conservation, management, and forest health monitoring strategies for forest tree species. We are taking an ecological and genetic approach to better understand the interaction of landscape characteristics (geology, climatic gradients, soil properties) and evolutionary processes (gene flow, selection) on ecologically important plant traits (water-use efficiency, disease resistance, phenology, and growth) of western white pine across the LTBMU, to determine the adaptive potential of this forest tree.

Justification: Historical and current land-use (fire suppression and stand densification, historical logging), coupled with environmental change (introduced organisms and global climate change), threaten the biodiversity, sustainability, and functioning of forest ecosystems. At a landscape scale, the Lake Tahoe Basin serves as a model to study these biotic and abiotic influences on montane coniferous forest tree species. Forest trees dominate the terrestrial landscape of the LTBMU and provide vital ecosystem functions and services (watershed protection, biodiversity, food resources, wildlife habitat, sequestering of greenhouse gases, recreation, economic and aesthetic value), thus our ability to assess forest health and conserve biodiversity at all levels (genes, species, ecosystems) becomes critical.

Very few efforts have been made to collect and conserve upper montane tree species and is a necessary first step to conservation of forest resources in the Lake Tahoe region (we are seeking funds from the **Lake Tahoe License Plate Program** for the same work and approach for **sugar pine**, *Pinus lambertiana*). A SNPLMA Round 7 funded project by Vogler and Maloney **Natural and anthropogenic threats to white pines from lower montane forests to subalpine woodlands of the Lake Tahoe Basin: An ecological and genetic assessment for conservation, monitoring, and management**, has just completed a comprehensive cone (195 individual trees) and foliar (249 individuals) collection of *Pinus monticola* (western white pine). Seeds collected will be grown at the Placerville Nursery, and seedlings will be screened for disease resistance to the non-native pathogen, *Cronartium ribicola*, causal agent of white pine blister rust (WPBR). This is the first study of its kind to determine resistance frequency, at a landscape-level, for three species of white pine (sugar pine, western white pine and whitebark pine) across 3 elevation zones.

The next important phase of this project is to evaluate the adaptive genetic variation of ecologically important plant traits (e.g., water-use efficiency, phenology, growth, survival) in the white pine species of the Lake Tahoe Basin (sugar pine, western white pine and whitebark pine). *Pinus monticola* is an important component of upper montane forests and very little, if anything, is known about the amount of standing adaptive genetic variation and local adaptations. Climate-sensitive, high-elevation forests and species are critical to study, to evaluate the interactions and effects of both natural and anthropogenic influences (Parmesan and Yohe 2003). Montane environments and the high degree of heterogeneity (geology, climate, topography and biotic interactions) associated with these systems, provide a complex adaptive landscape. Thus,

our ability to evaluate adaptive genetic variation in natural forest tree populations will allow us to potentially detect the sensitivity (e.g., narrowly versus broadly adapted) of these species and populations to environmental change, as well as improve our ability to identify the vulnerability of populations of *P. monticola* to WPBR or climatic changes (e.g., warming and extended drought periods). Initial evaluation of the distribution of adaptive genetic variation for traits such as disease resistance, water-use efficiency, and phenology, across the LTBMU, may help elucidate patterns observed in WPBR occurrence, MPB- and drought-mediated mortality across the region.

Through years of research on forest tree adaptations, it is now possible to identify genes associated with complex traits such as disease resistance, water-use efficiency, drought adaptation, phenology, cold adaptation, wood quality, and growth in natural forest populations (see <http://dendrome.ucdavis.edu/crsp/>). Such information can help prioritize conservation, reforestation and restoration strategies, as well as forest health monitoring (Neale 2008). Identifying patterns of adaptive variation at the landscape level, will in the future constitute a valuable, cost-effective tool, to design conservation and management strategies for forest tree species (Gonzalez-Martinez et al. 2006), as well as protect and conserve valuable forest resources, offering ecological, environmental, and economic benefits.

Background/Goals/Objectives: Historical and current land-use (fire suppression and stand densification) coupled with environmental change (introduced organisms and global climate change) pose significant challenges to montane forests. Current stressors to white pine species in the Lake Tahoe Basin include, the non-native pathogen WPBR (Maloney 2000, Maloney et al. in review), and climate-driven outbreaks of mountain pine beetle (MPB) (see Table 1). The interactions of climatic warming, MPB-outbreaks, and WPBR will have a number of cascading biotic and abiotic effects that will have both negative population and genetic consequences, influencing how white pines respond to changing environmental conditions.

Table 1. Natural and anthropogenic stressors of western white pine in the LTBMU (*Maloney, Jensen and Vogler, unpublished SNPLMA Round 7 data*). L = low; M = moderate; H = high.

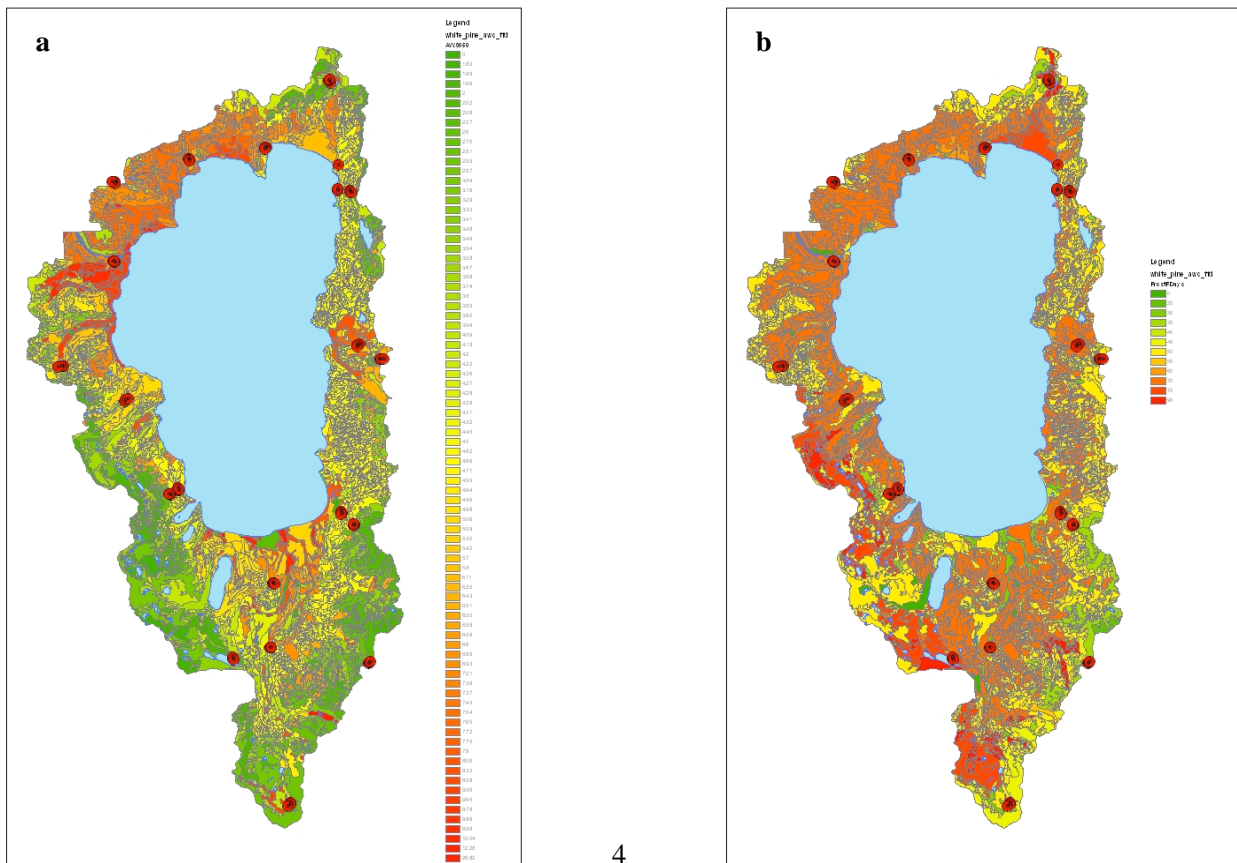
		Natural and anthropogenic stressors				
Population	Basin location	WPBR (%)	*MPB-mortality (%)	Climate	Historical logging	Fire suppression
Incline Lake	NE	13	11	NA	L	L
Flume trail	E-NE	14	8	NA	L	L
Montreal Cyn	E	9	9	NA	M	L
Heavenly	E-SE	0	28	NA	L	L
Armstrong Pass	SE	2	7	NA	L	L
Meiss Meadow	S	4	1	NA	L	L
Echo Lake	SW	6	5	NA	L	L
Jake's	W-SW	5	3	NA	L	L
Blackwood Cyn	W	44	15	NA	L	L
Mt Watson	NW	21	9	NA	M	L

* = Approximately 90% of the observed MPB-mediated mortality has occurred in the last 7 years.

NA = climate effects are still to be determined, data not available (Vogler & Maloney SNPLMA Round 7).

Adaptive traits by environment interactions. Environmental heterogeneity in temperature, precipitation, geology, soil properties, elevation, along with landscape features (topography), influence genetic variation, structure, and local adaptations in forest tree species. **Figure 1** provides evidence of the spatial heterogeneity in two key environmental drivers (available water capacity and frost-free days) across the Lake Tahoe Basin. Parent material (volcanic versus granitic in origin, alluvial influences) and precipitation gradients, characterize available water capacity (AWC), and strongly control plant-soil interactions. An adaptive trait such as water-use efficiency will be influenced by this environmental effect (e.g., highly water-use efficient genotypes might be associated with low AWC sites as a result of local adaptation to soil moisture limitation) and is an important trait to evaluate given climatic changes and predictions of extended drought periods (West et al. 2007; Neale 2008; Gonzalez-Martinez et al. 2008). Elevation and temperature gradients, as well as, landscape features, can dictate the number of frost-free days in a given location, influencing the phenology (growth initiation/bud flush and growth cessation/bud set) of trees. For example, in Figure 1b, many of the high-elevation locations (e.g., Freel Peak and East Peak area) around the LTBMU have relatively short growing seasons (low number of frost-free days) and likely initiate winter dormancy days, if not weeks, before trees lower in elevation (see Howe et al. 2003, for a review of cold adaptation in forest trees).

Figure 1. Available water capacity in the top 0-50 inches (a) and number of frost free days (b). **Color legend: green = low values, yellow = intermediate values, and red = high values.** Location of 20 study populations of western white pine (10) and sugar pine (10) in red circles. Data and maps provided by the Natural Resources Conservation Service (NRCS) and W. Loftis, South Lake Tahoe, CA.



Adaptive traits and forest health. Plants have evolved mechanisms that afford species protection from biotic (e.g., pathogens, insects) and abiotic (e.g., drought, air pollutants, heat) stressors, whether through direct or indirect genetic controls (Burdon 1987; Panek et al. 2002; Dodd et al. 2008). The major-gene for resistance is a direct genetic control that confers immunity against *C. ribicola* (Kinloch et al. 1970; Kinloch et al. 1999). A phenological trait, such as early onset of winter dormancy (bud set), may be an indirect mechanism that protects white pines (particularly in high, cold, and dry environments) from *C. ribicola*. If individuals initiate winter dormancy in August, when basidiospores are generally thought to start releasing, early bud set genotypes may not be receptive hosts, due to stomatal closure that inhibits pathogen entry.

In California, MPB activity is often triggered by protracted droughts periods (see CFPC reports 1970 - 2006). There is evidence of recent MPB activity and MPB-mediated mortality within the Lake Tahoe Basin (see Table 1), that is clearly higher than what has been observed in upper montane forests 10 years ago (mean western white pine mortality < 1%; range = 0-4%, Maloney 2000). Very little is known about the biology of MPB in high-elevation forests in California (S. Smith and B. Bentz, pers. comm.). Host and environmental interactions, in CA, seem to be different than in other locations in western North America. The California Mediterranean climatic regime is a strong selective force influencing drought adaptation and tolerance of many of our forest tree species. Because MPB preferentially attacks drought-stressed trees, there may be a link between water-use efficiency and host suitability to this native insect. Preliminary correlations show a moderate to strong relationship between high MPB-mediated mortality and higher soil available water capacity ($r^2 = 0.62$, Maloney et al. unpublished data). Trees adapted to higher water availability may be less water-use efficient and drought tolerant, therefore vulnerable to MPB-attack than trees growing on sites with lower AWC, which may be more water-use efficient and drought tolerant, thus less suitable to MPB.

Leveraged by genomic discoveries in white pine species (<http://dendrome.ucdavis.edu/whisp/>), a comprehensive seed and foliar collection, and an ecological and environmental dataset (Vogler and Maloney SNPLMA Round 7), we can begin to better understand the interaction of environmental influences on evolutionary processes and local adaptation of western white pine across the Lake Tahoe Basin. Genotypic and phenotypic analyses will allow us to determine the potential of this forest tree species to respond to current and future environmental change. Our objectives are to determine:

Objective 1. Determine patterns of adaptive genetic variation in genes controlling ecologically important plant traits (disease resistance, water-use efficiency, phenology, and growth) across the Lake Tahoe Basin.

Objective 2. Determine the underlying genes that control adaptive phenotypic traits using an association genetics approach.

Approach/Methodology/Location of Research:

Objective 1. Determine patterns of adaptive genetic variation across the Lake Tahoe Basin for genes controlling ecologically important plant traits. DNA will be isolated from needle samples

from 249 trees over 10 populations at the high-throughput facility of the Neale Lab-UC Davis. A large number of single nucleotide polymorphisms (SNPs) have been discovered through the re-sequencing of the western white pine genome at candidate gene loci for disease resistance, water-use-efficiency, phenology, and growth (<http://dendrome.ucdavis.edu/whisp/>), providing an abundant supply of SNPs available for genotyping in the California white pines. The 384 candidate gene SNPs, will be placed on a SNP chip designed by Illumina, with genotype assays performed at the UC Davis Genome Center, (http://www.genomecenter.ucdavis.edu/dna_technologies/illumina.html). We will use standard methods of within and between population genetic structure. (F_{st} and STRUCTURE, Pritchard et al. 2000) as well as correlate genotypic data with environmental data (e.g., AWC, frost free days, temperature, precipitation).

Objective 2. Determine the underlying genes that control adaptive phenotypic traits using an association genetics approach. The association genetic approach requires SNP genotyping and whole plant phenotyping for a large number of unrelated families (see Neale and Savolainen 2004). Once seed is processed at the Placerville nursery, approximately 72 seeds per family (for 195 families) will be stratified, germinated, and seedlings cultured and maintained at the Institute of Forest Genetics, Placerville, CA, for phenotypic evaluations. All families will be replicated in 2 soil moisture treatments: (1) control and (2) soil moisture deficit treatment. In year 2, after germination, progeny from all families will be phenotyped for phenology (dates of bud flush and bud set), water-use efficiency (stable carbon isotope ratio determined from needle tissue), height-growth, survival and disease resistance (this work is already slated to be done at the Placerville Nursery, Vogler and Maloney SNPLMA Round 7). Analytical analyses of the stable carbon isotope ratio ($\delta^{13}C$) will be performed at the Stable Isotope Facility at UC Davis (<http://stableisotopefacility.ucdavis.edu>). Once all phenotypic data has been collected it will be associated with SNP genotypic data (from Objective 1) using methods described in Gonzalez-Martinez et al. (2008).

Location of research. All material has been collected from populations/stands in the LTBMU. Genetic analyses and genotyping will be done at the Neale Lab (UC Davis) and at the UC Davis Genome center, (http://www.genomecenter.ucdavis.edu/dna_technologies/illumina.html). All phenotypic evaluations and greenhouse studies will be done at the Institute of Forest Genetics (IFG), Placerville, CA. Analytical analyses of the stable carbon isotope ratio ($\delta^{13}C$) will be performed at the stable isotope Facility at UCD (<http://stableisotopefacility.ucdavis.edu>).

Relationship of research with previous research and studies. The results of this study will be incorporated and linked with the work being done by Vogler and Maloney (SNPLMA Round 7). To measure the relative importance of an adaptive trait (disease resistance, water-use-efficiency, phenology, growth) we will correlate this genetic information with measures of tree fitness such as reproductive output, recruitment dynamics and survival (data being collected by Vogler and Maloney). All genetic and environmental data will be related to observed patterns in WPBR distribution, MPB activity, mortality, and seedling/recruitment dynamics in the LTBMU.

Strategy of Engaging with Managers. Demographic models being developed by Vogler and Maloney (SNPLMA 2007) will provide information to resource managers in the Lake Tahoe

Basin (LTBMU, NRCS, State Parks, NDF, TRPA, Lahontan, CTC, CDF, Ski Industry) about current population status (e.g., stable, declining, or growing) on federal and non-federal lands. Populations showing signs of decline (due to WPBR, MPB, climate) will be recommended for restoration. Genetic risk and forest health assessments for disease resistance, phenology, and drought tolerance will provide valuable information about suitable and local plant material to deploy (within Basin seed-transfer guidelines) in restoration and reforestation projects. Mitigating the impacts of a non-native pathogen and climate may be needed to maintain forest health, in some locations, in the Lake Tahoe Basin. Stands that have high frequencies of major-gene resistance to WPBR will be recommended for genetic conservation and protection, to maintain natural evolutionary processes of gene flow across the Lake Tahoe landscape.

Deliverables/Products. Our research will provide significant and previously unavailable information about the amount of adaptive genetic variation and adaptive potential of a forest tree species in the LTBMU. Develop a genetic database and diagnostic tools for ongoing evaluation and monitoring for Lake Tahoe Basin resource managers. This information and diagnostic tools will be key resource for managers in developing restoration, reforestation and conservation strategies.

Schedule of Events

	Activity	Year 1	Year 2	Year 3
1a	SNP genotyping (Neale Lab & UCD Genome Center)	X		
1b	Stratify, sow and culture western white pine seeds/seedlings at IFG	X		
1c	Perform sequence analysis, and measures of diversity, neutrality, and structure (Neale Lab-UCD)	X		
1d	Generate well interpolated climate data (UCD)	X		
1e	Continue working with soil survey data (UCD – NRCS)	X		
1f	File quarterly reports (IFG)	X		
2a	Continue culturing, maintaining western white pine seedlings and record keeping (IFG)		X	
2b	Determine % germination (UCD – IFG)		X	
2c	Year 1 phenology and 1-year height growth measurements (UCD – IFG)		X	
2d	File quarterly reports, attend meetings (IFG, UCD, Neale Lab)		X	
3a	Continue culturing, maintaining western white pine seedlings and record keeping (IFG)			X
3b	Year 2 phenology, 2-year height growth, and water-use-efficiency measurements (UCD – IFG)			X
3c	Carbon stable isotope analyses ($\delta^{13}\text{C}$)/water-use efficiency, use as a diagnostic tool (UCD)			X
3d	Genotype –phenotype association studies (Neale Lab – UCD)			X
3e	Genetic database development (Neale Lab – UCD)			X
3f	Data analysis (environmental, ecological, and genetic associations), GIS maps (UCD)			X
3g	Publish results (IFG, UCD, Neale Lab)			X
3h	File quarterly reports, attend meetings (IFG, UCD, Neale Lab)			X

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