

## STUDY PLAN: SIERRA NEVADA RESEARCH CENTER

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### 1. Title

**Genetic variability of *Pinus albicaulis* at treeline in relation to patch age, vegetative layering, and climate variability.**

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### 2. Problem Reference

Sierra Nevada Research Center RWUD – Problem 3: Climate and Landscape Change

### 3. Literature Review & Background

Whitebark pine (*Pinus albicaulis*) is a dominant component of the upper treeline of the Sierra Nevada in California. At the upper treeline, this species grows in a prostrate form, termed krummholz. By stem layering, this form is highly persistent, living more than 1700 years (King and Graumlich 1998). Vegetative spread of krummholz suggests that patches might contain only a single genotype. However, seed of this species is wingless and bird-dispersed (Tomback et al. 1990; Tomback and Kramer 1980), primarily by the corvid, Clark's nutcracker (*Nucifraga columbiana*). This bird has been observed to collect multiple seed from one or more cones, fly to another location, and cache the seed for later consumption (Tomback and Kramer 1980), which affects the genetic structure of the species by seed germinated from seed caches (reviewed in Bruederle et al. 2001; Bruederle et al. 1998; Furnier et al. 1987). The expected genetic structure from this dispersal is that multiple individuals might exist in patches, these arising from seed caches that would be related by ancestry, an expectation confirmed by observed data: Rogers et al. (1999) found in fact krummholz patches contain multiple genets, and that relationships among genets within patches ranged between half- to full-siblings. Moreover, genetic diversity was greatest at the lee side of the apparent dominant wind direction, suggesting periodic recruitment either by seedfall or bird caching. In addition, within-patch genetic diversity tended to increase with patch size. Local, fine-scale, and among site structure found in this work was consistent with the meta-population structure and stand dynamics observed in the sister taxon, limber pine (*P. flexilis*) in the Colorado Front Range (Rebertus et al. 1991).

The analysis by Rogers, et al. (1999) indicated that genetic diversity exists within krummholz patches and thus patches increase in size by both clonal and seed propagation. The objectives of that study, however, were not to determine the extent of diversity mapped within patches, nor diversity related to patch size and age, nor the relationships by ancestry among genets. Moreover, patch sizes in that study were all greater than 100 m<sup>2</sup>. In the study we describe below, we examine the genetic relationships and structure of krummholz ranging in size from seedling clumps to patches of 60 m<sup>2</sup> size.

### 4. Objectives

- 1) What is the spatial diversity of genets within krummholz patches?
- 2) Are the number of genets in a krummholz patch related to patch size and age? What is the relatedness of the genets within patches and does the relatedness vary by patch size and age?
- 3) Is patch size and diversity related to climatic events?

## 5. Methods

We selected two sites within the krummholz zone in the eastern Sierra Nevada between Lee Vining Canyon and Virginia Lakes (the same general area as in Rogers et al. 1999): Warren Bench and Dunderberg Peak (Fig. 1).

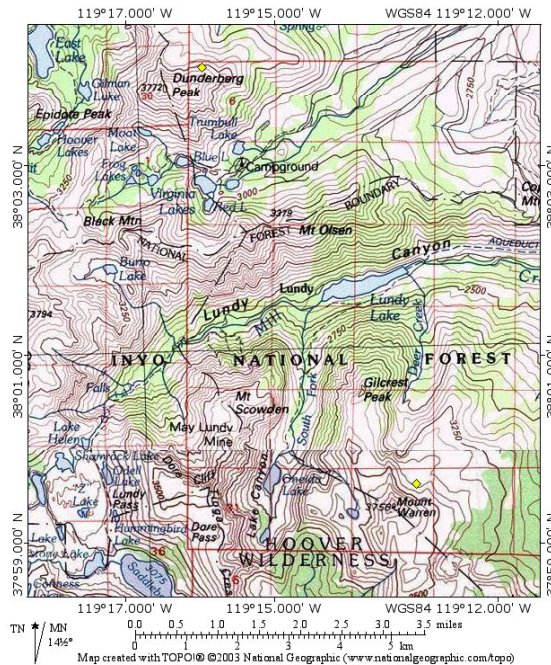


Figure 1. Map of the study site locations in the area of Lundy Canyon, west of Mono Lake, CA, noted by yellow diamonds.

Starting with the Rogers et al. (1999) lower limit on krummholz patch size we sampled 5 patch size classes in a decreasing geometric progression series of approximately 1/4 of the largest size class:

- 1) 6.1 m (20ft) X 9.14 m (30ft) (55.74 m<sup>2</sup>; 600 sq ft)
- 2) 3.05 m (10ft) X 4.57 m (15ft) (13.94 m<sup>2</sup>; 150 ft<sup>2</sup>); 1/4 class 1
- 3) 1.83 m (6ft) X 1.83 m (6ft) (3.34 m<sup>2</sup>; 40 ft<sup>2</sup>); 1/15 class 1
- 4) 0.91 m (3ft) X 0.91 m (3ft) (0.84 m<sup>2</sup>; 10 ft<sup>2</sup>); 1/60 class 1
- 5) seedling clumps

Sampling consisted of clipping a branch of live foliage within a specific grid location for each sample patch for use in biochemical analyses. Foliage was collected from three seedling clumps and two patches for size classes 1-4 per site. At the seedling class, as many samples of foliage as existing stems were taken. In the patch class 4, we sampled in a 0.3 m (1 ft) X 0.3 m (1 ft) grid; in the three larger patch classes, we sampled in a 0.61 m (2 ft) X 0.61 m (2 ft) grid. In each case, foliage was collected from the center of each sample grid. Patch age was estimated by increment core samples near the basal stem of each patch; seedling ages were estimated from internode counts.

As was done in our previous work (Rogers et al. 1999) sample genotypes were measured by allozyme assays, using standard methods (Conkle et al. 1982). Our laboratory routinely obtains 10 to 20 polymorphic loci in allozymes. Moreover, statistical behavior in these data is good (Westfall and Conkle 1992). In patches that are genetically polymorphic, genotypes will be mapped over the grid. The standard genetic statistics packages, BIOSYS (Swofford and Selander 1981) and GDA (Lewis and Zaykin 2000), will be used to summarize the data. Genetic relatedness, estimated

by the Queller and Goodnight (1989) method used in previous work (Rogers et al. 1999; Schuster and Mitton 1991) will be computed by the Goodnight software, Relatedness.

## 6. Application of Research Results

Results will indicate genetic diversity in whitebark pine related to the age and persistence of the krummholz form at treeline. These will contribute to management planning by US Forest Service and National Park Service managers of subalpine communities and contribute to anticipated population response to climate change.

Presentations of results are anticipated to be in a report to the Inyo National Forest and in standard journals in genetics and at meetings of geneticists, conservation biologists, and land managers.

Additional statistical analyses will be done by SAS (SAS Institute Inc. 1989) and JMP (SAS Institute Inc. 2003). Further data analyses and graphical presentations will be done in Mathematica (Wolfram Research Inc. 2003). Mapping of sample locations will be done in ArcView (Environmental Systems Research Institute Inc. 1996).

## 7. Safety and Health

Standard procedures determined by the SNRC Safety Committee will be followed (see SNRC intranet). Field and Office Job Hazard Analyses and Emergency Evacuation Procedures on file for Millar research team pertain to and adequately cover safety procedures for this project.

## 8. Environmental Analysis Considerations (FSM 1950)

Except for removal of some seedlings, plants will not be destructively sampled. Applicable permissions and collaboration with the Inyo National Forest has been obtained. Laboratory hazardous waste disposal will be done by standard procedures.

## 9. Personnel Assignment, Time of Completion, and Cost

Constance I. Millar, Research Geneticist. Principal investigator. Oversight and supervision for all aspects of the study, including project design, justification, grant applications, field work, data analysis, communication, quality control, field and office safety, and publication/dissemination

Robert D. Westfall, Quantitative Geneticist. Co-principal investigator. Responsible for contributing to planning, field collections, and data analysis

Diane L. Delany, Geneticist. Responsible for laboratory and data collection and analysis.

John C. King, Dendrochronologist. Responsible for sample collection and analysis.

Time needed for completion:

Fieldwork: completed

Laboratory analysis: completed

Data analysis: data summary, completed; statistical analysis, 1 month

Manuscript preparation and review: 3-6 months

Remaining cost: Salary for Millar, Westfall, and Delany; King donates his time. Software used is either already obtained or is public domain.

## Literature Cited

- Bruederle, L. P., D. L. Rogers, K. V. Krutovskii, and D. V. Politov. 2001. Population genetics and evolutionary implications. Pp. 137-153 in D. F. Tomback, S. F. Arno and R. E. Keane, eds. *Whitebark Pine Communities. Ecology and Restoration*. Island Press, Covelo, CA.
- Bruederle, L. P., D. F. Tomback, K. K. Kelly, and R. C. Hardwick. 1998. Population genetic structure in a bird-dispersed pine, *Pinus albicaulis* (*Pinaceae*). *Canadian Journal of Botany* 76:83-90.
- Conkle, M. T., D. H. P, L. B. Nunnally, and S. C. Hunter. 1982. Starch gel electrophoresis of conifer seeds: a laboratory manual. USDA FS General Technical Reprint PSW-64. Pp. 18. Pacific Southwest Forest & Range Expt. Stn., Berkeley, CA.
- Environmental Systems Research Institute Inc. 1996. ArcView GIS. Using ArcView GIS. Environmental Systems Research Institute, Inc, Redlands, CA.
- Furnier, G. R., P. Knowles, M. A. Clyde, and B. P. Dancik. 1987. Effects of avian seed dispersal on the genetic structure of whitebark pine populations. *Evolution* 41:607-612.
- King, J. C., and L. J. Graumlich. 1998. Stem-layering and genet longevity in whitebark pine (*Pinus albicaulis*). Pp. 27. Laboratory of Tree Ring Research, University of Arizona, Tucson, AZ.
- Lewis, P. O., and D. Zaykin. 2000. Genetic Data Analysis: Computer program for the analysis of allelic data. Free program distributed by the authors over the internet from the GDA Home Page at <http://lewis.eeb.uconn.edu/lewishome/software.html>.
- Queller, D. C., and K. F. Goodnight. 1989. Estimating relatedness using genetic markers. *Evolution* 43:258-275.
- Rebertus, A. J., B. R. Burns, and T. T. Veblen. 1991. Stand dynamics of *Pinus flexilis*-dominated subalpine forests in the Colorado Front Range. *Journal Of Vegetation Science* 2:445-458.
- Rogers, D. L., C. I. Millar, and R. D. Westfall. 1999. Fine-scale genetic structure of whitebark pine (*Pinus albicaulis*): Associations with watershed and growth form. *Evolution* 53:74-90.
- SAS Institute Inc. 1989. SAS/STAT™ User's Guide, Version 6, Fourth Edition, Volume 1. SAS Institute Inc., Cary, NC.
- SAS Institute Inc. 2003. JMP® Statistics and Graphics Guide. SAS Institute Inc., Cary, NC.
- Schuster, W. F., and J. B. Mitton. 1991. Relatedness within clusters of a bird-dispersed pine and the potential for kin interactions. *Heredity* 67:41-48.
- Swofford, D. L., and R. B. Selander. 1981. Biosys-1. Univ. Illinois, Urbana.
- Tomback, D. F., L. A. Hoffman, and S. K. Sund. 1990. Coevolution of whitebark pine and nutcrackers: Implications for forest regeneration. Symposium on whitebark pine ecosystems: ecology and management of a high-mountain resource, Mar. 29-31, 1989, Bozeman, MT Gen. Tech. Rep. INT-270:
- Tomback, D. F., and K. A. Kramer. 1980. Limber pine seed harvest by Clark's nutcracker in the Sierra Nevada: Timing and foraging behavior. *Condor* 82:467-468.
- Westfall, R. D., and M. T. Conkle. 1992. Allozyme markers in breeding zone designation. *New For.* 6:279-309.
- Wolfram Research Inc. 2003. Mathematica, Version 5.0, Champaign, IL.