A biosystematic survey of Sierra Lodgepole pine (Pinus contorta var. murrayana [Critchfield]) populations in the transverse and peninsular ranges of Southern California

Richard Gobin Everett

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A BIOSYSTEMATIC SURVEY OF SIERRA LODGEPOLE PINE

(Pinus contorta var. murrayana [Critchfield]) POPULATIONS IN THE
TRANSVERSE AND PENINSULAR RANGES OF SOUTHERN CALIFORNIA

A Thesis
Presented to the
Faculty of
California State University,
San Bernardino

In Partial Fulfillment
of the Requirements for the Degree
Master of Science
in
Biology

by
Richard Gobin Everett

June 1997
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June 5, 1997
Abstract

Lodgepole pine (*Pinus contorta* Douglas) is the most widespread conifer in North America, ranging from the Canadian boreal forest to northern Mexico. The western montane portion of the species distribution, from the Cascades of Oregon south through the transverse and peninsular mountain ranges of Southern California to the San Pedro de Martir mountains of Baja California, is occupied by the subspecies Sierran lodgepole (*Pinus contorta* var. *murrayana* Critchfield). This subspecies is an important as a primary overstory component in alpine and sub-alpine communities, and is the subject of this work. Although described as a distinctive subspecies (*Pinus contorta* ssp. *murrayana* Balf.), there is speculation as to its differentiation as an individual subspecies. Among descriptions of four subspecies using both isoenzyme and morphometric surveys of the species, the existence of a *Pinus contorta* ssp. *murrayana* subspecies-specific band of the isoenzyme alcohol dehydrogenase (ADH), ADH-2, has been noted. This study tests the null hypothesis that variations in the sampled anatomical, morphological, and chemotaxonomic traits of southern California populations of *Pinus contorta* var. *murrayana* are identical to these same traits over the subspecies entire range. This is tested by: (1) sampling and quantifying gross morphological data of the subspecies; (2) examining foliar characteristics; and (3) testing for the presence or absence of the subspecies-specific isoenzyme, ADH-2.

Similarities in leaf morphology, cone morphology, and the presence of a subspecies specific isoenzyme confirm the varieties *murrayana* identity with the Sierra
populations. Notwithstanding likely geographic isolation from Sierra Nevada populations since the Pleistocene, significant genetic isolation of the southern California populations has not occurred. This is likely due to having either a significantly large population that maintains an equilibrium of genetic diversity, or, there is some likelihood of genetic flow from the Sierra Nevada populations. Long distance wind pollination, or a large population size, may have aided in maintaining genetic diversity since the spread of the species from Pleistocene refugia. Null alleles, or alterations of ADH during germination in specialized environs may affect estimates of genetic identity and outcrossing using this allele. Although outcrossing is indicated in all but two of ten sampled populations, affirmation of gene flow between populations can only be inferred.
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INTRODUCTION

Lodgepole pine (*Pinus contorta* Douglas) is North America's most widespread conifer, spreading from the northernmost limits of the Canadian boreal forest to as far south as northern Mexico (Critchfield, 1957). Because of its ability to establish itself in a broad range of environments, the species is well noted for its very broad ecological amplitude (Critchfield 1985). The high elevation western montane portion of the species distribution, from the Cascades of Oregon south through the transverse and peninsular mountain ranges of Southern California to the San Pedro de Martir mountains of Baja California, is occupied by the subspecies Sierran lodgepole (*Pinus contorta var. murrayana* Critchfield [Hickman, 1993]). This subspecies is an important as a primary overstory component in alpine and sub-alpine communities (Griffin and Critchfield, 1976), and is the subject of this work.

Taxonomic History

Lodgepole pine was documented as a distinct tree species by European cultures in 1805. Widespread use of the tree for lodgeframing by Native Americans is the source of the common name of the tree. During the Lewis and Clark Expedition in August 1805, lodgepole pine was encountered and noted as a distinct tree species as the party climbed toward the headwaters of the Madison and Beaverhead rivers near present-day Lemhi Pass on the Montana-Idaho border in August 1805 (Moulton, 1983). However, Clark's first recorded observation of the tree which allowed an identification of the species did not occur until the Expedition's encampment at Traveller's Rest (Lolo Creek, Montana) on 12 September 1805. Clark makes a reference to the coastal
subspecies of the tree, *Pinus contorta var. contorta*, during the 1805 to 1806 winter encampment on the Pacific coast (Moulton, 1989; Devoto, 1953).

The first notation of botanical recognition of *Pinus contorta* was made by the Scottish botanist David Douglas in 1825, when he encountered the coastal form (*Pinus contorta var. contorta*) along the Oregon and Washington coasts (Critchfield, 1957). It was not until 1852 that John Jeffrey collected the Sierra Nevada form of Lodgepole pine, first in the Siskiyou Mountains, and then in the Sierra Nevada. The following year Jeffrey's sponsoring group, the Oregon Association, issued a circular with a description of the pine by Balfour, naming it "*Pinus Murrayana Oreg. Com.*" (Murray [1853] in Critchfield, 1957). The Sierran Lodgepole was subsequently collected and re-described a number of times, until Sudworth described the Sierran Lodgepole as *Pinus contorta var. latifolia* (Sudworth, 1871), grouping it with the Rocky Mountain variety, then later describing it as a separate variety, *Pinus contorta var. Murrayana* (Sudworth 1880).

**Current Taxonomic Description**

The genus *Pinus* is divided into two large subgenera, or sections: the Haploxylon (soft pines) and Diploxylon (hard pines). These subgenera are further divided into Subsections, Groups, and then species. At the species level further subdivision can occur into subspecies, variety, or race. In Critchfield (1957), Mirov (1967), Hitchcock and Cronquist, (1973), Harlow et al., (1979), and Wheeler (1981), consider *Pinus contorta* a Diploxylon, a member of the subsection Contortae, and as a species is further subdivided into four varieties: var. *contorta* (the Beach Pine), var.
*latifolia* (Rocky Mountain lodgepole), var. *murrayana* (Sierra lodgepole), and var. *bolanderi* (the Mendocino Plains lodgepole). McMinn and Maino (1938) hold the Sierra lodgepole to be identical to the Rocky Mountain race. However, the cones of *murrayana* are loosely serotinous, unlike the cone serotiny typical of var. *latifolia*. This taxonomic character, combined with the electrophoretic work of Wheeler (1981), Epperson (1983), and Critchfield's (1985) discussion on the migration and divergence of the species from its congenera, Jack Pine (*Pinus banksiana*), are convincing arguments that var. *murrayana* is a valid taxonomic assignment.

Lodgepole pines possess distinctive blue-green needles, usually two to a fascicle, and they are usually twisted along the long axis, ("contorted"), giving the tree its Latin specific name *Pinus contorta*. The needles are strongly persistent, often remaining on the tree for two to three years. Variety *murrayana* also has two to three needles per fascicle, as well as a distinctive orange to grey bark, with loosely appressed scales, which are typical of all other varieties of the species. All varieties establish in areas of primarily winter precipitation, short growing seasons, and cold temperatures (Rundel et al, 1988). Soils do not appear to limit establishment, as lodgepole can be found in deep soils, or among poorly weathered rock (Thorne, 1988; Sheppard, 1984).

**Typical Communities in California**

**Northern California and the Sierra Nevada**

The Klamath, Cascade, and Sierra Nevada Ranges form the northern portion of the Sierra lodgepoles' distribution in California; within this area the tree is an important
component of a number of plant communities (Munz and Keck, 1949). The terrain the
tree inhabits in the volcanic, metasedimentary, and ultrabasic (Sawyer and Thornburgh,
1988) soils of the southern Cascades and Klamath areas are portions of what is refered
to the Sierran floristic province (Munz and Keck, 1949). The Sierra Nevada is a
series of granitic blocks 400 miles long and provides a southward continuation of the
mountainous terrain favored by lodgepole.

Sierra lodgepole is noted as a vegetation component along a elevational
gradient, (beginning at 1800 m in the northernmost parts of its range and 2400m in the
southern portion) in the Sierran red fir forest as described by Munz and Keck (1949)
and Munz (1974). This is the equivalent of the Abies magnifica zone of Sawyer and
Thornburgh (1988). Red fir is the major climax component of the community, but
Sierra lodgepole is an aggressive early and intermediate successional seral component
(Sawyer and Thornburgh, 1988). According to Sawyer and Thornburgh (1988), Sierra
lodgepole is a minor climax component as elevation increases through the Tsuga
mertensiana zone, and finally becomes a major climax component on xeric slopes.

Sierra lodgepole continues in a role of early to mid-seral canopy component as a
function of increasing elevation and decreasing latitude, but also increases its ecological
amplitude and distribution by exploiting mesic sites not occupied by red fir forest
(Rundel et al, 1988). Sierra lodgepole serves as a pioneer species in patchy burned
areas of the red fir forest. After establishment, the tree acts as a nurse plant for less
sun tolerant secondary successional components, most often Abies magnifica (Rundel et
al, 1988). On the eastern slope of the Sierra, lodgepole begins to intergrade with the
upper elevation stands of *Pinus jefferyi*. As elevation increases stands become more open and dominated by Sierra lodgepole. It usually occurs in extensive even-aged stands (Rundel, et al, 1988), dominating ridgelines and open basins, or invading meadows or seep areas (Helms and Ratliff, 1987; Helms 1987). Successful establishment in these areas seems to be closely related to lack of precipitation (Helms, 1987), or decreasing microsite moisture (Helms and Ratliff, 1987). Elimination of grazing may enhance recruitment by decreased herbivory (Rundel, et al, 1988). Establishment also occurs in small patchy areas of fire killed stands, or avalanche chutes (Butler, 1979). Above these stands of relatively pure lodgepole, the species begin to intergrade with a more subalpine plant association, composed of *Tsuga, Pinus aristitada, Pinus flexilis,* and *Juniperus*. Krummholtz lodgepole are not uncommon in these areas. Towards the more southerly portions of the Sierra Nevada the lodgepole does not establish readily from seed.

**Southern California**

High elevation dry slopes and moist meadows between 1900 to 3300 m in elevation is typical of Sierra lodgepoles' habitat in southern California (Munz, 1974; Thorne, 1988). The drier slopes are xerophytic and vegetatively depauperate compared to similar subalpine forests in the Sierra Nevada (Thorne 1988). Moist meadows, which include mesic, northerly slopes and areas of perennial overland water-flow, form isolated islands and downslope trending stands of favorable habitat for the tree, and mirrors typical habitat found in the Sierra Nevada (Helm, 1987; Helm and Ratliff 1987). Pure stands of Sierra lodgepole do form, especially on northern slopes
(Minnich, 1976), but are limited by terrain and local environment. Areas of cold air drainage also allows the tree to persist in more xeric plant communities not otherwise hospitable to the species, such as Holcomb Valley and Onyx Summit (Minnich, personal communication; Everett, personal observation). Recruitment of juveniles throughout southern California during the late 1980's and early 1990's has been slow or non-existent, and may be due to persistent drought of the period (Everett, personal observation). Sierra lodgepole survive in timberline habitat, and form low mats of krummholz along with community cohorts of *Pinus flexilis* and *Juniperus* sp. Establishment of the species after fire is difficult and long; snags are persistent, and recruitment from adjoining stands is slow (Sheppard, 1984).

**Study Area**

**Montane Physiography and Climate.** The San Gabriel, San Bernardino, and San Jacinto transverse and peninsular mountain ranges of Southern California rise 3450 m above surrounding deserts situated to the north and east of the ranges, and coastal mediterranean lowlands to the south and west (Arno, 1984). Although the orogenies of these three mountain ranges are somewhat different, all consist of similar fault-blocked basement rock, and possess soils typical of these derivations. Sample sites in the San Gabriel mountains were combinations of Pelona Schist and Permian-Triassic granitic rock; both the San Bernardinos and San Jacinto ranges are mosaics of Precambrian igneous and metamorphic complexes (California Department of Mines and Geology, 1978). The summers are cool and dry, and winters cold, when most of the annual precipitation in excess of 30 inches falls in the form of snow (NOAA, 1992),
mimicking the climate of the Sierra Nevada. Many outliers of the Sierran Nevadian floristic provinces are found within these Southern California ranges (Axelrod, 1976) as portions of higher-elevation islands of alpine and sub-alpine vegetation.

Hypothesis


This study tests the null hypothesis that variations in the sampled anatomical, morphological, and chemotaxonomic traits of southern California populations of *Pinus contorta* var. *murrayana* are identical to these same traits over the subspecies entire range. This is tested by: (1) sampling and quantifying gross morphological data of the subspecies; (2) examining foliar characteristics; and (3) testing for the presence or absence of the subspecies-specific isoenzyme, ADH-2.
STUDY DESIGN, MATERIALS AND METHODOLOGY

Study Site Selection

Final stand locations, and subsequent individual trees were selected using the following procedures. (1) A literature review was conducted of locations and extent of Southern California lodgepole pine stands, according to Critchfield and Little (1966), Griffin and Critchfield (1972), Minnich (1976), and Barbour and Smith (1988); (2) U. S. Forest Service aerial photographs of 1:16500 scale were utilized to delineate stand location; (3) Timber inventory and sale information, also from the U.S. Forest Service, was consulted for stand locales; (4) On-site surveys of Sierra lodgepole stands were employed to delineate access, total stand size, percent composition of lodgepole in the stand (in excess of 50 percent of the standing trees), tree maturity, and timing of cone set. Initial estimates defined a possibility of as many as forty stands located in the study area; ten stands were selected as representative locations; (5) Preliminary reconnaissance was conducted of selected stands to evaluate final suitability of the ten stands for sampling. Figure 1 and Table 1 describe the locations and physiography of selected sample stands.

Field Collections and Measurements

Ten representative Pinus contorta var. murrayana individuals from each stand were selected for sampling. These individuals were full growth, full sunlight, forest overstory dominants or codominants that had achieved sexual maturity and possessed mature set cones. Standardized field data sheets were used to record individual tree data and stand information. The stand locations were first mapped by field
triangulation, and the location recorded by both standard Land Plat survey systems, and the Universal Transverse Mercator grid (Figure 1, Table 1). Site-specific physiographic variables were also recorded prior to departing the sampled tree or stand. Percent slope and aspect were read directly from a Suunto Azimuthal compass and clinometer. Elevation was determined from barometric altimeters or USGS 7.5' topographical quadratic maps, and recorded for both individuals and entire sites. Representative photographs of each stand are presented in Figures 2 through 11.
Figure 2.-- Representative stand photograph, Bluff Lake, San Bernardino National Forest, San Bernardino Mountains.
Figure 3.— Representative stand photograph, Butler Peak, San Bernardino National Forest, San Bernardino Mountains.
Figure 4.-- Representative stand photograph, Holcomb Valley, San Bernardino National Forest, San Bernardino Mountains.
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Figure 8.-- Representative stand photograph, Tahquitz Peak, San Bernardino National Forest, San Jacinto Mountains.
Figure 9.— Representative stand photograph, Wellman Cienaga, San Jacinto State Park, San Jacinto Mountains.
Figure 10.-- Representative stand photograph, Wildhorse Meadows, San Bernardino National Forest, San Bernardino Mountains.
Figure 11.-- Representative stand photograph, Wright Mountain, boundary of Angeles and San Bernardino National Forest, San Gabriel Mountains.
**Tree Sampling**

**Tree Age**

Individual tree age was sampled using an 18" (45.7 cm) long, 0.189" (0.48 cm) internal diameter Haglof Increment borer. The increment core was inspected for number of growth rings and the age recorded. The core was placed into a 5 mm internal diameter glass tube and sealed. The ten year incremental growth was taken from the first 2.54 cm of the bore sample (the outer 2.54 cm of xylem, exclusive of vascular cambium and phloem).

**Tree Morphometrics**

Tree height in feet was taken by establishing a 66 foot (one chain; 20.12 meters) baseline on a level line away from the tree, and measuring the angle from the base of the tree to the top of the tree. The measuring instrument, a Suunto topological and percent clinometer, automatically performed the trigonometric conversion to height employing a standard 66 foot (1 chain) baseline. All plant measurements were converted to SI units in the field. Crown class, crown ratio, and general crown condition were estimated from the end of the baseline, using U.S. Forest Service estimation technique for Regions 1 and 4 (Dilworth, 1982).

Tree diameter at breast height (DBH) was sampled 4.5 feet (1.37 m) from the base of each tree with a metric/standard DBH tape.

On-tree cone position and needle parameters were sampled according to methods in Critchfield (1957) and Wheeler (1985). Target collection goal was a minimum of 100 cones from each individual tree. Reflex angle of the cone, the angle
described by the cone in relation to the branch on which it developed, was recorded to
the nearest degree prior to removal from the tree. If this task proved difficult because
of inaccessible cone position, the measurement was accomplished after the branch was
cut away from the tree. Cones were removed and stored in paper sacks after reflex
angle measurement, height on the tree, and on-tree cone azimuth were documented.

Needles were removed from the tree for transportation to the laboratory for
further measurement. Foliage was sampled from the outer crown of the tree,
representing needle growth from the past 5 to 7 years, plus strobili and cone primordia.
A sub-sample of a minimum of 20 needles removed from the branch by their fascicles
and placed into a vial, and preserved in a formalin-formaldehyde-acetic acid solution.
Characteristic branch samples were pressed as vouchers, and the remainder of collected
foliage was stored frozen at 0° C for use during later resin canal measurements. All
voucher specimens are deposited in the Herbarium of the California State University
San Bernardino.

Each individual tree was tagged in the field in a nondestructive manner, first
placing biodegradable polyethylene flagging on the tree, and then permanently marked
with an embossed aluminum nursery tag containing a alphanumeric code identifying
the tree and stand. The tree was then photographed. A full tree-height photograph was
taken, normally of the southern exposure of each sampled tree. Additional exposures
were made in order to document unusual tree characteristics. Frame numbers from the
film rolls were recorded onto each tree's data sheets. Photographs have been used for
both documentation and identification of the sampled tree, assuming the individual
needs to be located again in the field.

**Laboratory Measurements**

Collected field samples were processed at laboratory facilities at California State University San Bernardino, and Rancho Santa Ana Botanical Gardens, in the following manner: Measurements of cone and foliar characteristics were made, an electrophoretic test for a known subspecies specific isoenzyme was performed, and the samples were stored for confirmational testing. Cones were removed from the field and stored at room temperature. Needles destined for laboratory work were placed in cold storage (+4°C) until needed for laboratory processing.

**Cones**

After air-drying for six months, the cones were weighed. The cones were separated by tree and measured in 3 axises: length along cone axis, width along a diameter bisecting the cones axis (width one), and a second width measurement at a diameter perpendicular to the first width measurement (width two). The cones were weighed individually at the time of dimensional measurement, and then weighed as a group (per tree).

**Isoenzyme Study**

The chemotaxonomic test, modified from Conkle et al, (1982), Soltis et al, (1986) and Reiseberg et al, (1988), was designed to test for the isoenzyme alcohol dehydrogenase (ADH), of which one isozyme, ADH-2, is subspecies-specific (Wheeler, 1983). Starch slab gel electrophoresis of the seed megagametophyte was used to detect the isoenzyme.
Seeds were removed from the cones, cleaned, and stored at -20°C. The seed was then prepared for removal of the megagametophyte for electrophoresis. Stratification involved: (1) taking the seeds out of cold storage; (-20°C); (2) washing them in a fungicide (Ortho Multi-purpose Daconil 2787); (3) breaking the seed coat with a 5 minute immersion in 30% hydrogen peroxide solution; and (4) placing them in 25 mm Petri dishes. Petri dishes were readied beforehand with sterile sand, overlayed with a layer of Whatman #4 filter paper, saturated with the fungicide solution, and prepared as a seedbed.

Germination was initiated by placing seed into the Petri dish, at ambient room temperature, and exposure to indirect daylight (an 8 hour light, 16 hour dark cycle). When the radicle of the seedling reached 2 to 7 mm in length after germination, the megagametophyte (n or 1n) tissue and root were removed from the seedcoat. The megagametophyte was then removed from the embryo (2n) tissue by gently prying the megagametophyte apart with a pair of sharpened #4 Dumont forceps at the contact margin between the megagametophyte and the embryo. The megagametophyte was pulled out and away from the embryo. The megagametophyte was placed into a 1.5 ml Bio-Rad microcentrifuge tube in 0.7 to 1.0 ml of distilled water and stored at 4°C until use in electrophoresis.

Electrophoresis. The measurements of alcohol dehydrogenase in the megagametophyte tissue was performed using a standard starch gel electrophoretic technique. Starch (Starch Art #w526) for the gel was prepared by boiling in gel buffer while swirling vigorously and vacuum degassing for approximately four minutes. The
starch was then poured into rectangular plexiglass molds and allowed to set for one hour. Wells for the megagametophyte tissues were cut lengthwise 3.2 cm from the long side. If not used immediately, the gel was refrigerated. The megagametophyte was removed from cold storage and placed into a grinding pestle block, and ground with 1 drop grinding compound and Tris-extract (ph 7) with a glass grinding rod. A filter paper wick (0.5 x 1.0 cm) was then soaked with this extract. The wick was moved to the well of the electrophoretic gel and placed into the well after blotting excess extract away from the wick, and making sure the wick extended to bottom of well. San Gorgonio megagametophyte tissues, used as reference tissue, were placed in the leftmost well as a standard. Gel trays were rinsed first with tapwater, then distilled water. Sponges used in the buffer tanks were cleaned and rinsed. The electrode buffer was poured into the tanks, within 3 cm of filling. Clean sponges were placed into the electrode tanks on both sides. The gel was placed onto the gel tray and covered with Saran wrap, leaving 2.5 to 3.0 cm of exposed gel on either side. The buffer-dampened sponges were drawn up from both electrode tanks and placed on top of the gel. A flat weight of safety glass is placed on top of the Saran wrap, preventing the gel from curling. Electrical systems are connected, and ran at 50 volts / 55 milliamps for an minimum of two hours. Power sources were monitored and adjusted to maintain 55 milliamps during a run. After the bands had stopped migrating, the gel was cut into thin stainable slices with a piano wire cutting jig. The gel was stained for ADH and placed in a warm dark incubator at 35° C for a minimum of 1 hour. After removal of the sliced and stained gel from the incubator, the gel was inspected and scored for
allelic banding on a light table. Each gel was photographed after scoring. Banding results, allele frequencies, and F statistics were then developed using Rancho Santa Ana Botanical Garden's Genetics and Populations software (POPGAP).

Needle Anatomical Study and Resin Canals

From a single branch subjectively drawn from each tree, five needles were selected for anatomical study. The selection included older needles (inside of branch) and newer needles (outside of branch). From each two-needle fascicle, a single needle was halved by a transectional slice perpendicular to the needle's length using a # 21 scalpel and a cork cutting surface. From the distal half of the needle, a thin cross section was prepared using the same slicing equipment, and mounted onto a 2.54 cm x 7.62 cm glass slide. This slide was inspected visually with an Mideo Systems 7x-40x dissection microscope for number of resin canals per needle. The cross section was then photographed via a 25" (68.5 cm) video display with a signal from a video camera coupled to the Mideo dissection scope, onto 100 ASA Kodachrome film using a Canon EOS 620 camera 70-210 mm zoom telephoto at f 4.5 automatic exposure, mated to a Canon Technical Back E. The proximal end of the needle was placed under the dissection microscope abaxial end up, over a 2.54 cm x 7.62 cm dimension millimeter grid, and the number of stomata per millimeter on the abaxial needle surface were counted and recorded.

Statistics

Descriptions of statistics and methodologies are described in Appendix 1. Individual tree data, stand data, local population data, and comparative species data
was subject to statistical analysis including descriptive statistics, nonparametric statistics, and principal component analysis using Supercalc 5, Microsoft Excel 4.0, MINITAB, SAS 6.11, SigmaPlot 1.0, and SigmaStat 2.02 software on accessible IBM compatible microprocessors in the California State University, San Bernardino Department of Biology, a personal 486-50mhz IBM compatible personal computer, or a OpenVMX AXP Operating System mainframe computer at University of California, Riverside.

The Mann-Whitney-Wilcoxon Rank Sum (Johnson, 1984; Neave and Worthington, 1988), was used to test the hypothesis that sampled stands in southern California are similar to against species traits from Sierra Nevada populations ($H_0$ = no difference). This was performed using MINITAB (Release 9.1 OpenVMS AXP, 1992) software available on the University of California, Riverside mainframe. This procedure used Sierran population data culled from Critchfield (1957, 1980), and Koch (1987). These comparative characteristics were originally collected in a manner identical to field collections for this work. The traits used were height, diameter at breast height, bark width, crown ratio, cone reflex angle, cone length, cone width, number of needles per fascicle, number of resin canals, needle width, and the number of stoma per mm$^2$.

Discriminant canonical analysis (Jackson, 1991; Joliffe, 1986) of 18 stand-by-stand tree and abiotic site variables was performed using SAS/STAT software in a Windows 3.1 environment. This allowed a method of attributing variations in the dataset to canonical variables developed from the dataset and placement of these
canonical variables into a multidimensional space. Further manipulation in SigmaPlot 1.0 allows three-dimensional representation of individual primary components developed from the stand-by-stand variables.
RESULTS

Tree Morphometrics

Morphometric analysis shows a broad amplitude of tree dimensional data (Table 2). The mean for stand height was $11.67 \pm 2.02$ m, ranging from a low of $8.08$ m at Pine Mountain, to a high of $14.3$ m at Holcomb Valley. Diameter breast height means vary from the population wide mean of $47.14 \pm 10.65$ cm, with a low of $32.23$ cm at Wellman Cienaga to largest average girth of $66.00$ cm at San Gorgonio. Bark width averaged $4.3 \pm 1.44$ mm, with thinnest bark width at Holcomb Valley ($3.2$ mm) and thickest at San Gorgonio ($6.0$ mm). The ten-year-radial-increment growth estimator, population mean $12.2 \pm 5.2$ mm, was lowest at Butler Peak ($2.3$ mm) and greatest at Bluff Lake ($17.7$ mm). Mean age has a moderately high variability throughout the population, the mean of $114.49 \pm 57.01$ years, bracketed by Holcomb Valley's relatively young stand ($70.1$ years) and Tahquitz Peak's aged stand ($231.0$ years). Within populations, crown class and crown ratios of all trees were similar, with mean crown class for all stands $1.32 \pm 0.16$ (lowest at both Holcomb Valley and Tahquitz Peak, 1.1; and highest at Butler Peak, 1.7), and mean crown ratios $7.99 \pm 0.59$. (Tahquitz Peak crowns were thinnest at 7.0, whereas Holcomb Valley had the highest average crown, 9.0).

Cone Morphometrics

Mean stand and population cone morphometric data are displayed in Table 3. Mean reflex angle of all cones from the entire population was $106.31 \pm 4.75^\circ$, with lowest mean stand reflex angles at Pine Mountain ($100.07^\circ$) and the greatest cone reflex.
angle at Bluff Lake (117.2°). Mean cone length was 43.18 ± 9.8 mm, with shortest mean cone length found at Holcomb Valley (18.44 mm) and longest mean cone length at Wildhorse Meadows (45.36 mm). Mean cone width was 30.17 ± 4.76 mm, with Holcomb Valley stand means the smallest (19.16 mm) and Wellman Cienaga possessing the greatest mean widths (38.35 mm). Population mean cone weight was 5.13 ± 1.3 g, with lightest stand means at Holcomb Valley (2.45 g), and heaviest mean cone weight at Wellman Cienaga (7.52 g).

**Needle Morphometrics**

Needle morphometric data included population and stand means for number of needles per fascicle (n=100), number of resin canals per needle (n=500), needle width (n=500), and number of stomata per square millimeter (n=500) (Table 4). The mean number of needles per fascicle varied little between stands, with a population mean of 2.01 ± 0.17; any differences are not significant. Low stand means of 2.0 needles per fascicle were found at Bluff Lake, Butler Peak, Holcomb Valley, Onyx Peak, Throop Peak, Tahquitz Peak, and Pine Mountain; the high stand means of 2.04 needles were located at San Gorgonio and Wellman Cienaga. Mean number of resin canals per needles did demonstrates greater variability, with a population mean of 1.75 ± 0.271, the low stand mean being 1.22 resin canals per needle at Bluff Lake, whereas the highest stand mean of 2.10 per needle was found at Wildhorse Meadows. Population mean needle width was 1.80 ± 0.11 mm; the stand low mean width was found at Onyx Peak (1.71 mm), and the stand high mean width was at Wellman Cienaga (1.98 mm). Stomata per millimeter² exhibited a population mean of 57.69 ±
4.8. In this case, the lowest density was at Wildhorse Meadows (50.9 per mm$^2$) and Holcomb Valley possessed the highest density (64.68 per mm$^2$).

**Isoenzyme Assay**

Alcohol dehydrogenase 2 (ADH-2), a proposed sub-species specific isoenzyme, appeared as a monomorphic band in all gels; ADH-1 and ADH-3 appeared as polymorphic bands. The inbreeding coefficient, "F", a test of the probability of homozygosity by descent, was developed using isoenzyme data from the one tested locus, Alcohol dehydrogenase (Table 5). Results indicate two stands, San Gorgonio ($F = +0.379$) and Pine Mountain ($F = +0.396$), are inbreeding (homozygous). All other stands possess negative "F" values, indicative of outcrossing (heterozygous) populations. Genetic Identity values were also developed (Table 6); most stands were related at or higher than 0.99, the lowest relation was between Tahquitz Peak and Butler Peak (0.795), and four locales were identical at these loci (Wildhorse Meadows, Throop Peak, Wellman Cienaga, and Onyx Peak).

**Nonparametric Statistics**

Comparison of the southern California Sierra Lodgepole stands with other stands from the Sierra Nevada is possible with the use of nonparametric statistics. Employing eleven variables (Table 7) from both Sierra Nevada populations and Southern California populations in a Mann-Whitney-Wilcoxon test , $T_L$ equalled 60, ($T_{critical} = 30$). Criteria for rejection requires $T_L$ must be less than or equal to $T_{critical}$. In this case, the null hypothesis is not rejected. A 'Z' test of the Mann-Whitney-Wilcoxon test statistic yields a Z score of -0.0328, ($Z_{critical} = -1.65$), in which case the
null hypothesis is not rejected. This indicates that the medians of the tested characteristics of these two populations are statistically similar, hence the Sierra and southern Californian populations are similar.

**Discriminant Canonical Analysis**

Discriminant canonical analysis of 18 variables from all ten stands seeks to describe variability within the dataset by assignment of the variables into principal components (Appendix 1). Results for the first three principal components are graphically displayed in Figure 12. The first principal component, likely to be elevation, explains 77.6% of variance within the dataset. Second and third principal components (likely aspect and slope) accounted for another 21% of the variance (98.43%). The remaining 1.67% is explained by the sum of all remaining tree variables.
Figure 12 – Three-dimensional plot, first three Principal Components, all stands, *Pinus contorta* var. *murrayana*, San Gabriel, San Bernardino, and San Jacinto ranges

- PCA Location, First v. Second v. Third Principal Components

- Tahquitz Peak
- Throop Peak
- Wright Mountain
- Onyx Peak
- Bluff Lake
- San Gorgonio
- Wildhorse Meadows
- Holcomb Valley
- Wellman Cienaga
- Butler Peak
- Elevation
- Slope
- Aspect
DISCUSSION

Gene Flow Between Populations

The movement of pollen, and therefore of genes, from one population to another can be considered gene flow (Slatkin, 1985a). This definition can be expanded to include the movement of both individuals, and entire populations (Slatkin, 1987). This definition can also be expanded to include the movement of extranuclear segments of DNA, and the creation or elimination of entire populations (Slatkin 1985a, 1987).

Knowledge of common methods of modelling and assessment of the effects of pollen gene flow on the genetics of populations in forest trees (especially temperate conifers) is applicable in discussing related phenomena in the Sierra lodgepole pine. These methodologies have implications for explanation of results of the between-stand gene flow indicated in the results of the F-tests, and the taxonomic similarities due to maintained gene flow between populations. Further, implications may impact on management and conservation strategies.

Gene Flow

Gene flow includes all the mechanisms resulting in the movement of genes from one population to another (Slatkin, 1985a). Migration of individuals and subsequent exchange of genetic information between populations is also considered gene flow. Within species genetic flow is felt to determine the variability of local genetic changes within populations (Slatkin 1985a, Ellstrand 1992). The effects of lack of genetic flow include inbreeding depression, and lowering of genetic variation; conversely, outbreeding depression and hybridization are phenomena occurring during periods of
high gene flow (Ellstrand, 1992). These consequences of gene flow are dependent
upon the size of the population, or the rates of gene flow. Higher gene flow should
increase effective population size, $N_e$ (Jain and Bradshaw, 1966). Gene flow may also
alter evolutionary phenomena in populations. Haldane (1930) noted that gene flow is
capable of countering various degrees of selection forces; selection, $s$, in a given
generation is neutralized by a rate of $m$ per generation of gene flow. Gene flow,
especially migration, may also be adequate to counter the effects of random genetic
drift within a population (Wright, 1931). As little as one migrant per generation is
sufficient to cause differentiation due to drift (Wright, 1948; Slatkin, 1987).
Accordingly, gene flow can perform a significant function in the genetic fate of
populations (Ellstrand, 1992) by the introduction and maintenance of genetic variability
within populations (Hamrick, 1987).

The magnitude of gene flow among natural populations has been the subject of
much discussion. Initial historic assumptions were that gene flow among populations
of a species is high, and helped maintain the both the genetic similarity of populations,
and the concept of "biological species" groups of organisms either capable of, or
actually interbreeding (Mayr, 1942). This belief has been contested by Ehrlich and
Raven (1969), noting that similarities shared by geographically isolated populations are
due primarily to natural selection, and that gene flow is limited or nonexistent in these
cases. Levin and Kerster (1974) estimated gene flow in most plant species as less than
one percent per generation within populations, and quote Bradshaw (1972) to the regard
that "...Effective population size in plants is to be measured in meters and not
kilometers." This indicates that the scale of gene flow, at least at the level of the individual, may be restrained to the point of at which it may be inconsequential compared to natural selection. However, the importance of gene flow has been re-estimated; Ellstrand (1992) has measured gene flow levels commonly exceeding one percent, and often as high as ten percent. In a wind-pollinated species such as the Sierra lodgepole pine, maintenance of genetic flow between isolated populations, as they are within the Transverse and Peninsular ranges of southern California, is essential in maintenance of species homogeneity, and might explain the overall lack of large-scale genetic and taxonomic variation seen in the sampled populations.

Estimation Of Gene Flow

Gene flow can be estimated two ways: direct estimation which depends on actual observations of moving individuals or gametes, or indirect estimates which employ allele frequencies which assess the amplitude of gene flow among separate populations. Because of the difficulty in establishing gene flow via pollen across the geographical barriers present in southern California, any inference of gene flow between Sierra lodgepole populations must be indirectly derived. The check for ADH-2 as a subspecies confirmation allows a method of indirect estimation of gene flow within the sampled populations of Sierran lodgepole.

Allele frequencies may be used in samples from differing populations to estimate gene flow within these groups (Slatkin 1985a) In natural populations, average gene flow can be estimated three ways (Slatkin and Barton, 1989). One method is Wright's $F_{ST}$, dividing the inbreeding coefficient within a population $F_r$, into subunits
due to nonrandom mating within local populations \( F_{is} \). Assuming two alleles per locus, and unlimited number of subpopulations, then:

\[
F_{st} = \frac{Var(p)}{\beta(1-p)}
\]

where \( \hat{p} \) is the average frequency of one of the two alleles in the population and \( Var(p) \) is the variance of allele frequencies within the subpopulations (Slatkin and Barton, 1989). After estimating \( F_{st} \), \( Nm \), the number of immigrants, can be estimated using the equation:

\[
Nm = \frac{1}{4} \left( \frac{1}{F_{st}} - 1 \right)
\]

Slatkin and Barton (1989) pose several questions regarding problems with estimating gene flow in this manner, including: 1) The possibility that \( F_{st} \) is altered when there are more than two alleles; 2) The potential for mutation and selection at this particular locus altering \( F_{is} \); 3) Can this \( F_{st} \) be equated with other fitness expectations in other populations? 4) How does geographical arrangement of sample locations alter expectations? 5) How long does a population need to allow \( F_{st} \) to approach equilibrium? and 6) What is the best way to estimate \( F_{st} \) from data?

A second method used to estimate \( Nm \) is that of maximum likelihood, where the distribution of the allele frequencies is a function of the rate of gene flow. Once a beta distribution is assumed, then a maximum likelihood estimate is constructed for the neighborhood size, \( e \) or \( Nm \). Presuming a beta distribution of two alleles, the average
frequency may be calculated as follows:

\[
\phi(p) = \frac{\Gamma(4Nm)}{\Gamma(4NmP)\Gamma(4Nmq)} p^{Nm_P - 1} q^{Nm(1-P) - 1}
\]

where \( q = 1 - p \). Adjusting the equation to allow for sampling error and sampling with replacement results in:

\[
L = \frac{2n!}{\hat{P}^j (1-\hat{P})^i} \frac{\Gamma(4Nm)}{\Gamma(4NmP)\Gamma(4Nmq)} p^{Nm_P - 1} q^{Nm(1-P) - 1}
\]

where \( j = 2n - i \), "j" being a recessive allele, and "i" a dominant. This method has the benefit of being able to calculate a range of values of gene flow, which can be used to test differing values of gene flow.

The third method of estimating gene flow is different than other methods based on \( F_{ST} \). The rare allele method of Slatkin (1985b) is based on the average number of alleles which are found in only one sample. Slatkin (1985b) related that the expected number of alleles to be found in the single sample is roughly linearly to \( \log_{10}(Nm) \):

\[
\log_{10}[\hat{p}(1)] = a \log_{10}(Nm) + b
\]

where both \( a \) and \( b \) are dependent on the number of individuals sampled from the population.

Both Slatkin (1985b), and Slatkin and Barton (1989) find the above equation useful for determining \( Nm \) from data garnered from electrophoresis; they both feel that \( Nm \) is only slightly associated with either mutation or selection acting upon a given locus.
Employed in this work is Wright's $F$, as an initial assessment of gene flow between the sampled Sierra lodgepole populations. The use of only one isoenzyme is rather limiting, and felt to be an introductory survey of gene flow within these populations. In these wind-pollinated populations, outcrossing is likely the norm; and it is unusual to see inbreeding indicated in the Wright Mountain and San Gorgonio populations. However, without more extensive electrophoretic testing such as in Wheeler and Guries (1985) or Epperson (1983), better estimation of gene flow, and solid confirmation of any out- or in-crossing within these populations, is difficult.

Potential Gene Flow

The deposition of pollen, seeds, or other units of dispersal is known as potential gene flow (Levin and Kerster, 1974). This can be contrasted to actual gene flow, which is the rate of actual fertilization or establishment of new individuals in a population as a function of distance from the original genetic source. The pollen of most plant species occurs by either wind-borne means (anemochory) or is vectored by animals (zoochory). Pollination by animals is more efficient than wind pollination (Faegri and van der Pijl, 1979; Regal, 1982). The number of pollen grains which need to be produced are generally lower, and probability of seed set higher, in animal pollinated plants. However, animal vector pollen and its effects on gene flow are not typical of many coniferous temperate forest species, but rather a provence of angiosperm tropical forest ecosystems (Aide, 1986; Hamrick and Murawski, 1991). Wind pollination is more typical of areas of low species diversity and diverse climates, and of temperate and boreal forests, prairies, and savannahs (Levin and Kerster, 1974).
Within the subalpine and alpine areas of southern California wind pollination is the normal case for all of the coniferous species found in these areas; given the general homogeneity of the Sierra lodgepole populations, this is also the likely mechanism for intra-population gene migration.

**Pollen Gene Flow**

Gene flow has been categorized by plant population geneticists as limited, idiosyncratic, or geographically extensive (Ellstrand 1992; Ellstrand and Elam, 1993). The most common view of gene flow is that of Levin and Kerster (1974), that it is limited. A median viewpoint is that gene flow is idiosyncratic (Ellstrand, 1992), and may vary from exceedingly low to extremely high, and varies temporally or among individuals and populations. Gene flow is considered to be very far-reaching by forest population geneticists (Muona, 1990). Current evaluations of gene flow by pollen support the median view (Ellstrand, 1992). Given the stand to stand distances involved in southern California, and the possibility of gene flow from Sierran populations, geographically extensive pollen gene flow is suggested here.

Generally, four different methods are utilized in gauging gene flow by pollen (Ellstrand, 1992): 1) pollen dispersal from point sources; 2) pollen dispersal from both point and non-point sources; 3) deducing gene flow from population genetic structure (the only method available for use in this work); and 4) paternity analysis of progeny in source and sink populations.

Pollen dispersal evaluates pollen flow by indirect measurement of pollinator foraging distances, of pollen analogues, or by direct measurement of highly
polymorphic or artificially marked pollen. Measurement of pollen dispersal involves
the monitoring of simulated populations fixed with a genetic marker, surrounded by
populations with an alternate gene. Flow is measured, as a function of distance from
the fixed allele population, by successful fertilization and fruiting of progeny collected
from the surrounding populations. The use of radio-isotopes in measuring
amenochrony cannot be employed because of the lack of control of the marker.

Deduction of gene flow from extant population genetic organizations can be
performed by a number of previously discussed methods. Estimation of $N_m$ or $F$
using private alleles (Slatkin and Barton, 1989), or other estimates of the number of
immigrants per generation using allelic frequency differences may be employed to
measure gene flow by pollen. Although employing only one allele, this was employed
using available ADH data in this study.

Paternity exclusion is noted as the portion of seeds within a population which
have drawn their parental genetic contribution from sources outside the population.
This information is developed by multilocus genotyping of all reproductive individuals
within a population; knowledge of the maternal gametophytic genotype, compared with
the genotype of its progeny allows determination of the paternal gametic contribution
(Ellstrand, 1992).

**Wind-mediated Gene Flow in Forest Trees**

Gene flow estimates, especially of $N_m$, from isoenzyme datasets have been
calculated for many species (Hamrick, 1987; Govindaraju 1988a, 1989; and Ellstrand
1992). These gene flow estimates deviate substantially among plant species from very
low (much less than 0.1 $Nm$) to considerably higher ($Nm$ in excess of 10 immigrants per generation).

In general, wind pollinated trees demonstrate higher levels of pollen-mediated gene flow than trees with other modes of pollination. (Levin and Kerster, 1974; Farris and Mitton, 1984; Govindaraju, 1988a, 1989). With the levels of outcrossing suggested by the ADH data, this is suggested to be a likely model of what occurs within the southern California Sierran lodgepole. Most of the estimates have been obtained by allelic frequency methods. Sample sizes, and population sizes and densities have differed among studies, so results may be difficult to compare, but in general the estimates for temperate forest trees, especially other conifers, have been high. For example, Govindaraju (1989) estimated, by reviews of other workers employing using private alleles and outcrossing rates, gene flow in 14 species of forest trees. Wind-pollinated species, including a number of species of pines and oaks, had high levels of gene flow, with Black Pine ($Pinus nigra$) having an $Nm$ as high as 21.83. Data from $Pinus ponderosa$ (Farris and Mitton, 1984) and $Pinus flexilis$ (Schuster, et al, 1989) also indicate that these wind-pollinated species have high levels of gene flow.

These estimates may also prove low; Govindaraju, (1988b) notes that techniques using private alleles and outcrossing rates to evaluate gene flow are possible underestimates, and may not represent actual gene flow levels in natural populations. An interesting exception to this high $Nm$ is that of the Torrey pine (Ledig and Conkle, 1983), which was calculated to have little or no genetic flow. In most cases, as is postulated here with the Sierra lodgepole, the levels of gene flow are high enough to
overcome genetic drift. Generally, there is little differentiation between populations of forest trees due to high levels of gene flow, routinely via pollen dispersal (Muona, 1990). Although genetic diversity is also high in tropical forest trees and temperate angiosperms, this is likely due to animal vectors, and not wind pollination (Hamrick and Murawski, 1991). With statistically significant morphological similarities within the southern California populations, long-distance pollen dispersal is likely as the causitive agent in maintaining the populations resemblance to each other.

Comparative intraspecific studies of wind-pollinated species indicate that although gene flow is generally high, many factors can lead to considerable variation in gene flow rates. Differing phenologies associated with an elevation gradient apparently have restricted gene flow in *Pinus flexilis* in Colorado (Schuster, et al, 1989). Ten polymorphic loci were assessed for allelic frequency differences between populations and used to calculate $N_m$. Interpopulation gene flow was found to be restricted; elevation differences greater than 400 m generally did not have synchronous pollen release times, and eight of ten loci had significant differences in gene frequencies. However, $N_m$ was estimated to be as high as 11.1 individuals per generation between upper and lower tree line populations. This was felt to be an example of stepping-stone gene flow between transitional populations, augmented by gene flow from avian seed transport (Schuster, et al, 1989).

Other variations in gene flow may be due to differences in population densities. During periods of pollen release, individuals in populations with high densities will be exposed to pollen clouds with comparably minor amounts of their own pollen,
preventing selfing (Farris and Mitton, 1984). Use of maximum likelihood data drawn from allelic frequency work for Ponderosa pine in Farris and Mitton (1984) indicates a outcrossing rate of $t = 0.81$ in low density stands, and outcrossing rates of 0.96 for normal density stands. Outcrossing rates in some of the other North American conifer species have been estimated to be 0.98 in Douglas fir (Neale and Adams, 1985) and 0.99 in Pinus contorta; (Epperson and Allard, 1984). Rates of outcrossing in this work did not reach such high rates. Assessment of $N_m$ using only one allele is likely to yield a biased or inaccurate estimation, unlike works which employ higher numbers of polymorphic loci.

Distribution of sub-populations and geographic isolation may also be important in bringing about variation in gene flow. Govindaraju (1989) indicates that single populations, or sets of populations, may have distinctively different gene flow levels when compared to other populations within a species range; each of these species may be bound by a stepping-stone system of gene flow. It is likely that one, or few, populations within a species range may be influencing total amounts of gene flow throughout the species range. In Pinus torreyana; sub-populations are isolated enough to curtail gene flow altogether (Ledig and Conkle, 1983). Although likely isolated from the Sierra Nevada populations since the end of the Pleistocene, initial indications from this work is that, in the possible 120 generations since the beginning of the Holocene significant isolation has not occurred. This is due to having either a significantly large population that maintains an equilibrium of genetic diversity, or, there is genetic flow from the Sierra Nevada populations.
Homozygosity, Inbreeding and the Sierra Lodgepole Pine

Three lines of evidence have been used in testing the hypothesis of similarities within southern California Sierra lodgepole: 1) classical morphological data; 2) specific needle tissue histology; and 3) isoenzyme work. The isoenzyme data requires comparison with data from both the Sierra Nevada populations, and data from other subspecies.

This isoenzyme work tested for a subspecies-specific band of alcohol dehydrdogenase (ADH, E.C. # 1.1.1.1), ADH-2, as noted in Wheeler (1981), and Wheeler, et al (1983). ADH-2 has a high degree of variation, half of which occurs among subspecies (like Sierra lodgepole): "ADH-2 is unique in ssp. murrayana by the presence of a relatively rare allele occurring here in high frequencies. (Wheeler, 1983)"

ADH-2 bands were present in collected samples. Banding results were also scored and analyzed for the "F" statistic: the inbreeding coefficient. Two stands of trees indicated a high degree of homozygosity possibly indicating inbreeding among those subpopulations (Pine Mountain, in the San Gabriel Mountains possessed F=0.396, and San Gorgonio, in the San Bernardino Mountains, F=0.379).

The possibility of the existence of null alleles, which would not produce the stainable bands sought during electrophoresis, could have altered the overall scoring of ADH banding for these two tree stands, or any other stand in the population. This would have been achieved by not indicating polymorphism (heterozygosity) at the investigated loci, lowering the number of bands, so increasing the number of perceived homozygotes. There are several works, including Chan and Burton (1992) which
review the possible advantages to plants for heterozygosity in the ADH loci. Barnett and Naylor (1969) found alcohol dehydrogenase activity and ethanol utilization in germinating longleaf and slash pine seeds. ADH activity increases in pine embryos prior to germination, peaks at germination, then slowly decreases in seedlings. In the megagametophyte, the tissue used in this particular isoenzyme study, ADH activity remains constant until the deterioration of the megagametophyte during germination, where activity increases. Feret and Bergmann (1976) note that alcohol dehydrogenase is apparently a dimer controlled by a single locus with co-dominant alleles in most plant systems. These ADH systems also form heterozygote enzymes, and use of megagametophyte tissue has shown ADH characteristics are heritable. Dehydrogenase is alterable during different parts of the plants growth, therefore differing electrophoretic studies using the same plant materials may also develop contradictory results. Kinlaw et al. (1990) found three ADH cDNAs in Pinus radiata. Two of the three cDNAs correspond to one ADH locus, and a third cDNA belongs to a second ADH locus. Although it is acknowledged that understanding of ADH regulation in conifers is minimal, it at least appears that it is similar to angiosperm regulation (used by seeds, seedlings, and flooded plants). Restriction fragment polymorphism implied a complex of ADH genes that may be larger or more numerous than angiosperms. Incidental statements of note in Kinlaw et al. (1990) regarding ADH use during flooding is of interest: 1) citing Hook (personal communication) and noting that ADH is an important component in flooding tolerance; and 2) ADH has a role in anaerobic stress responses (root flooding). These responses may be of metabolic importance in
seedling establishment of Sierra lodgepole in the moist environments it is found at in southern California. In Newman and Van Toai (1991), the ADH genetic system is reviewed, and ADH's contributions to stress responses noted. ADH production is induced during flooding in seeds and seedling of many plant species (conifers are notably absent in this list), and induction of ADH varies with seedling age and organ development. Newman and Van Toai (1991) also provide confirmational notation of ADH as a three isoenzyme system, and a discussion of homodimer and heterodimer formation within this system: ADH1-ADH1 and ADH2-ADH2 homodimers, and an ADH1-ADH2 heterodimer, and thus the possible source of intrinsic error within the isoenzyme staining system. The loci are thought to reside on two different chromosomes, at least in angiosperms. Knowles and Grant (1985) found that one of the polymorphic enzymes used in Rocky Mountain lodgepole (var. contorta) was ADH, which consisted of a three allele system. Isoenzyme variations were inherited as single-gene traits, and spatial heterogeneity contributed more to variations in genetic composition than did temporal (age) heterogeneity.

Null alleles may mask genetic differences between populations to workers unable to stain for the allele, or unwilling to perform inheritance testing called for in Allendorf et al, (1982). In this work, the possibility of the existence of null alleles poses questions regarding the accuracy of gel interpretation. Allendorf et al (1982) found Ponderosa pines and Red pines have mean frequencies of null alleles of 0.31 % and 0.28% (at 29 and 27 loci, respectively); null alleles seem to be rare. Not only are they rare, but they are maintained by selection-mutation balance. However, ponderosa
pines have large amount of polygenic and isoenzyme variations compared to red pines, and that, combined with similar amounts of null alleles, indicate null alleles are not really contributing to polygenic variation. Significantly, Allendorf et al, (1982) includes a description of null alleles and the effects on protein polymorphism: a "null/active" heterozygote is indistinguishable from a homozygous "active" allele, thus very hard to detect. Null phenotypes can only be detected in large samples or when the null allele is in a high frequency.

Although conifers and, most notably, lodgepole pine, are extremely well studied regarding genetic systems, gene flow, management, and utilization, there is a paucity of work dealing with null alleles in the systems, and possible consequences of these subtly present alleles in the pine's genetic make-up. There seems to be an advantage for plants to have ADH available in quantities besides the needed amounts for basic biochemical and physiological functions, especially during seed germination, and in flooded root conditions that may give rise to anaerobic conditions. In employing ADH as an genetic characteristic in isoenzyme studies, care must be taken to realize that null alleles may be present in small, but notable amounts, and increasing sampling errors in smaller sampling schemes. The same heterozygosity that may be extant in the ADH system, may, in some cases, be also allowing some selective advantages with growth, cone production, or establishment in microhabitat.

In the specific case of *Pinus contorta* var. *murrayana* (Critchfield), Sierra lodgepole, the possibility of the null allele may indicate three possibilities: 1) the inbreeding populations noted in these isoenzyme measurements may not indicate
inbreeding at all, if there are null, non-protein-expressive genes in the populations; 2) increased heterozygosity of ADH, and expression of it during germination, may allow increased survival in preferred habitats in the higher elevation southern California areas: wet meadows and soggy, snow-packed northern exposures, which have a high degree of soil saturation or flooding during peak germination times of the Sierra lodgepole; and 3) if there is undetected heterozygosity in ADH or in other loci, there may be an undetected advantage to these populations as far as growth, fecundity, and fitness.

Fire Ecology and Sierra Lodgepole

Although the Rocky Mounain lodgepole is one of the best studied species of conifer in North America because of its high commercial importance, the Sierra lodgepole and its ecology has been relatively neglected. Rundel et al, (1988, page 587) note: "No fire studies of lodgepole pine [ecology] and other subalpine forests in the Sierra Nevada have been made."

Wheeler, (1985), Arno (1980), and Critchfield (1957) note the differences in fire regime in the species: the Rocky Mountain variety is keyed into a fire-climax situation: many stands are perpetuated by repeated large scale fire events, and lodgepole finds it difficult to establish anywhere without the effects of fire on the usually serotinous cones. The Sierra Nevada variety of lodgepole lacks a dependably serotinous cone, and many of the collected cones for this work showed little or no serotiny: and stand stocking never quite gets to the dog-hair thickness of the Rocky Mountain variety. This lowers overall fuel loads and dependence on fire regimes for
the disturbance needed for perpetuation of stand dynamics. Sheppard (1984) notes the contributions of low stand densities and rocky soils to lower fire frequency. All of the sampled stands typified low stand stocking numbers per hectare, scattered among rocks of drier soils, and rarely showed damage by fire. The long term influences of fire in these ecosystems may ultimately influence the distribution of the variety.

However, fire is an integral element of the Sierran lodgepole forest: Kilgore (1972) notes the importance of fire in regards to vegetation associations in the Sierran forest, and how it relates to at least four functions of the community (Rundel, 1988): 1) maintenance of fire-dependent community components; 2) perpetuation of non-equilibrium uneven-aged stands; 3) nutrient cycling; and 4) a natural prophylactic treatment of forest pathogens and insects. Sheppard (1984) notes that within the San Jacinto area, fires are a frequent visitor: fires caused by lightning may have burned areas smaller than 0.4 ha every one to two years, did not spread, and did not burn hot enough to kill the thinned-bark lodgepole: hence an almost stable fire-perpetuated equilibrium. Recruitment into new areas would have to be the result of patchy dynamics (Rundel et al, 1988), after very long-term fuel load accumulation, or during a rare high-intensity fire event. What may happen if this factor is eliminated from the system?

Rundel (1988) has noted that in Vankat (1970) states the Sierra Nevada lodgepole densities and stand cover have increased during the last fifty years. This may be due to the suppression of a natural fire regime - it is postulated that the increased fuel loads may lead to highly cataclysmic fires on a landscape scale. With successful
elimination of a natural fire regime in southern California forests, this is a likely possibility with some of the purer, over-mature stands in this area. Another possibility is the establishment of Red fir as an seral understory component, and ultimately competition. In southern California, White fir, Sugar pine, Limber pine or another mixed conifer community component displaces lodgepole from its niche if fire is eliminated. This may be an exception in small microsites where lodgepole is the only successful pioneer, as in Bluff Lake, or where lodgepole can escape fire, as in the low density stands of Tahquitz Peak.

**Implications for Management and Conservation**

Extensive gene flow in forest trees may be a source of problems in forest management and silviculture. Forestry has the generalized mission of maintenance and perpetuation of the diversity and genetic plasticity of natural populations, while seeking to make rapid and applicable gains in genetic improvement of desirable stock. Depending on the long-term goals of management personnel, gene flow can act as a stabilizing influence on larger natural populations, or as a source of interference in the small-scale effects typical of tree breeding programs which attempt to select for and perpetuate desirable characteristics.

A drawback affecting forest trees in locales with very high immigration and gene flow, especially at some of the higher $Nm$ levels noted ($>10$), is the possibility of outbreeding depression (Ellstrand, 1992; Ellstrand and Elam, 1993). In instances of decreasing population size, such as those subjected to intensive anthropic disturbances (harvesting or slash and burn agronomics), or natural disturbances (fire, disease), the
risk of outbreeding depression increases if the number of immigrants from sympatric populations remains constant.

Generally, inbreeding depression is not a problem in wind-pollinated species except in cases of small population size, low population densities, or with angiosperm species capable of high-selfing rates (Muona, 1990). Selfing does occur in wind-pollinated trees, but may be due to mating between close relatives or family groups (Shaw and Allard 1982 in: Muona, 1990), however, chance inbreedings usually do not survive beyond the seedling stage (Farris and Mitton, 1984; Neal and Adams, 1985). Although incrossing is indicated in two stands, San Gorgonio and Pine Mountain, this data is suspect in light of having only one isoenzyme tested, rather than a suite, and the aggressively wind-pollinated nature of Sierra lodgepole.

Seed orchards (defined here as areas of intensive artificial selection for desirable timber characteristics) are often inundated with pollen from nearby breeding populations, or are not isolated from genetic conservation sites (Muona, 1990). Contamination of desirable seed stock, either in situ or located within seed orchards, by either intraspecific or interspecific gene flow from adjoining congeners is an ongoing problem in selective tree breeding programs (Ching, 1982; Ellstrand and Elam, 1993). Progeny resulting from crosses between extant populations and transported nursery stock can result in offspring that are poorly adapted for the provence for which they are intended (Muona, 1990). With the introduction of Sierra Nevada provenance lodgepole seedlings onto sites on the Big Bear District of the San Bernardino National Forest, both poor survival of non-acclimated seedlings, and future contamination of local
populations by these imports is possible.

Levels of differentiation between managed and unmanaged populations will vary widely depending on species and growth conditions. Genetic diversity and continued natural selection will be required as protection against abiotic and biotic stresses, as well as anthropogenically generated stress. Complete comprehension of the variability of breeding systems and genetic of forest trees, and the selection forces which have created these populations, is needed to manage natural populations and design effective programs for breeding of selected stock. This knowledge of the modality of gene flow in these organisms forms baseline information also needed for genetic conservation.
CONCLUSIONS

Sierra lodgepole pine (*Pinus contorta* var. *murrayana* Critchfield [Hickman, 1993]) is a phenotypically plastic conspecific of the widespread Rocky Mountain lodgepole: the variety is widely established in the montane areas of California, and within the higher elevation conifer and subalpine plant communities of the area. Similarities in leaf morphology, cone morphology, and the presence of a sub-species specific isoenzyme confirm the varieties *murrayana* identity with the Sierra populations. Notwithstanding likely geographic isolation from Sierra Nevada populations since the Pleistocene, significant genetic isolation of the southern California populations has not occurred. This is likely due to having either a significantly large population that maintains an equilibrium of genetic diversity, or, there is some likelihood of genetic flow from the Sierra Nevada populations. Long distance wind pollination, or a large population size, may have aided in maintaining genetic diversity since the spread of the species from Pleistocene refugia. Null alleles, or alterations of ADH during germination in specialized environs may affect estimates of genetic identity and outcrossing using this allele. Although outcrossing is indicated in all but two of ten sampled populations, affirmation of gene flow between populations can only be inferred, and not supported, using this data.

Although not as closely tied to fire as part of its successional regime, Sierra lodgepole still apparently needs this disturbance in order to maintain itself within the plant communities. Non-obligatory cone serotiny, differing fire regimes, and the perpetuation of a equilibrium oriented community are strategies evolved by this variety
of lodgepole to exist in California habitats.

**Suggestions for Further Study**

In order to strengthen this work, gross plant morphology would need to be downplayed in favor of the increasingly quantitative methodologies available for identification of components of the differing portions of the plants genome. Further work would include much more rigorous isoenzyme study, and would include all feasibly stainable isoenzymes, allowing more accurate and enhanced estimates of gene flow, and in- or out-crossing. Chloroplast DNA work could easily enable identification of either parental or maternal lineage depending on the approach of the investigation. If included in a range-wide work, cDNA could aid in either supporting or denying current biogeographical thought regarding migration of the species in a post-Pleistocene environment. Another method of investigating stand-by-stand relationships of the trees includes chemotaxonomic identification of foliar terpenes.

Although it may seem that there are more interesting and rewarding facets of lodgepole genetics, history and biochemistry, other than null alleles, their existence indicates the need for a more encompassing work. Developing a way to accurately and empirically record alleles that remain undetected within a given electrophoretic system is frustrating and unexciting to all but the most dedicated worker. However, a review of the literature, however obliquely related to Sierra lodgepole, that the existence of null alleles, their contribution to heterozygosity and their further effects on survivability and fitness, may be an important future step to take in understanding the mating systems and complex genetics of the Sierra lodgepole.
Although difficult to investigate at the landscape level needed, the study of mechanisms of gene flow over longer distances, and its success or failure to continue gene flow in these isolated populations would also help determine both critical population breeding sizes, and rates of genetic turnover within the species. Sierra lodgepole is not immediately threatened in manners which would elicit the concerned responses of conservation special interest groups, but the continued encroachment of the edifices of civilization makes the understanding of maintenance of its genetic equilibrium and flow an important future concern for both management and perpetuation of this variety of tree.
Tables
## Table 1: Geographical information by stand locale

<table>
<thead>
<tr>
<th>Stand Names</th>
<th>Elevation m</th>
<th>Aspect</th>
<th>Percent</th>
<th>UTM Coordinate</th>
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<tbody>
<tr>
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<td>2313</td>
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<td>0.0</td>
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<tr>
<td>Butler Peak</td>
<td>2599</td>
<td>340°</td>
<td>31.0</td>
<td>3790500 m N, 499050 m E</td>
</tr>
<tr>
<td>Holcomb Valley</td>
<td>2194</td>
<td>064°</td>
<td>2.6</td>
<td>3796100 m N, 506000 m E</td>
</tr>
<tr>
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<td>2730</td>
<td>270°</td>
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<td>3787250 m N, 527000 m E</td>
</tr>
<tr>
<td>San Gorgonio</td>
<td>2783</td>
<td>095°</td>
<td>17.3</td>
<td>3775500 m N, 516000 m E</td>
</tr>
<tr>
<td>Throop Peak</td>
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<td>135°</td>
<td>18.5</td>
<td>3802000 m N, 426000 m E</td>
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<tr>
<td>Tahquitz Peak</td>
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<td>58.4</td>
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<tr>
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<td>3783000 m N, 521400 m E</td>
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<td>Pine (Wright) Mountain</td>
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<td>239°</td>
<td>29.8</td>
<td>3797000 m N, 441100 m E</td>
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</tbody>
</table>

*000°: Flat terrain

*Universal Transverse Mercator, zone 11, 1927 North America Datum
Table 2: Tree morphometric means by stand.

<table>
<thead>
<tr>
<th>Stand Names</th>
<th>Height m</th>
<th>DBH cm*</th>
<th>Bark Width cm</th>
<th>10 YRb Age</th>
<th>Crown Class</th>
<th>Crown Ratio</th>
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<td>17.7</td>
<td>69.0</td>
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<td>3.8</td>
<td>2.3</td>
<td>80.3</td>
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<tr>
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<td>3.2</td>
<td>15.2</td>
<td>70.1</td>
<td>1.1</td>
</tr>
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<td>38.47</td>
<td>4.8</td>
<td>8.6</td>
<td>107.8</td>
<td>1.4</td>
</tr>
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<td>126.3</td>
<td>1.3</td>
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<td>4.1</td>
<td>8.8</td>
<td>231.0</td>
<td>1.1</td>
</tr>
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<td>15.8</td>
<td>56.6</td>
<td>1.3</td>
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<tr>
<td>Wildhorse Meadows</td>
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<td>5.6</td>
<td>16.7</td>
<td>85.1</td>
<td>1.4</td>
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<td>Pine Mountain</td>
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<td>53.42</td>
<td>3.8</td>
<td>8.0</td>
<td>191.2</td>
<td>1.3</td>
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<td>4.3</td>
<td>12.2</td>
<td>114.4</td>
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</table>

* DBH: Diameter Breast Height
Table 3: Cone morphometric means by stand.

<table>
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<tr>
<th>Stand Name</th>
<th>Cone Reflex Angle</th>
<th>Mean Aspect Cone</th>
<th>Cone Length mm</th>
<th>Width 1 mm(^a)</th>
<th>Width 2 mm(^b)</th>
<th>Mean Width</th>
<th>Mean Weight gm</th>
</tr>
</thead>
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<tr>
<td>Bluff Lake</td>
<td>117.2</td>
<td>230.07</td>
<td>40.48</td>
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<td>31.36</td>
<td>31.44</td>
<td>5.34</td>
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<td>Butler Peak</td>
<td>107.31</td>
<td>166.74</td>
<td>41.59</td>
<td>29.64</td>
<td>29.68</td>
<td>29.66</td>
<td>5.09</td>
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<tr>
<td>Holcomb Valley</td>
<td>102.75</td>
<td>180.36</td>
<td>29.48</td>
<td>18.44</td>
<td>19.87</td>
<td>19.16</td>
<td>2.45</td>
</tr>
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<td>110.45</td>
<td>151.47</td>
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<td>32.23</td>
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<td>105.69</td>
<td>201.67</td>
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<td>30.16</td>
<td>30.13</td>
<td>5.18</td>
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<tr>
<td>Throop Peak</td>
<td>107.05</td>
<td>186.96</td>
<td>42.34</td>
<td>27.03</td>
<td>29.18</td>
<td>28.11</td>
<td>4.89</td>
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<td>Tahquitz Peak</td>
<td>103.75</td>
<td>188.56</td>
<td>39.43</td>
<td>30.49</td>
<td>30.60</td>
<td>30.55</td>
<td>4.62</td>
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<tr>
<td>Wellman Cienaga</td>
<td>102.43</td>
<td>174.21</td>
<td>68.40</td>
<td>38.30</td>
<td>38.40</td>
<td>38.35</td>
<td>7.52</td>
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<tr>
<td>Wildhorse Meadows</td>
<td>106.47</td>
<td>182.39</td>
<td>45.36</td>
<td>32.69</td>
<td>32.16</td>
<td>32.43</td>
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<tr>
<td>Pine Mountain</td>
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<td>163.60</td>
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<td>29.52</td>
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<td>182.6</td>
<td>43.18</td>
<td>30.02</td>
<td>30.30</td>
<td>27.47</td>
<td>5.13</td>
</tr>
</tbody>
</table>

\(^a\) Mean first width measurement  
\(^b\) Mean second width measurement
Table 4: Needle morphometric means by stand.

<table>
<thead>
<tr>
<th>Stand Name</th>
<th>Mean Number of Fascicles</th>
<th>Mean Number Resin Canals</th>
<th>Needle Width (mm)</th>
<th>Number of Stomata (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bluff Lake</td>
<td>2.00</td>
<td>1.22</td>
<td>1.86</td>
<td>52.58</td>
</tr>
<tr>
<td>Butler Peak</td>
<td>2.00</td>
<td>1.8</td>
<td>1.81</td>
<td>63.64</td>
</tr>
<tr>
<td>Holcomb Valley</td>
<td>2.00</td>
<td>1.34</td>
<td>1.58</td>
<td>64.68</td>
</tr>
<tr>
<td>Onyx Peak</td>
<td>2.00</td>
<td>1.78</td>
<td>1.71</td>
<td>52.46</td>
</tr>
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<td>1.8</td>
<td>1.87</td>
<td>60.78</td>
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<tr>
<td>Throop Peak</td>
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<td>1.88</td>
<td>1.83</td>
<td>58.56</td>
</tr>
<tr>
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<td>1.82</td>
<td>56.18</td>
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<td>2.1</td>
<td>1.79</td>
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<td>1.96</td>
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<td>1.75</td>
<td>1.80</td>
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</table>
Table 5: Southern California transverse and peninsular Ranges Sierra lodgepole stand inbreeding coefficients 'F*', all ADH loci.

<table>
<thead>
<tr>
<th>Stand Name</th>
<th>n</th>
<th>Expected</th>
<th>Observed</th>
<th>Stand &quot;F&quot;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bluff Lake</td>
<td>10</td>
<td>0.330</td>
<td>0.556</td>
<td>-0.685</td>
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<tr>
<td>Butler Peak</td>
<td>10</td>
<td>0.308</td>
<td>0.370</td>
<td>-0.201</td>
</tr>
<tr>
<td>Holcomb Valley</td>
<td>5</td>
<td>0.140</td>
<td>0.200</td>
<td>-0.429</td>
</tr>
<tr>
<td>Onyx Peak</td>
<td>3</td>
<td>0.167</td>
<td>0.333</td>
<td>-0.994</td>
</tr>
<tr>
<td>San Gorgonio</td>
<td>9</td>
<td>0.298</td>
<td>0.185</td>
<td>+0.379</td>
</tr>
<tr>
<td>Throop Peak</td>
<td>9</td>
<td>0.158</td>
<td>0.185</td>
<td>-0.171</td>
</tr>
<tr>
<td>Tahquitz Peak</td>
<td>6</td>
<td>0.162</td>
<td>0.278</td>
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<td>1</td>
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<td>Wildhorse Meadows</td>
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<tr>
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<td>8</td>
<td>0.174</td>
<td>0.125</td>
<td>+0.3961</td>
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</tbody>
</table>

* Wrights 'F': 1-(observed heterozygosity/expected heterozygosity)
Table 6: Nei's Genetic Identity values, all stands.

<table>
<thead>
<tr>
<th></th>
<th>Bluff Lake</th>
<th>Butler Peak</th>
<th>Holcomb Valley</th>
<th>Onyx Peak</th>
<th>San Gorgonio</th>
<th>Throop Peak</th>
<th>Tahquitz Peak</th>
<th>Wellman Cienaga</th>
<th>Wildhorse Meadows</th>
<th>Pine Mountain</th>
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<tbody>
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<td>Bluff Lake</td>
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</tr>
<tr>
<td>Pine Mountain</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### Table 7: Mann-Whitney-Wilcoxon test, mean population traits, southern California transverse and peninsular ranges versus Sierra Nevada Sierra lodgepole populations

<table>
<thead>
<tr>
<th>Trait</th>
<th>Southern California</th>
<th>Sierra Nevada</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height</td>
<td>11.675</td>
<td>13.26</td>
</tr>
<tr>
<td>Diameter Breast Height</td>
<td>47.140</td>
<td>50.60</td>
</tr>
<tr>
<td>Bark Width, mm</td>
<td>4.340</td>
<td>3.56</td>
</tr>
<tr>
<td>Crown Ratio</td>
<td>0.680</td>
<td>0.58</td>
</tr>
<tr>
<td>Cone Reflex Angle</td>
<td>106.340</td>
<td>99.00</td>
</tr>
<tr>
<td>Cone Length</td>
<td>43.120</td>
<td>45.00</td>
</tr>
<tr>
<td>Cone width</td>
<td>30.165</td>
<td>31.00</td>
</tr>
<tr>
<td>Needles per fascicle</td>
<td>2.030</td>
<td>2.00</td>
</tr>
<tr>
<td>Number of Resin Canals per Needle</td>
<td>1.746</td>
<td>1.95</td>
</tr>
<tr>
<td>Needle Width</td>
<td>1.804</td>
<td>1.93</td>
</tr>
<tr>
<td>Stomata per mm²</td>
<td>57.720</td>
<td>39.00</td>
</tr>
</tbody>
</table>

Mann-Whitney Confidence Interval and Test

- **C1** N = 11 Median = 11.68
- **C2** N = 11 Median = 13.26

**Point estimate for ETA1-ETA2 is 0.03**

95.1 pct c.i. for ETA1-ETA2 is (-28.96, 28.21)

T = 127.0

Test of null hypothesis is significant at 1.0000

Cannot reject at alpha = 0.05

- **C2** N = 11 Median = 13.26
- **C1** N = 11 Median = 11.68

**Point estimate for ETA1-ETA2 is -0.03**

95.1 pct c.i. for ETA1-ETA2 is (-28.21, 28.96)
Table 7: continued

\[ T = 126.0 \]

Test of null hypothesis is significant at 1.0000

Cannot reject at alpha = 0.05

using:

\[
\left( n_1 \right) \left( n_2 \right) \frac{(n_1)(n_1 + 1)}{2} - W_1 = T
\]

for both tails of the test, then range for \( T, T_L \) to \( T_H \), is 60, and 61. \( T_{\text{critical}} \) is ≤ 30, do not reject.

z-Score Test, employing \( T \) (Johnson, 1984): z-score statistic:

\[
\frac{T_L - \mu_T}{\sigma_T}
\]

hence:

\[
\frac{60 - 60.5}{15.22} = -0.0328
\]

\( z_{\text{critical}} = -1.65 \), do not reject.
Appendix
Appendix 1: Statistical Methodology

Quantification of alterations in allelic frequencies within populations of the same trees are influential tools for students determined to study the changes and the factors causing these changes. Comparison of mean allele frequencies from small sample populations and this statistic's incorporation into Wright's "F" statistic (the Inbreeding Coefficient) are one method commonly used to evaluate gene flow in between communities. However, use of mean allele frequencies from small samples can be misleading because of the radical changes that occur in some small sample statistics without a normal distribution and the buffering effects of a large sample size (Mettler, et al, 1988). A small sample with large variances may restrict comparison with larger populations because of the mathematical enlarging of the difference of each sample from the mean.

Common methods employed to compare means two classic statistics utilize the sample variances: Students "t" test, and Fischers "F" statistic. To oversimplify and standardize: the variance is the average of $n$ squared deviations from a mean, also known as the mean square (Sokal and Rolhf, 1981):

$$\text{Variance} = \frac{\sum y^2}{n}$$

where $y$ is the sum of the differences from a sampled mean, squared). Both statistical tests rely on an assumption that a normal distribution of sample points about the
sampled mean is present. Student's "t" is a method of comparing a mean from a smaller population (n less than, or equal to 32) with a population whose n exceeds 32. Student's "t" may be expressed as:

\[ t = \frac{\bar{X} - \mu}{s - \sqrt{n}} \]

Where "t" is the Student's statistic,

\[ \bar{X} \]

X-bar is the smaller population mean,

\[ \mu \]

\[ \mu \] is the larger population mean, s is the standard deviation of the smaller population, and n is the population of the smaller population (Wonnacott and Wonnacott, 1977).

Fischers F-statistic is a bit more direct mathematically, where the comparative statistic is a ratio of two larger sample population ratios (n greater than 32):

\[ F = \frac{s_1^2}{s_2^2} \]

where \( F \) is the sample variance ratio (the F-statistic),

\[ s_1^2 \]
is variance of a sampled population, and

\[ s_2^2 \]

is the variance of the population to be compared with the first population. This is expressed as a ratio nearing one when the variances are similar, and departing towards zero as differences in variances increase (Sokal and Rolhf, 1981).

Both of these statistics may be infrequently used to compare mean allele frequencies, especially mean heterozygosity per locus. However, in small samples these statistics are subject to problems of larger swings in variance because of the fewer number of samples: the smaller the sample, the lower \( n \) is, and it's ability to act as a divisor for the square of sum of the sample is diminished. Standard deviations, and therefore variances, do not describe the variation that occurs in a population without a standard distribution (Mettler et al, 1988). The problem then is that variance that may be considered "normal" for the larger population gains additional weighting in a small sample size, and makes comparison difficult for that small population. Other methods are commonly used to compare allelic frequencies without either Student's "t" or Fischer's F-statistic.

In dealing with allelic frequencies a common, well documented way of summarizing population data is Wright's F-statistic, rigidly demonstrated in Weir and Cockerham (1984). Other techniques used to quantify genetic variation, including percentage of polymorphic loci, average number of alleles per locus, and mean heterozygosity per locus (Mettler et al, 1988). Can mean heterozygosities be used as
another indice to compare populations if one of the sampled populations does not have enough data to describe a "normal" curve? Preferably not, and care must be executed to account for sample size (Weir and Cockerham 1984). If the abovementioned methods for testing means among populations are invoked, the same hazard regarding small "normal" distributions occurs. Somewhat more elaborate methods for comparison have been developed to compare populations with similar genetic indices (Mettler et al., 1988), based on assumptions more familiar to workers in populations genetics (random mating, the probability that any allele drawn at random from a population will be identical to another allele drawn at random from another population, etc.).

One method that overcomes the problem of populations having different indices is Nei's Index (Nei, 1973; mis-cited in Mettler 1988), which concentrates on exploring the probability of two alleles from different populations drawn at random are identical. Nei's formula for the probability of identity for one locus is:

\[ I_L = \frac{j_{xy}}{\sqrt{j_x j_y}} \]

where

\[ j_{xy} = \sum x_i y_i \]

and where \( x_i \) is the frequency of a given allele (i) in population \( x \), \( y_i \) is its frequency in population \( y \) (Sneath's Index, the probability of two alleles being drawn at
random),

\[ j_x = \sum x_i^2 \]

is the sum of squared allele frequencies in population x, and

\[ j_y = \sum y_i^2 \]

the sum of squared allele frequencies in population (Mettler et al, 1988).

Nei's index normalizes Sneath's index (the numerator) with a geometric mean of the two sampled populations, and concerns the probabilities that an allele from population one has an identical probability of being sampled as does the same allele from population two (the denominator). This normalization surmounts the problem variances within small sample size (Nei 1973; Mettler et al, 1988). This formula does not account for differing base pair groups giving rise to codon variations within the same allele; this is apparently dealt with in Nei (1975), as cited in Mettler (1988).

Nei's index is still a valuable investigative tool being applied to population allele frequency information garnered from electrophoretic data (e.g: Slatkin & Voelm 1991, where the index is used to investigate \( F_a \)). However, with the huge strides made in molecular techniques used to investigate genetic diversity, other techniques that do not necessarily rely on Nei's index are being used extensively. Spatial Autocorrelation of electrophoretic data (Epperson, 1989; Sokal and Oden, 1978a and 1978b) compares differing allele frequencies and absolute geographic differences to characterize genetic flow in populations, sometimes independently of heterozygosity indices. Chloroplast
and mitochondrial DNA can be mapped and used for comparison in lieu of isoenzyme data (Neale and Sederhoff, 1988), although in Martin & Simon (1990), Nei's index is employed in mitochondrial DNA genetic distancing. Nei's index is still a currently serviceable implement in comparing population allele frequencies regardless of sample size; it can be applied even in the case of single allele populations of less than ten.
Literature Cited


