THE GEOGRAPHIC AND ECOLOGICAL PATTERNS OF GENETIC VARIATION IN THE *ABIES GRANDIS-ABIES CONCOLOR* COMPLEX

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Authorization to Submit Dissertation

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Abstract

Abies grandis and *Abies concolor* occupy many of the forests of western North America and are of great ecological and economic importance to the region. *Abies grandis* is generally a seral component of mesic, low to mid elevation forests of the Pacific Northwest and the northern Rocky Mountains with a mild maritime climate. *Abies concolor* is generally a dominant tree of much more xeric, mid to high elevation forests of the central and southern Rocky Mountains with a few small populations in the mountains of northern Mexico. Although these taxa are regarded as ecologically, morphologically and genetically distinct in these regions, they form an expansive hybrid zone in between from the Transverse Range in southern California to the north-central Cascade Mountains of Washington State and the Mountains of north-central Idaho. Due to the complex geographic and ecological patterns of phenotypic and genetic variation of these populations, their description, identification, delineation and taxonomic treatment remain largely unresolved. In this study, molecular genetic and morphometric analysis are used to determine the paternal and maternal lineage and genetic composition of hybrid and pure populations across the entire geographic region. Additionally, analysis of GIS-based environmental data layers is used to assess the differences that characterize the environmental niche of pure and hybrid populations. These results should inform a more uniform taxonomic treatment and facilitate further genetic and ecological study of this complex.

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Chapter 1

Phylogenetic Position and Incongruence of the *Abies grandis-Abies concolor* Complex

Abstract

Grand fir (*Abies grandis* (Douglas) Lindley) and concolor fir (*A. concolor* (Gordon & Glendinning) Hildebrand) are widely distributed throughout many of the forests of western North America. Although they are regarded as morphologically and ecologically distinct, they intergrade over an expansive region between the southern and inland extent of the range of grand fir and the northern extent of the range of concolor fir. The intricate patterns of phenotypic variation of the complex have confounded the classification and delineation of these taxa for over a century. The present study analyzes chloroplast (cp) and mitochondrial (mt) DNA sequences to determine the paternal and maternal lineage of pure and intergradient populations spanning the entire complex. All intergradient populations east of the Cascade Mountains are dominated by individuals with an incongruent combination of a grand fir cp haplotype and a concolor fir mt haplotype, thus, providing evidence that the intergrading complex comprises an extensive hybrid zone spanning several thousand km^2. Populations of concolor fir of the Transverse range of southern California are characterized by the incongruent combination of a cp haplotype of the Sierra Mountains and an mt haplotype of the southern Rocky Mountains, which suggests the existence of a second hybrid zone between Sierra Mountain and Rocky Mountain forms of concolor fir. Populations containing cp/mt incongruence are correlated with specific morphological characters that aid in the identification of hybrid populations in the field. The most likely

source populations of both species in the hybrid zone and their associated migration routes are discussed. We hypothesize that hybridization between the two has allowed grand fir to occupy a more xeric ecological niche than pure grand fir. More thorough ecological characterization of pure and hybrid populations await further study.

Introduction

Grand fir (*Abies grandis* (Douglas) Lindley) and concolor fir (*A. concolor* (Gordon & Glendinning) Hildebrand) are major components of forests throughout much of western North America, yet little is known about the complex patterns of ecological and phenotypic diversity that characterize their collective range. Grand fir is a generally seral tree of mesic forests from low to middle elevation of the Pacific Slope, Cascades and Northern Rocky Mountains, while concolor fir is a generally dominant tree of relatively more xeric forests from middle to high elevation in the central and southern Rocky Mountains and the montane regions of California and locally in northern Mexico (Liu, 1971). Although the two species are regarded as morphologically and ecologically distinct in the coastal and northern extent of the range of grand fir and the southern extent of the range of concolor fir there is an expansive region in between from central Idaho and adjacent Oregon to the Sierra Mountains where they intergrade (Figure 1). This zone of intergradation is characterized by highly variable intermediate phenotypes that make the identification and delineation of distinct taxa dubious (Daniels, 1969).

Phenotypically pure grand fir of the northern coast has distinctly 2-ranked, pectinate shade leaves with a shiny-green adaxial surface without any rows of stomates (although a

few stomates may occur at the base of the apical notch), a prominent full length groove and a rounded apex with a notch .1-.3mm in depth. The outer bark of mature trees is generally gray in color, plated and hard with a distinctly reddish-purple periderm (Daniels, 1969). Grand fir of the Cascades and northern Rocky Mountains is more variable than that of the coast with a small portion that have yellow periderm and some have more abundant adaxial stomates near the apex and apical notches that are not quite as deep (Daniels, 1969; Lacaze and Tomassone, 1967).

The two regional forms of concolor fir, Rocky Mountain and Sierra Mountain, have been variously regarded as slightly variant forms of a univalent species, *Abies concolor*, (Farjohn, 1990; Harlow and Harrar, 2001) or as distinct enough to warrant separate varietal or specific status. Liu (1971) recognized the Sierra Mountain form as *Abies concolor* var. *lowiana* and the Rocky Mountain form *Abies concolor* var. *concolor*. Hunt (1993) recognized the Sierra Mountain form as *Abies lowiana* and the central and southern Rocky Mountain form as *A. concolor* with apparent disjunct populations in the southern Cascade Mountains, Siskiyou Mountains and mountains of southern California. Throughout the remainder of this paper the treatment of Liu (1971) will be used for these regional varieties of concolor fir.

A. concolor variety *concolor* has upswept leaves with an average of 10 or more rows of adaxial stomata averaged for all quartiles of the leaf, adaxial groove usually absent but may have a minute basal groove and a pointed to acute apex often without a notch but may have a minute notch up to .05mm depth. The outer bark of this regional form is furrowed and grayish. The inner bark has a prominent corky periderm and is predominately yellow but many populations sampled in this region had a significant percentage of trees with red

periderm (Daniels, 1969). *A. concolor* variety *lowiana* of the Sierra Mountains is more variable in all foliage characters than the Rocky mountain form. In many respects the phenotypes of the northern Sierras are intermediate between concolor fir of the Rocky Mountains and grand fir (Daniels, 1969; Liu, 1971). Foliage phenotypes in the southern Sierras resemble those found in the central Rocky Mountains (Daniels, 1969; Hunt, 1993; Hamrick and Libby, 1972). The outer bark of the Sierra Mountain form is grayish and furrowed with extensive cork periderm that is invariably yellow. Typically this regional form has 2-ranked but not pectinate shade leaves with an average of 9 rows of adaxial stomata averaged for all quartiles of the leaf, a partial to full length adaxial groove and a variable apex from acute to rounded with an apical notch absent or up to 0.15mm deep. Sun leaves are obscurely 2-ranked to upswept.

Previous studies of the patterns of phenotypic variation within the zone of intergradation have been based on the evaluation of morphological characters (Daniels, 1969; Frederick, 1977; Zobel, 1973) and monoterpenes (Houkal, 1977) that are presumed to be genetically controlled with the hybrid index method of Anderson (1949). These studies characterized the intergradient populations as generally intermediate between grand and concolor fir but with higher levels of variability than occur in either pure zone. Intergradient phenotypes range from nearly pure grand and concolor fir to phenotypes with combinations of characters of both species and or character states that are intermediate to both species, often with contrasting phenotypes in close proximity to each other. The relative influence of character states associated with grand and concolor fir within this region are generally positively and negatively correlated with latitude, respectively. The margins of the zone of

intergradation are not discrete but are indeterminately delimited by discontinuities in morphological characters, which vary locally with the landscape and appear to emanate into the otherwise pure zones at a much lower frequency (Daniels, 1969; Houkal, 1977; Zobel, 1973). Daniels placed the northern boundary at approximately 45° N based on abrupt transitions from populations with predominantly red periderm and relatively few adaxial stomates to significant portions of populations with yellow periderm and leaves with more abundant adaxial stomtaes to the south. Libby placed the southern boundary at approximately 42° N based on an abrupt transition to populations with more abundant adaxial stomates and 100% yellow periderm to the south in the Sierra Mountains, though bark tallies as far south as Humboldt County yielded a significant percentage of trees with red periderm.

These studies suggest the grand fir-concolor fir intergrading complex comprises an extensive hybrid zone spanning several thousand square kilometers from central Idaho to at least northern California. Additional support for this explanation comes from common garden studies in which seed from pure sources resulted in pure phenotypes and seed from intergradient sources resulted in intergradient phenotypes (Hamrick, 1966; Hamrick and Libby, 1972; Lacaze and Tommasone, 1967). Furthermore, controlled cross-pollinations between an *A. grandis* paternal parent and *A. concolor* variety *concolor* maternal parent resulted in highly viable seeds (Critchfield, 1988) and the same natural crosses have occurred in several arboreta (Gathy, 1957; Klaehn and Winieski, 1962; Scheplitz, 1956).

Although the hybrid origin hypothesis for intergradient phenotypes has gained some acceptance regionally for populations in southwestern Oregon and northwestern California,

the genetic composition of the putative hybrids has only been inferred and their taxonomic placement and delineation remain unresolved. The general state of taxonomic confusion remaining over the complex is evident in the many discrepancies present in the contemporary taxonomic treatments by Hunt (1993), Liu (1971) and Farjon (1990). Although they all acknowledge the existence of grand fir-concolor fir hybridization in northwestern California and southwestern Oregon, there are many discrepancies regarding the morphological description, delineation and taxonomic placement of intergradient populations characteristic of this region. In more practical terms this general state of taxonomic confusion over the zone of intergradation transfers directly to the realm of natural resource managers of this region. Without the ability to even identify the species being managed, a clear understanding of the genetic-environmental interaction fundamental to effective silvical application seems out of reach. There have been several recent studies utilizing chloroplast and mitochondrial DNA markers of the highly variable and taxonomically unresolved populations of *Abies* of Mesoamerica, and intergrading populations of western North America to assess their diversification and distribution (Aguirre-Planter *et al*., 2012; Jaramillo-Correa *et al*., 2008; Oline, 2009).

One approach to assess the potential role of secondary contact in the origin of these complexes is to compare differentially inherited genomes. In family Pinaceae the nuclear, mitochondrial and chloroplast genomes have different modes of inheritance; they are biparentally, maternally and paternally inherited respectively (Mogenson, 1996). This pattern of inheritance has been confirmed in *Abies* (Liepelt *et al.*, 2002; Vendramin and Ziegenhagen, 1997; Ziegenhagen, 1995). Incongruence of paternally inherited species-

specific chloroplast (cp) and maternally inherited mitochondrial (mt) DNA markers has been used to reveal natural hybridization between *Abies homolepis* and *A. veitchii* (Isoda, 2000) and to verify ancestry of controlled crosses of *Abies alba* with *A. nordmanniana*, *A. pinsapo* and *A. cephalonica* and to assess relative gene flow through seed and pollen dispersal among regional populations of *A. alba* (Liepelt, 2002). These studies provide the framework to inform more precise taxonomic treatment, effective management practices and support further genetic and ecological studies of these populations. This study analyzes the sequences of three chloroplast (cp) intergenic spacers and three mitochondrial (mt) introns to determine the paternal and maternal lineages respectively of intergradient and pure populations throughout the grand fir-concolor fir complex in a robust phylogenetic framework.

These molecular results are referenced to the results of a morphometric analysis based on a suite of differentiated foliage characters to demonstrate that the grand fir-concolor fir intergrading complex comprises two extensive hybrid zones and two regions of pure populations (Daniels, 1969). Specific morphological characters that aid in the identification of hybrid populations in the field are discussed. The most likely source populations of concolor fir genes in the hybrid zones and the associated historical migration routes are evaluated and the extent of introgression into the pure zones surrounding the hybrid zones is also examined.

Methods

The study area encompasses the entire *Abies grandis-Abies concolor* complex. Sampling localities span the putative hybrid zone of central Idaho, northwest California, northeast Oregon, southwest Oregon and include reference populations of phenotypically pure species throughout their collective range (Figure 1). At each location, (Table 1), 4-6" of a foliage-bearing branch tip within 3 meters of the ground was collected from 4-15 individuals that were randomly selected. Additional samples of *Abies* representing the major clades recovered in an earlier phylogenetic study of the genus based on *rbcl* DNA sequences (Suyama *et al.,* 2000) were obtained along with one sample of *Keteleeria evelyniana* to serve as the outgroup (Table 2). Approximately 100 mg of fresh or 40 mg dry foliage was removed from each sample and ground with mortar and pestle in liquid nitrogen. Total genomic DNA was extracted and purified using Qiagen DNeasy Plant Minikits (Qiagen, Valencia, CA). Extracted DNA was then quantified using a Beckman DU 640 spectrophotometer (Beckman Coulter) and stored in elution buffer at -20º C. Foliage samples were then pressed and deposited in the University of Idaho Stillinger Herbarium.

DNA sequences of three chloroplast intergenic spacers that have been highly variable in *Abies, trn*R-*trn*N *and trn*L-*trn*F (Suyama et al., 2000) and in other conifers *psb*A-*trn*H (Sang et al., 1997) were sequenced to determine the paternal lineage. Three mitochondrial introns were sequenced, *nad1* intron 2, *nad5* intron 1 and *nad5* intron 4 to determine the maternal lineage. These introns have long repeat motif regions that have relatively high substitution rates flanking them compared to the rest of the genome (Wu, 1998). Primers used in the study are listed with annealing temperatures, (Ta°C) in Table 3. Polymerase chain reaction

(PCR) was carried out for all cp spacers using 20 ng of total genomic DNA. Each 25 ul reaction contained 10mM tris-HCl (pH 9.0 at 25º C), 50 mM KCl, 0.1% Triton X-100®, 20 ug BSA, 4mM $MgCl_2$, 400 uM dNTPs, 0.4 uM each primer and 0.5 units *taq* DNA polymerase (Promega). PCR amplification for all cp spacers was carried out in a Tetrad2 Thermal Cycler (MJ Research) with the following thermal profile: 94° C for 1 minute followed by 30 cycles of 94° C for 30 s, (Ta°C) for 30 s, 72° C for 1 min., followed by 2 min. at 72° C.

PCR was carried out for all mt introns using 20 ng of total genomic DNA. Each 25 ul reaction contained 15mM tris-HCl (pH 8 at 25° C), 50mM KCl, 2 ug BSA, 4mM $MgCl_2$, 400 uM dNTPs, 0.4 uM each primer and 0.5 units Ampli*Taq* Gold (Applied Biosystems).

PCR amplification was carried out with the following thermal profile: 94° C for 5 min., followed by 30 cycles of 94° C for 30 s, (Ta° C) for 30 s, 72° C for 1.5 min., followed by 72° C for 2 min. Remnant primer and nucleotides were removed from PCR product in 7ul reactions consisting of 5ul of PCR product and 2ul of ExoSAP-IT® (USB Corporation, Cleveland, Ohio). This reaction was placed in a Tetrad2 Thermal Cycler (MJ Research) for 15 minutes at 37° C and then 15 minutes at 80° C.

Sequencing reactions included 2ul of BigDye v3.1® (Applied Biosystems, Foster City, CA) and 2ul of cleaned PCR product in 10ul volumes with 1.25mM $MgCl_2$ and 50mM tris-HCl (pH 9.0). Sequencing reactions were carried out in a Tetrad2 Thermal Cycler (MJ Research) with the following thermal profile: 93° C for 3 min., followed by 25 cycles of 30 s at 93° C, 30 s at 50° C and 2 min. at 60° C. Sequencing reaction products were cleaned by ETOH precipitation and sequenced on an ABI 3130 capillary sequencer (Applied Biosystems, Foster City, CA). Sequences were analyzed with ABI Sequencing Analysis software (Applied

Biosystems, Foster City, CA). Sequences were aligned using Sequencher 4.5 (Gene Codes, Ann Arbor, MI).

Results

A total of 800 nucleotides of the intergenic spacer region *trn*R-*trn*N, 580 nucleotides of the intergenic spacer *psb*A-*trn*H and 500 nucleotides of the intergenic spacer *trn*L-*trn*F were sequenced for all 195 samples from the grand fir-concolor fir complex and all 14 samples of the out-group. An additional three samples obtained from controlled crosses established between a grand fir paternal parent and Rocky mountain concolor fir maternal parent at the Institute of Forest Genetics (Critchfield, 1988) were sequenced at these three spacer regions to confirm paternal inheritance of cp genome in these taxa. The program J Model Test was used to determine the best fitting substitution model and F81 had the highest score for *psbA-trnH* and *trnL-trnF* and JC had the highest score for *trnR-trnN* using BIC. The sequences were concatenated and a binary matrix was added to code for unambiguous indels using the method of Simmons, (2000).

Maximum-parsimony analysis was performed on the concatenated cp sequence data with a binary matrix, using Paup version 4.0 (Sinauer Associates, Sunderland, Massachusetts) with a heuristic search, the TBR branch-swapping algorithm and the branches collapsed option. A Bayesian analysis was performed on Mr Bayes 3.21 with the concatenated sequences and a partitioned binary matrix. The recovered phylogenies have the same topology of the ingroup with five main groups overall, although node support was generally much better with the Bayesian analysis, Figure 2.

Group I includes only the endemic *A. bracteata* that has long been considered the most basal in the genus and has been classified in the section *Bracteata* by Farjon and Rushforth (1990). Group II includes *A. alba* of southern Europe and Mediterranean region classified in section *Abies* by Farjon and Rushforth (1990). Group III consists of sub-alpine firs; *A. frima* of Asia and the North American taxa *A. balsamea* of the boreal region*, A. fraseri* of the Appalachian Mountains and *A. lasiocarpa* of the sub-alpine forests of western North America. They are classified in the sections *Momi* and *Balsamea* respectively by Farjon and Rushforth, (1990). Group IV includes *A. amabilis* and *A. procera* of the Cascade and Pacific Coast Ranges of North America. They are classified in sections *Amabilis* and *Nobilis* respectively by Farjon and Rushforth (1990). Group V is strongly supported and includes three strongly supported sub-groups: The first sub-group comprises two haplotypes of apparently unresolved Mesoamerican taxa; haplolotype I includes two samples of *A. religiosa* and two of *A. guatemalensis* and haplotype II includes a single sample of *A. vejarii*.

The second and third sub-groups of group V include all of the pure and intergradient populations of the grand fir-concolor fir complex and will be described in further detail (Figure 3). The second sub-group of group V comprises a lineage of concolor fir of the central and southern Rocky Mountains with three haplotypes and a lineage of concolor fir of the Sierra and Transverse Mountains with two haplotypes. Cp-type ABCO I is the only haplotype found in populations 19 and 20 of the southern Rocky Mountains and cp-types ABCO II and ABCO III occur in population 18 of the central Rocky Mountains. Cp-type ABLO I is the only haplotype found in population 17 of the Transverse Mountains and it accounts for over half of the samples in population 16 of the central Sierra Mountains. Cp-type ABLO I

also occurs at a much lower frequency in intergradient population 11 of northeast Oregon and in intergradient populations 10 and 12 of central Idaho. Cp-type ABLO II is common in population 16 of the central Sierras and is found in one individual of intergradient population 11 of northeast Oregon.

The third sub-group of group V, the grand fir lineage, includes three cp-types, ABGR I-III , that occur primarily in grand fir populations of the Pacific Coast and the northern Rocky Mountains and seven cp-types that occur primarily in intergradient populations, HYB I-IIV. Cp-types HYB I-VII cluster together within subgroup three and occur primarily in the intergradient populations, but also occur in nearly pure populations of grand fir along the margins of the zone of intergradation. The chloroplast haplotype frequencies and Nei's unbiased gene diversity statistic, *H*, (1987) are summarized for each population in Table 4. Populations throughout the regions characterized by pure grand fir phenotypes have the lowest levels of gene diversity, *H* = 0-.34, while nearly pure grand fir populations are slightly higher, *H* =.449-.7 and intergradient populations exhibit the highest values, *H* = 0.531-0.813. Concolor fir gene diversity is higher in the central Rocky Mountains, *H*=.444 than the southern Rocky Mountains, *H*=0. In the Sierra Mountains gene diversity was higher in the north, *H*=.494, than in the south, *H*=0.

A total of 850 nucleotides of the first intron of *nad*5, 750 nucleotides of the fourth intron of *nad5* and 1550 nucleotides of the second intron of *nad1* were sequenced for all 195 samples of the grand fir-concolor fir complex. Samples representing the major clades recovered in the earlier phylogenetic studies mentioned above along with one sample of K*eteleeria evelyniana* to serve as an out-group were also sequenced. An additional three

samples obtained from controlled crosses established between a grand fir paternal parent and Rocky mountain concolor fir maternal parent at the Institute of Forest Genetics (Critchfield, 1988) were sequenced at these three intron regions to confirm maternal inheritance of the mt genome in these taxa. The sequences of the out-group, *Keteleeria evelyniana*, were too divergent to align and were not included. The remaining sequences were concatenated and a binary matrix was added to code for unambiguous indels using the method of Simmons, (2000). Maximum-parsimony analysis was performed on the concatenated mt sequence data with a binary matrix, using Paup version 4.0 (Sinauer Associates, Sunderland, Massachusetts) with a heuristic search, the TBR branch-swapping algorithm and the branches collapsed option. A Bayesian analysis was performed on Mr Bayes 3.21 with the concatenated sequences and a partitioned binary matrix.

The recovered phylogenies have the same topology with distinct lineages but due to a lack of parsimony informative characters the relationship between the lineages remains unresolved (Figure 4). It should be noted that within the out-group *A. balsamea* and *A. fraseri* share a haplotype as do two samples of *A. lasiocarpa*. The *A. grandis-A. concolor* complex comprises four mt-types. Mt-type I, the only haplotype of *A*. *grandis,* is also shared with *A. amabilis* of the outgroup and is distinguished from all mt-types II-IV of concolor fir by two point substitutions in *nad5*i1 and two 4 nucleotide deletions in *nad1*i2 (Figure 5). Mt-type II is the predominant haplotype of *A. concolor* variety *concolor* of the Southern Rocky Mountains and population 17 of the Transverse Range in Southern California and is distinguished from mt-types III and IV by the length of the deletion in *nad5*i4 (Figure 5). Mt-type II is also shared with the out-group taxa, *A. religiosa* and *A. guatemalensis* of

Mesoamerica. Mt-type III is the haplotype of *A. concolor* variety *concolor* in the central Rocky Mountains and is distinguished from mt-types II and IV by a 107 nucleotide deletion in *nad5*14 and a single nucleotide deletion in *nad*1i2. MT-type IV is the predominant haplotype in the central Sierras and all of the intergradient populations of central Oregon and adjacent Idaho and is distinguished from mt-types II and III by a lack of any deletions in *nad5*i4. Mt-type IV is the only mt-type that occurs in the intergradient populations east of the crest of the Cascade Mountains and occurs at a lower frequency in a population of nearly phenotypically pure grand fir in Latah County, Idaho. All of the coastal and northernmost interior populations of grand fir are fixed for mt-type I, as are two putative hybrid populations from Klamath County, Oregon and Trinity County, California (Figure 7).

Although the regions characterized by pure morphological phenotypes are dominated by cp/mt-types that are congruent, most of the putative hybrid zone characterized by intergradient phenotypes is dominated by individuals with cp-types that are strongly supported in the *A. grandis* clade in combination with the mt-type IV, which is the predominant *A. concolor* variety *lowiana* mt-type of population 16 of the central Sierras. There are three exceptions to this general pattern: 1) The putative hybrid populations 14 and 15-HYB, which have intergradient phenotypes but have congruent combinations of cp-types nested in the *A. grandis* clade and the *A. grandis* mt-type I; 2) Population 5-ABGR* characterized as nearly phenotypically pure grand fir includes 2 individuals with cp-types that are nested in the *A. grandis* clade in combination with the concolor fir mt-type IV; 3) The cp-type ABLO I with primary occurrence in the Sierra Mountains also occurs in combination with the congruent mt-type IV in the intergradient population 11 of northeast

Oregon and twice each in intergradient populations 10 and 12 of central Idaho. The cp-type ABLO II with primary occurrence in the central Sierra Mountains is also found in one sample from intergradient population 11 of northeast Oregon.

It is also of major interest that all of the samples in population 17 of the Transverse Range in southern California have the incongruent combination of a cp-type ABLO I with the mt-type III of the southern Rocky Mountains. All three of the samples of controlled F1 hybrids with an *A. grandis* paternal parent and an *A. concolor* variety *concolor* of the Southern Rocky Mountains maternal parent have the incongruent combination of *A. grandis* cp-type ABGR I and mt-type *A. concolor* II of the southern Rocky Mountains, which confirms paternal and maternal inheritance for the cp and mt genomes respectively in this complex.

Discussion

Overview

In the phylogeny recovered from cp intergenic spacers *trnL-trnF, trnR-trnN* and *psbA-trnH,* the *A. grandis-A. concolor* complex comprises discrete and strongly supported *A. grandis* and *A. concolor* lineages that occupy group V along with a discrete lineage of unresolved Mesoamerican taxa. The overall topology of this phylogeny is very similar to the phylogeny based on the cp sequences *rbcL, rps18-rpl20,* and *trnL-trnF* (Aguirre-Planter *et al.*, 2011), and a phylogeny based on ITS sequences (Xiang *et al.*, 2009). Although, both of these studies showed strong support of group V, section Grandis (Farjon and Rushforth, 1990), with representatives of the same taxa, they did not form discrete lineages. This is likely due to the use of the intergenic spacers *psbA-trnH* and *trnR-trnN* in the present study, which

have relatively higher substitution rates in this group than the more highly conserved regions sequenced in the previous studies. The resolution of the complex into discrete lineages in the present study permits a more detailed assessment of geographic patterns within the complex.

The phylogeny recovered from mitochondrial introns resulted in distinct lineages with little resolution describing the relationships between these lineages. This is largely due to the relatively low substitution rate of the mitochondrial genome of plants and relatively recent radiation of *Abies*. Although the *A. grandis-A. concolor* complex comprises 4 distinct haplotypes with 2 substitutions and two deletions that distinguish the only *A. grandis* haplotype, mt-type I, from all of the other haplotypes, it must be noted that mt-type II of the southern Rocky Mountains and southern Sierra Mountains is shared with both *A. religiosa* and *A. guatemalensis* samples of Mesoamerica. Jaramillo-Correa et al. (2008) found that this same haplotype was one of several shared among all of the differentiated Mesoamerican Taxa. This pattern outside the complex doesn't preclude its utility as an effective means of determining the maternal lineage within it. The mt-types are largely fixed among populations of the complex and the geographic pattern correlates well with recognized regional segregates of grand and concolor fir and is a very close match to the delineation of distinct morphological and ecological forms of these taxa by Hamrick and Libby (1972). However this pattern is sharply contrasted by the geographic pattern of cp-types, which indicate greater gene flow between regional populations from pollen dispersal than from seed dispersal. The overall pattern of cp-type diversity and cp/mt-type incongruence coincides with the geographic patterns of phenotypic variation comprising the

phenotypically pure zones and intergradient regions. The populations characterized by pure grand and concolor fir phenotypes are nearly all fixed for a few cp-types with relatively low cp-type diversity, *H* = 0-0.6 for pure grand fir and *H* = 0-0.400 for the regional segregates of *A. concolor* and are fixed for the matching mt haplotype.

Intergradient populations between the northern Sierra Mountains and the northern Rocky Mountains have much higher levels of phenotypic diversity and cp-type diversity, with cp-type diversity *H* = 0.607-0.952, and are dominated by the incongruent combination of a cp-type strongly supported in the *A. grandis* lineage and the A*. concolor* variety *lowiana* mt-type IV. This general pattern of cp-type/mt-type incongruence occurs in all intergradient populations east of the Cascade Mountains, but is notably absent from the populations of putative hybrids in Klamath County, Oregon and Trinity County, California. This apparent discord between the intergradient phenotypes of these populations and their identity as grand fir based on cp-types and mt-types is likely due to repeated backcrossing (introgression) with coastal grand fir which is in close proximity and may have a selective advantage under the present climatic conditions. Populations 15, 7, and 8 show only one CP-type, HYB III, due to difficulties determining a few haplotypes in samples from these populations that did not sequence cleanly. Haplotype diversity values were not calculated for these populations.

The key question is whether the apparent incongruent combination of a paternally inherited cp-type of the *A. grandis* lineage and the mt-type IV of *A. concolor* variety *lowiana* of the central Sierra Mountains that dominates the intergradient populations is due to hybridization or incomplete lineage sorting. Though the case is largely circumstantial,

combined evidence of geographic intermediacy and hyper-variable morphological intermediacy of intergradient populations correlated with incongruence of paternally inherited cp-type of *A. grandis* and maternally inherited mt-type IV of *A. concolor* variety *lowiana* suggests the intergradient populations are the result of crosses between an *A. grandis* paternal parent and an *A. concolor* variety *lowiana* maternal parent. These data suggest the zone of intergradation comprises an expansive hybrid zone.

The exclusively unidirectional nature of the cross revealed by cp/mt-type incongruence in the present study may indicate a strong reproductive barrier to the reverse cross. All of the verified hybrids resulting from controlled crosses have been produced by this same directional cross (Critchfield, 1988). Natural crosses that have occurred in arboreta have all been between a grand fir paternal parent and a Rocky Mountain concolor fir maternal parent (Gathy, 1957; Scheplitz, 1956).

One of the most confounding aspects of the hybrid zone is the indeterminate nature of its margins. Daniels (1969) and Lacaze & Tomassone (1967) placed the northern boundary of the zone of intergradation at approximately 45° N based on abrupt discontinuities in the occurrence of adaxial stomates on significant portions of leaves and the occurrence of yellow periderm in grand fir. Both earlier studies indicated that these character states increased clinally to the south and emanated to the north at a much lower frequency into nearly phenotypically pure grand fir of the Cascades and Inland Northwest. Daniels attributed this apparent emanation of concolor fir-like characteristics into the nearly pure grand fir to introgression of concolor fir genes into grand fir populations to the north. Houkal (1977) found that monoterpenes bornyl acetate, tricyclene, β-pinene and camphene

all vary clinally in populations along a latitudinal transect through five populations on a route from Bonner County in northern Idaho to Valley County in central Idaho. These clinal patterns are consistent with patterns of introgressive hybridization (Anderson, 1949).

The occurrence of *A. concolor* variety *lowiana*r mt-type IV in two individuals along with one individual with yellow periderm and cp-type H =0.7 in the nearly pure grand fir population 5 in Latah County, Idaho at 46° 45' 13" N suggests there is introgression further north than previously believed. This evidence illustrates the cryptic nature of the northern boundary of the hybrid zone. Hamrick (1966) placed the southern boundary of the intergrading complex at about 42° N based on abrupt discontinuities in the occurrence of more abundant adaxial stomata and invariably yellow periderm in concolor fir populations to the south. However, this relationship is obscured by the occurrence of morphologically intergradient populations much further south on the west slope of the Sierras. Our only population sampled from this region, intergradient population 15 in Trinity County, California 40° 46' 49" N, 122° 57' 39", was completely intergradient morphologically but had congruent cp-types and mt-types of the grand fir lineage. This apparent discord between morphological phenotype and cp-type/mt-type has been attributed to backcrossing with nearby coastal grand fir populations. It is therefore apparent that the southern boundary of the intergrading complex extends to at least 40° 46' 49" N along the west slope of the Sierras.

In many respects A. concolor variety *lowiana* of the central Sierra Mountains looks intermediate to intergradient populations to the north in the hybrid zone and the population from the Transverse Range of Southern California, population 17, which in turn appears to

be intermediate between *A. concolor* variety *lowiana* of the central Sierras and *A. concolor* variety *concolor* of the southern Rocky Mountains. It is interesting to note that this population has the incongruent combination of cp-type ABLO I which is clearly divergent from the ABCO cp-types and the mt-type II of *A. concolor* variety *concolor* of the southern Rocky Mountains. This same pattern of cp-type/mt-type incongruence was found in two individuals in population 16 of the northern Sierra Mountains. This pattern suggests there has been hybridization between *A. concolor* variety *lowiana* of the Sierra Mountains and *A. concolor* variety *concolor* of the central and southern Rocky Mountains at some time in the past when they may have been parapatric.

A significant outcome of the present study is the correlation of cp-type/mt-type incongruence with specific morphological characters that are easily discernable in the field at the population level. In the most comprehensive analysis of phenotypic variation of the complex to date, Daniels (1969) discovered that all of the concolor fir samples from the Sierras had yellow periderm and several rows of adaxial stomates, while all of the coastal grand fir had red-purple periderm and virtually no adaxial stomates. The presence of rows of adaxial stomata is evident in the field due to the presence of an adaxial glaucescent band. With one noteworthy exception, all of the populations evaluated in this study that included individuals expressing either character state associated with Sierran concolor fir (yellow periderm or a glaucescent band on adaxial leaf surface) and individuals expressing either character state associated with grand fir (red periderm or shiny green adaxial leaf surface) had some individuals with incongruent cp/mt-types. For example, populations along the northern boundary of the zone of intergradation frequently appear to be pure grand fir but

may include a rare individual with yellow periderm or a glaucescent band on the adaxial leaf surface. At least 20% of the individuals in these populations had incongruent cp and mt haplotypes.

The Evolution of the Grand Fir-Concolor Fir Complex

Throughout the mid-Miocene the region of western North America comprising the states of California, Idaho, Nevada, Oregon and Washington was covered by contiguous mixed hardwood and coniferous forest. By the end of the Pliocene, forests of western North America resembled contemporary forests of the region (Chaney, 1947). The orogeny of the central Cascades, 2-20 MYA, brought about a pronounced continental climate to the inland portions of this region, developing arid shrub steppe habitat in the rain shadow, which ecologically isolated previously contiguous coniferous forest from the Cascades to the Rocky Mountains by over 300 km (Graham, 1999). The advent of the Pleistocene and the advance of the Cordilleran ice sheet into what now is northern Washington, Idaho and Montana during the last glacial maximum, approximately 17-20 kya (Pielou, 1991), caused massive plant migration southward (Daubenmire, 1975). At the same time many species near the ice sheet probably migrated to lower elevations. It has been hypothesized that forested habitats may have moved down as much as 1000 m in elevation during this time period (Barnosky *et al.*, 1987). Climatic zones were compressed both in latitude and elevation, which brought formerly ecologically and geographically isolated taxa into contact (Warner *et al.*, 1982).

The present day hybrid zone between the northern Sierra Mountains and northern Rocky Mountains could have originated from relatively ancient contact events between

remnant populations of the ancestral taxa dating to the Pliocene (Houkal, 1977) or from more recent contact events resulting from the migration of the taxa into the region during the Pleistocene (Daniels, 1969). Daniels hypothesized the existence of a Pleistocene migration route comprising a nearly contiguous coniferous forest extending from the northern Sierras across the Siskiyou, Ochoco, Aldrich, Strawberry and Wallowa Mountains of Oregon to the mountains of central Idaho. This same migration route has been hypothesized for several species with Sierran affinities and disjunct populations occurring in central Idaho, including *Ceanothus prostratus*, *Mertensia bella* and *Calochortus elegans* (Lorain, 1988). This hypothesis is supported by the distribution of cp-types HYB I-V and *A.concolor* variety *lowiana* cp-types ABLO I and II. Cp-type HYB III occurs in the highest frequency in population 15 from Trinity County California and also occurs in every population coincident with this migration route. The rare cp-types HYB IV and V occur in population 14-HYB of the southern Cascades, and are shared with populations 12 and 13 further inland along the same route. None of these cp-types occur in the northern and coastal extent of the range of grand fir. Furthermore, *A. concolor* variety *lowiana* cp-types ABLO I and II also occur at a lower frequency in hybrid populations along this hypothesized route. The elevated level of cp-type diversity in populations along this hypothesized migration route offer further supporting evidence of this explanation. High haplotype diversity is usually indicative of regions that served as refugia or dispersal routes, (Petit *et al.*, 2003; Taberlet *et al.*, 1998). Moreover, this hypothesized migration route is consistent with the prevailing winds from southwest to northeast.

Although the distribution of cp-types and the levels of diversity support the existence of this migration route, it is very difficult to determine whether the patterns of phenotypic variation of the hybrid zone are due to the widespread gene flow of both taxa and subsequent contact or the establishment of stabilized hybrids that subsequently migrated into this region. The above explanation emphasizes the initiation of contact between the two taxa during the Pleistocene. However, it seems very likely that previous contact events occurred during the Pliocene as evidenced by the long branch length of hybrid cp-types that are exclusive to the region of central Oregon and adjacent Idaho and occur only with the incongruent mt-type IV of *A.concolor* variety *lowiana* of the northern Sierras.

The most noteworthy ecological difference between grand and concolor fir is their apparent affinity for moist and relatively more xeric conditions respectively. The distribution of phenotypically pure grand fir coincides with the mesic coniferous forest ecosystem, which is dominated in late succession by *Thuja plicata* and *Tsuga heterophylla*. Concolor fir is associated with much drier forest types and is even capable of persisting on exposed thin soils and bedrock. It has long been hypothesized that introgression of concolor fir into pure grand fir has allowed the latter to occupy a more xeric ecological niche (Johnson, 1995 and Zobel, 1974). The present study has shown that all of the genetic influence of grand fir to the south of the southern limits of the mesic coniferous forest ecosystem in the northern Rockies or east of the coastal distribution of this forest type is through hybridization with concolor fir. This apparent association of putative hybrids with drier forest types than are associated with pure grand fir offers further support of this hypothesis.

Summary

The emerging picture of the intergrading *A. grandis-A. concolor* complex is a dynamic balance between climate shifts correlated with range contraction and divergence and climate shifts correlated with range expansion and reticulation. This system of Mountains, the Cascade, Coast, Siskiyou, Klamath, Ochoco, Wallowa and Rocky Mountain ranges currently support regionally isolated populations separated by many miles of shrub steppe desert, but under cooler macroclimates, elevational zones were likely compressed and there was likely contact between formerly divergent populations. Almost every regional segregate in this complex from the southern Sierra Mountains to the nearly pure grand fir of the Inland Northwest shows some evidence of gene flow with its nearest neighboring segregate with some evidence of introgression into pure populations along the margins of these regions; through elevated haplotype diversity, cp-type/mt-type incongruence or hyper-variable morphological intermediacy. *A. concolor* variety *lowiana* of the central Sierra Mountains looks intermediate to intergradient populations to the north in the hybrid zone and the population from the Transverse Range, which in turn appears to be intermediate between *A. concolor* variety *lowiana* of the central Sierras and *A. concolor* of the southern Rocky Mountains. Thus, the Cascade and Sierra Mountains appear very much like a bridge between divergent populations for gene flow during climate shifts and at some time in the past there appears to have been a similar bridge between the Southern Sierra Mountains and southern Rocky Mountains. It is likely that recent and ancient hybridization events have been crucial to the evolutionary development of both grand and concolor fir and their adaptation to dynamic environments.

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Figures

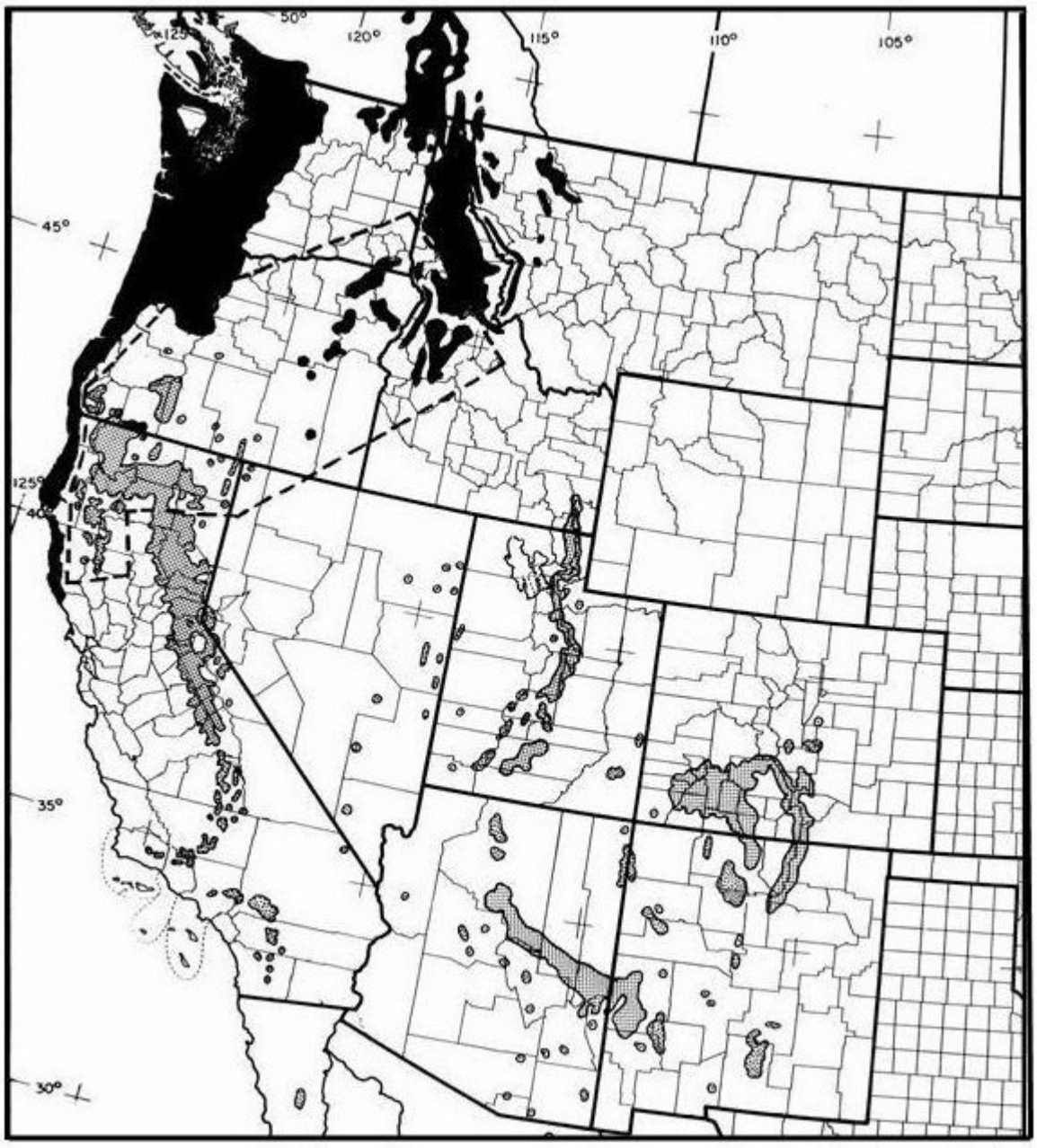

Figure 1.1 Map of the *A. grandis-A. concolor* complex; *A. concolor* populations represented by the stippled pattern and *A. grandis* shaded in black. The indeterminate boundary of intergradient populations is bordered by dashes. Adapted from E. L. Little (1971).

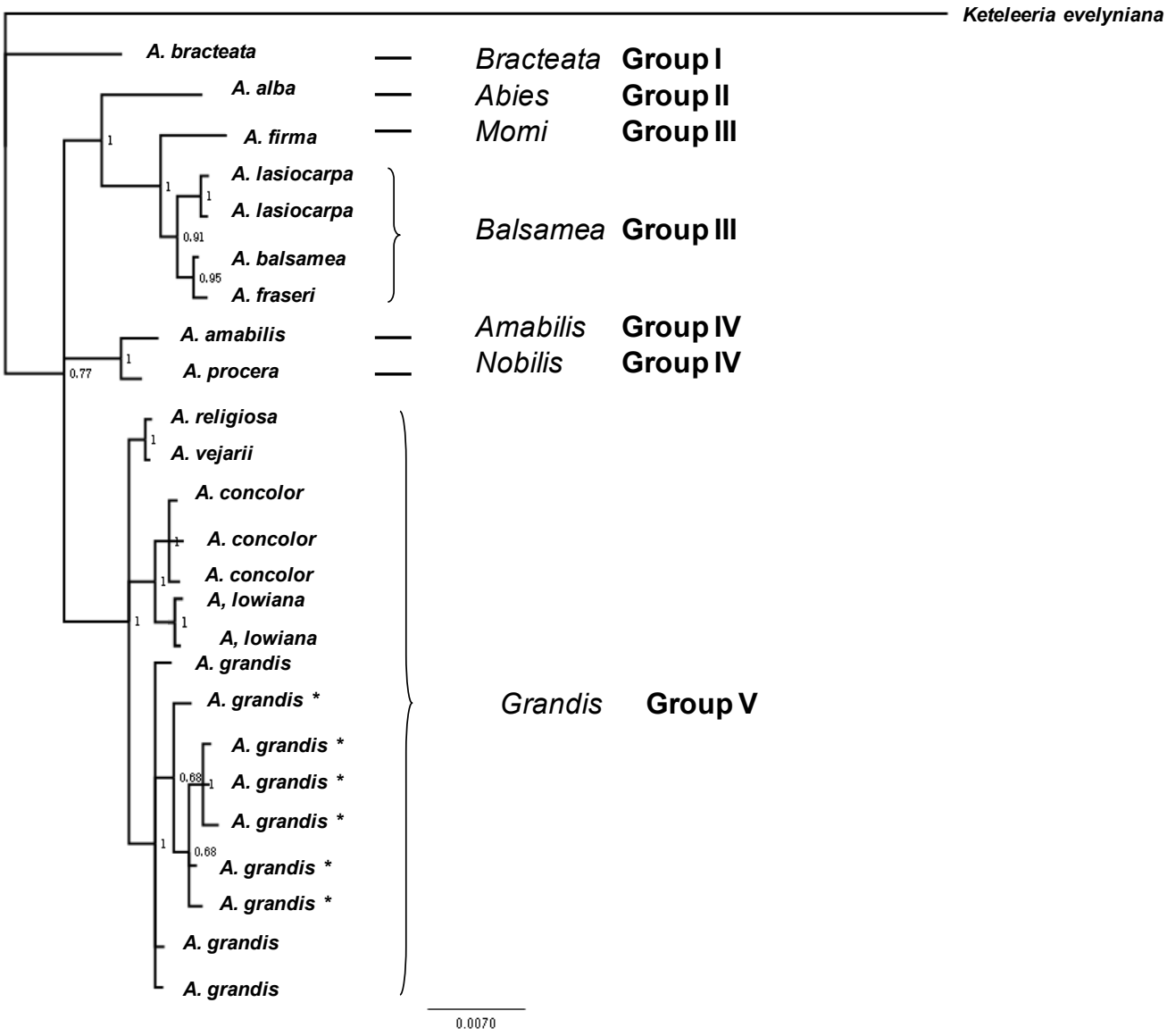

Figure 1.2 Phylogeny recovered from Bayesian analysis of chloroplast intergenic spacers *psbA-trnH*, *trnL-trnF* and *trnR-trnN*. Sections from Farjon and Rushforth (1990), and Groups used in the present study are depicted for each taxon. *A. grandis* * represent hybrid cp-types.

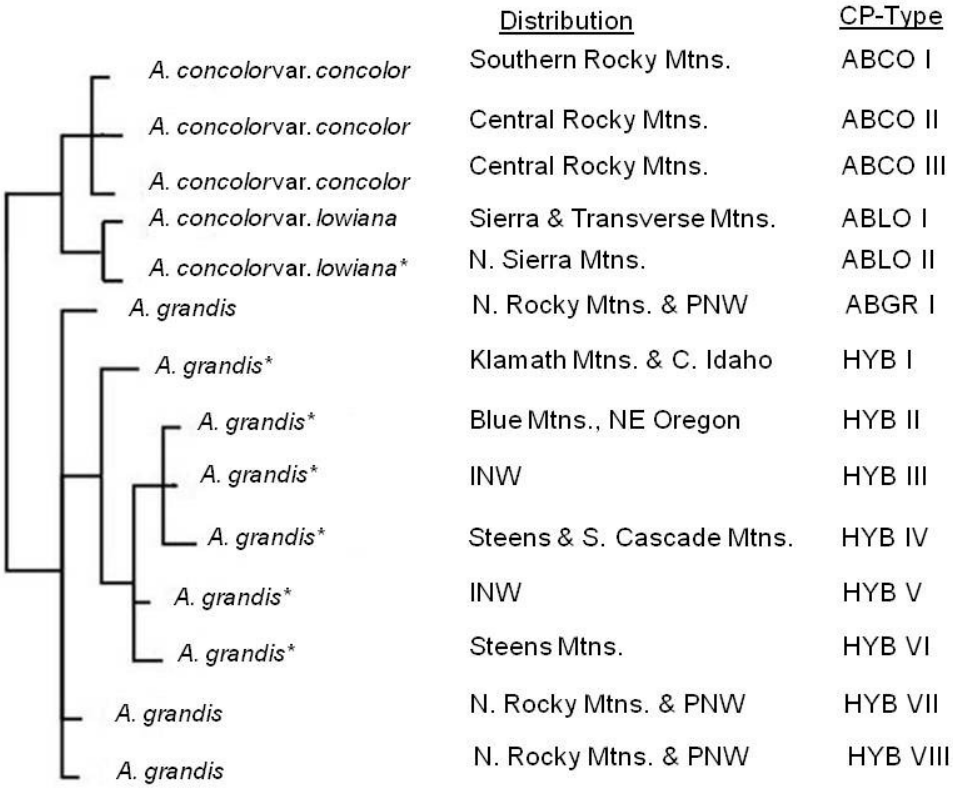

Figure 1.3 *A. grandis-A. concolor* complex cp-types with geographic distribution. ABLO represents *A. concolor* variety *lowiana*, ABCO represents *A. concolor* variety *concolor* and HYB represents intergradient populations. The Inland Northwest is abbreviated INW. The Blue Mountains occupy NE Oregon and SE Washington State. The Steens Mountains are located in southern Oregon. Cp-types listed as *A. grandis** are strongly supported in the *A. grandis* lineage, but occur most frequently in hybrid populations with the incongruent mt-type of *A. concolor* variety *lowiana* of the northern Sierra Mountains.

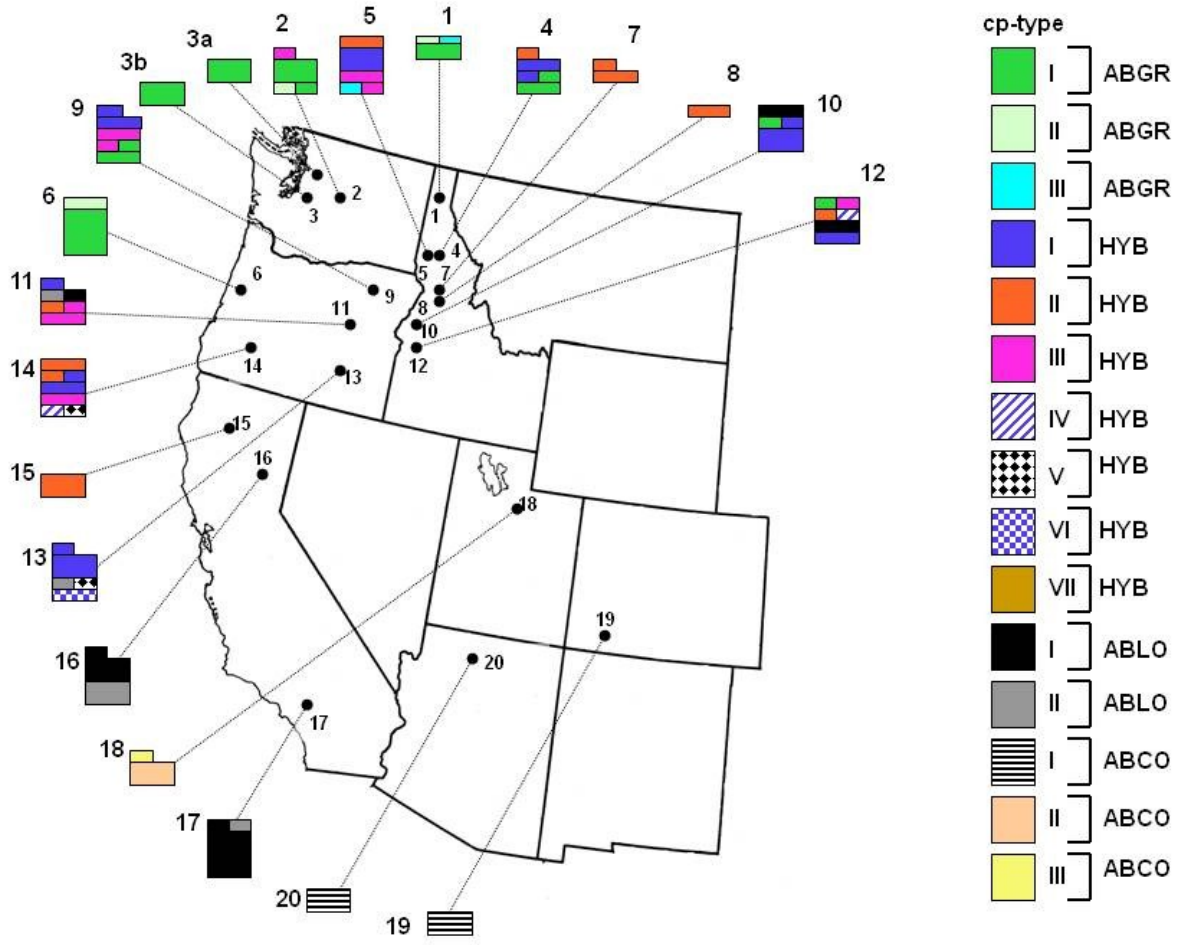

Figure 1.4 Geographic distribution of chloroplast haplotypes,(cp-types). ABCO represents an *Abies concolor* variety *concolor* haplotype; ABLO represents an *Abies concolor* variety *lowiana* haplotype; ABGR represents an *Abies grandis* haplotype; HYB represents haplotypes that occur primarily in intergradient populations, but also occur in nearly pure *Abies grandis*.

	Nad5i4	*Nad5i1*
MT-I	::::::::::::::::::::::::	GCGCGAGGGGAAGGTCCTGAAAGGTCA
MT-II	ATAGATAGATA::::TACATATATCT	GCGCGAGGGGAAGGTCCTTCAAGGTCA
MT-III	::::::::::::::::::::::::	GCGCGAGGGGAAGGTCCTTCAAGGTCA
MT-IV	ATAGATAGATAGATATACATATATCT	GCGCGAGGGGAAGGTCCTTCAAGGTCA
		**

	Nad1i2 indel 1	*Nad1i2* indel 2
MT-I	GGTTATACCCCCCTATCTATCTATAG	CGAACAACC::::CCCT::::CTTTTG
MT-II	GGTTATACCCCCCTATCTATCTATAG	CGAACAACCAACCCCCTAACCCTTTTG
MT-III	GGTTATA:CCCCCTATCTATCTATAG	CGAACAACCAACCCCCTAACCCTTTTG
MT-IV	GGTTATACCCCCCTATCTATCTATAG	CGAACAACCAACCCCCTAACCCTTTTG

Figure 1.5. Key to mitochondrial haplotypes based on the sequences of three mitochondrial introns, *nad5i4*, *nad5i1,* and *nad1i2*: MT-I represents the *Abies grandis* haplotype; MT-II represents the *Abies concolor* variety *concolor* haplotype primarily of the southern Rocky Mountains and Transverse Range; MT-III represents *Abies concolor* variety *concolor* of the central Rocky Mountains; and MT-IV represents the *Abies concolor* variety *lowiana* haplotype of the Sierra Mountains and hybrid populations of central Oregon and Idaho. Gaps are represented by : and substitutions are underscored with *. MT-I and MT-2 have 107 base pair deletions in *nad5i4*.

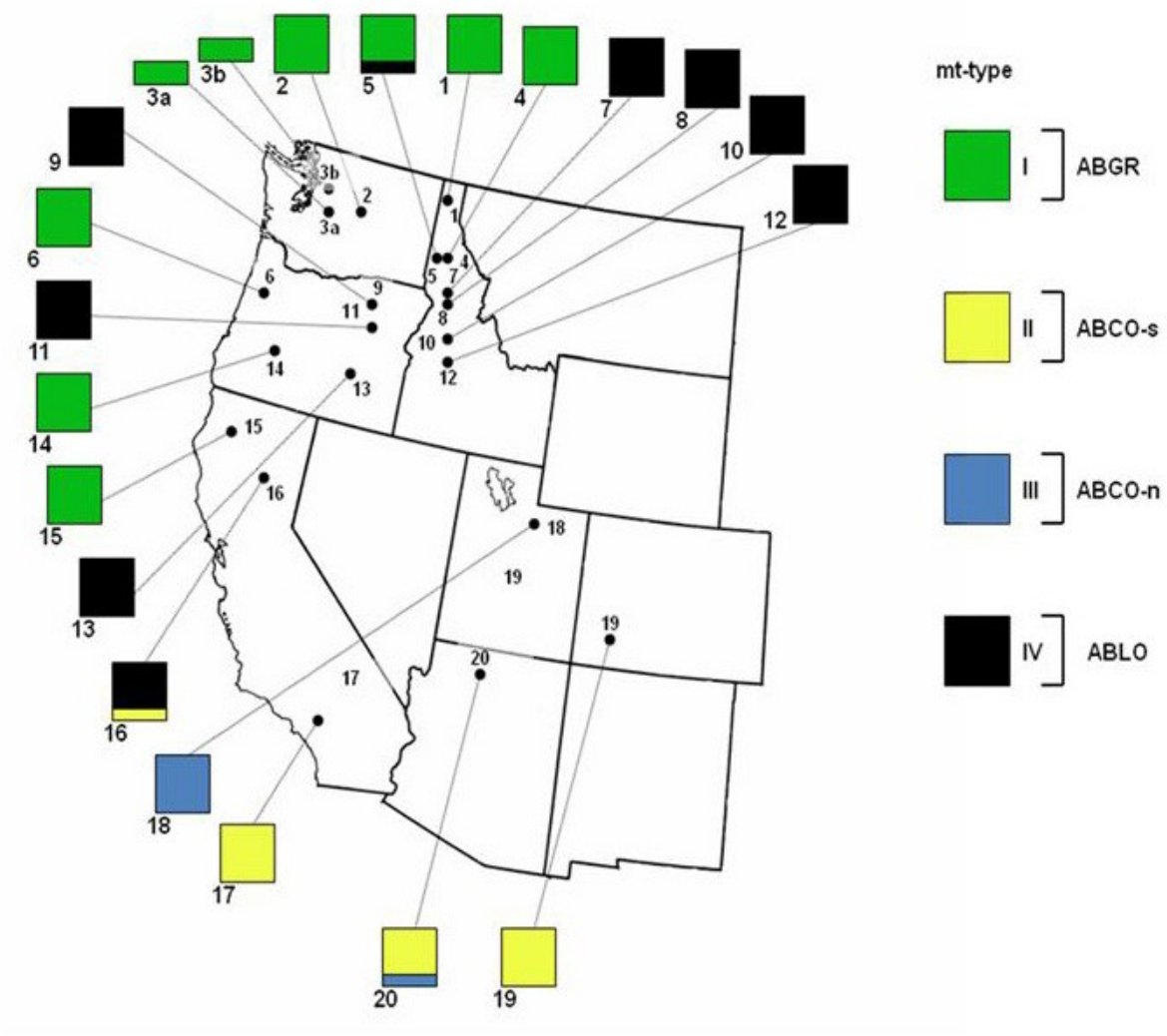

Figure 1.6 Geographic distribution of mitochondrial haplotypes (mt-types). ABGR represents *Abies grandis* ; ABCO-s represents *Abies concolor* of the southern Rocky Mountains; ABCO-n represents *Abies concolor* of the central Rocky Mountains; ABLO represents *Abies concolor* variety *lowiana* of the Sierra Mountains.

Tables

pop.	ID	Latitude	Longitude	Elevation (ft.)	State and County
TC	ABGR	48º 17' 28" N	116º 19' 42" W	2291	Bonner Co., ID
RL	ABGR	48º 02' 49" N	122º 13' 25" W	176	Snohomish Co., WA
SN	ABGRº	47º 23' 47" N	121º 23' 35" W	2575	Kittitas Co., WA
CL	ABGRº	46º 45' 47" N	116º 14' 49" W	2928	Clearwater Co., ID
LA	ABGRº	46º 45' 13" N	116º 54' 40" W	2712	Latah Co., ID
WS	HYB	45º 52' 37" N	116º 05' 47" W	4310	Idaho Co., ID
DR	HYB	45º 44' 58" N	116º 10' 05" W	4696	Idaho Co., ID
PR	HYB	45º 17' 11" N	118º 58' 04" W	2439	Umatilla Co., OR
BS	HYB	44º 55' 54" N	116º 09' 07" W	5388	Valley Co., Idaho
AL	ABGR	44º 39' 44" N	123º 14' 21" W	605	Benton Co., OR
IC	HYB	44º 15' 09" N	118º 41' 47" W	6800	Grant Co., OR
SID	HYB	44º 12' 56" N	116º 12' 42" W	5170	Valley Co., Idaho
SM	HYB	42º 46' 15" N	118º 43' 01" W	6362	Malheur Co., OR
KL	HYB	42º 45' 20" N	122º 04' 19" W	4371	Klamath Co., OR
LK	HYB	42º 23' 51" N	122º 11' 26" W	4961	Lake Co., OR
M	HYB	42º 23' 26" N	122º 25' 42" W	3461	Jackson Co., OR
GP	HYB	42º 16' 04" N	122º 36' 51" W	5800	Jackson Co., OR
WAH	HYB	42º 15' 39" N	120º 13' 59" W	6669	Lake Co., OR
WAL	HYB	42º 14' 02" N	120º 17' 35" W	5836	Lake Co., OR
KS	HYB	42º 13' 59" N	120º 46' 16" W	5456	Lake Co., OR
TB	HYB	42º 06' 57" N	122º 26' 31" W	4262	Jackson Co., OR
N	HYB	42º 04' 43" N	122º 47' 18" W	5435	Jackson Co., OR
S	HYB	42º 03' 05" N	122º 41' 35" W	4620	Jackson Co., OR
WE	HYB	40º 46' 49" N	122º 57' 39" W	4559	Trinity Co., CA
OW	ABCO	40º 36' 48" N	111º 07' 51" W	7543	Weber Co., UT
PL	ABLO	39º 54' 14" N	120º 32' 45" W	5827	Plumas Co., CA
WF	ABCO	38º 58' 43" N	111º 23' 50" W	8373	Sevier Co., UT
SS	ABCO	37º 33' 28" N	107º 49' 27" W	8421	La Plata Co., CO
FL	ABCO	35º 17' 08" N	111º 36' 52" W	7370	Coconino Co., AZ
BB	ABLO	34º 14' 29" N	116º 58' 28" W	6844	San Bernardino Co., CA

Table 1.1. All study populations are listed with field identification; ABGR represents pure populations of *A. grandis*, ABGRº represents nearly pure *A. grandis*, ABCO represents *A. concolor*, ABLO represents *A. concolor* variety *lowiana* (Liu, 1971) of the central and northern Sierra Mountains, and HYB represents populations that intergrade between *A. grandis* and *A. concolor variety lowiana*.

ID	Latitude	Longitude	El. (ft)	Location
Abies amabilis	44º 25' 17" N	121º 51' 25" W	4774	Linn, County, OR
Abies lasiocarpa-1	42º 52' 37" N	122º 17' 28" W	4940	Klamath County, OR
Abies lasiocarpa-2	44º 25' 46" N	121º 54' 47" W	4000	Linn, County, OR
Abies procera	43º 05' 22" N	122º 05' 30" W	5926	Klamath County, OR
Abies religiosa	19º 24' 00" N	101º 33' 36" W	8477	Cerro Burro, Mexico
Abies religiosa	15º 38' 36" N	91º 31' 09" W	10037	Soloma, Guatemala
Abies guatemalensis	15º 15' 34" N	92º 12' 46" W	9202	Niquivil, Mexico
Abiesguatemalensis	14º 31' 40" N	90º 08' 54" W	8684	Mataquescuinla, Guat.
Abies balsamea	N/A	N/A	N/A	Maine
Abies fraseri	N/A	N/A	N/A	Mitchell, NC
Abies vejarii	N/A	N/A	N/A	Tamaulipas, Mexico
Abies alba	N/A	N/A	N/A	U. of Washington Camp.
Abies firma	N/A	N/A	N/A	U. of Washington Camp.
Keteleeria evelyniana	N/A	N/A	N/A	U. of Washington Camp.
A. grandis X A. concolor (3)	N/A	N/A	N/A	Inst. of Forest Genetics

Table 1.2 Out-group taxa included in the phylogenetic study, but not involved in the *A. grandis-A. concolor* intergrading complex. The 3 samples of *A. grandis X A.concolor* are F1 hybrids obtained from controlled crossing studies at the Institute of Forest Genetics, Placerville California (Critchfield, 1988).

Region	Primer Sequence 5'-3'	(bp)	Ta	Primer Reference
	Chloroplast Genome			
*psb*A-*trn*H		≈580	52°C	T. Sang et al. 1997
	*psb*A-F GTTATGCATGAACGTAATGCTC			
	*trn*H-R CGCGCATGGTGGATTCACAAATC			
*trn*L-*trn*F		≈460	50°C	Y. Suyama et al. 2000
	*trn*L-F TTGGCTTTATAGACCGTGAG			
	*trn*F-R CCAGGAACCAGATTTGAACT			
*trn*R-*trn*N		≈850	53°C	Y. Suyama et al. 2000
	*trn*R-F GCCTGTAGCTCAGAGGATTA			
	trnN-R TCCTCAGTAGCTCAGTGGTA			
	Mitochondrial Genome			
*nad*1 Intron 1		≈1500	54°C	K. Isoda et al. 2000
	301-F GCAGCTCAAATGGTCTCTTACGA			
	302-R GGGAACAAGGGAATACCAAACC			
	303-F AAGTGATGATGATGCCCCTATTG			
	304-R CCTCCGCAAGGTTCTAAATTGG			
*nad*5 Intron 1		≈1000	52°C	X-Q. Wang et al. 2000
	*nad*5af-F GGAAATGTTTGATGCTTCTTGGG			
	*nad*5br-R CTGATCCAAAATCACCTACTCG			
*nad*5 Intron 4		≈750	52°C	J. Wu et al. 1998
	*nad*5in54 ATAAGTCAACTTCAAAGTGGA			
	*nad*5in34 CATTGCAAAGGCATAATGAT			

Table 1.3. Primers used to amplify the three chloroplast intergenic spacers and three mitochondrial introns.

Pop.	ID	Coordinates	State and County	N^{cp}	cp H^{nei}	N^{mt}	mtH^{nei}	CP/MT
TC-1	ABGR	48.291º N 116.328º W	Bonner Co., ID	3	.6	1	0	+
RL-3a	ABGR	48.047º N 122.224º W	Snohomish Co., WA	1	0	1	0	+
RL-3b	ABGR	47.075º N 122.893º W	Snohomish Co., WA	1	0	1	0	+
SN-2	ABGRº	47.396º N 121.393º W	Kittitas Co., WA	3	.524	1	0	+
CL-4	ABGR°	46.763º N 116.247º W	Clearwater Co., ID	3	.714	1	0	+
LA-5	ABGR°	46.754º N 116.911º W	Latah Co., ID	4	.75	2	.356	- +
PR-9	HYB	45.286º N 118.968º W	Umatilla Co., OR	3	.75	1	0	-
BS-10	HYB	44.932º N 116.152º W	Valley Co., Idaho	3	.607	1	0	+-
AL-6	ABGR	44.662º N 123.239º W	Benton Co., OR	2	.356	1	0	+
IC-11	HYB	44.253º N 118.696º W	Grant Co., OR	4	.810	1	0	- +
SID-12	HYB	44.216º N 116.212º W	Valley Co., Idaho	6	.952	1	0	- +
SM-13	HYB	42.771º N 118.717º W	Malheur Co., OR	4	.694	1	0	- +
KL-14	HYB	42.756º N 122.072º W	Klamath Co., OR	5	.760	1	0	+
WE-15	HYB	40.780º N 122.961º W	Trinity Co., CA	N/A	N/A	1	0	+
OW-18	ABCO	40.619º N 111.138º W	Weber Co., UT	2	.4	1	0	+
PL-16	ABLO	39.904º N 120.546º W	Plumas Co., CA	2	.556	2	.356	+
SS-19	ABCO	37.558º N 107.824º W	La Plata Co., CO	1	0	1	0	+
FL-20	ABCO	35.286º N 111.614º W	Coconino Co., AZ 1	1	0	2	.356	+
BB-17	ABLO	34.241º N 116.974º W	San Bernardino Co., CA	2	.2	1	0	-

Table 2.4. Cp-type and mt-type diversity in the *Abies grandis-Abies concolor* complex. ABGR represents pure *A. grandis* populations; ABGR° represents nearly pure *A. grandis*; ABCO represents *A. concolor* of the central and southern Rocky Mountains; ABLO represents *A. concolor* variety *lowiana* of the Sierra Mountains and HYB represents intergradient populations. Number of chloroplast haplotypes, N^{cp}; number of mitochondrial haplotypes, N^{mt}; Nei's haplotype diversity, H^{nei}; and cp-type/mt-type congruence are listed for each study population. + indicates congruence and - represents incongruence.

Chapter 2

Population Genetic Analysis of the *Abies grandis-Abies concolor* Complex

Abstract

Hybridization is an important evolutionary process with a spectrum of trajectories ranging from the reinforcement of reproductive boundaries to hybrid speciation. It has become increasingly clear that in many hybrid zones, environmental factors are important determinants of the relative fitness of hybrids and parental taxa. The effective identification of hybrids and delineation of hybrid populations is a major prerequisite to the assessment of correlations between hybrid and parental genotypes and ecological variables that may determine their relative fitness. In this study we investigate the spatial population structure of the *Abies grandis-Abies concolor* complex. Thirty populations spanning the entire complex were genotyped at 6 microsatellite loci and percentage ancestry was estimated using a Bayesian clustering program with an admixture model. These results were compared with a morphometric analysis based on a suite of six foliage characters. We demonstrate that all of the morphologically intergradient populations between 40° N in the Sierra Nevada Mountains and 45.8° N in the Rocky Mountains have admixed ancestry and are characterized by higher levels of allelic richness compared to pure populations. There is evidence of extensive introgression into populations of *A. concolor* south of this stabilized hybrid zone and some introgression was detected in nearly pure *A. grandis* as far north as 47.4° N in the Cascade Range and 46.7° N in the Rocky Mountains.

Introduction

Abies grandis and *A. concolor* are widely distributed throughout many forests of western North America. *A. grandis* inhabits mesic, low to middle elevation forests of the Pacific Northwest and northern Rocky Mountains, while *A. concolor* occurs further south in more xeric middle to high elevation forests in the southern Sierra Mountains and Mountains of southern California, central and southern Rocky Mountains and in the Mountains of northwest Mexico (Daniels, 1969; Liu, 1971). Although these taxa are considered to be ecologically and morphologically distinct within these regions, there is an expansive region in between where they intergrade, Fig 1. (Daniels, 1969; Frederick, 1977; Hamrick and Libby, 1972; Zobel, 1973).

Intergradient populations have been previously characterized chemically, (Houkal, 1977) and morphologically, (Daniels, 1969; Frederick, 1977; Hamrick and Libby, 1972; Zobel, 1973), as generally intermediate between pure *A. grandis* and *A. concolor* variety *lowiana* of the Sierra Mountains, but with higher levels of variability than the parental taxa exhibit. Phenotypes range from nearly pure *A. grandis* to nearly pure *A. concolor* to phenotypes with characters that are intermediate to the parental taxa or that have combinations of characters of the parental taxa. Utilizing the hybrid index method of Anderson (1949) these researchers have concluded that intergradient populations comprise an expansive hybrid zone. Further evidence of an expansive hybrid zone comes from a recent molecular study (Ott, 2006), which discovered that intergradient populations east of the crest of the Cascade Mountains, between 46° and 42° N were dominated by individuals with the incongruent combination of a paternally inherited chloroplast DNA marker of *A. grandis* and a maternally

inherited mitochondrial DNA marker of *A. concolor* of the central Sierra Mountains. The regional forms of *A. concolor* have been variously treated as a single species, (Farjon, 1990; Harlow and Harrar, 2001), as subspecies with the form of the southern Cascade, Siskiyou and Sierra Mountains designated as *A. concolor var. lowiana*, (Liu, 1971), or as separate species with the form of the Sierra Mountains designated as *A. lowiana*, and the form of the central and southern Rocky Mountains designated *A. concolor,* (Hunt, 1993), with apparent disjunct populations in the southern Cascade and Siskiyou Mountains and the Transverse Range of southern California (Figure 2). In many respects, *A. concolor* of the Sierra Mountains intergrades between the hybrid populations to the north and *A. concolor* of the Transverse Range, which more closely resembles *A. concolor* of the Southern Rocky Mountains (Hamrick and Libby, 1972).

Molecular data revealed incongruence of chloroplast DNA markers and mitochondrial DNA markers among a population of *A. concolor* of the Transverse Range of southern California (See Chapter 1), which had a chloroplast DNA marker of the Sierra Nevada form and the incongruent mitochondrial DNA marker of the southern Rocky Mountain form of *A. concolor*. Though incongruence of DNA markers with differential patterns of inheritance can be used to help identify hybrids, there are theoretical and practical limitations of this methodology. The retention of ancestral polymorphisms through divergence events (incomplete lineage sorting) can result in similar patterns (Wendel and Doyle, 1998). Additionally, incongruence of cytoplasmic markers doesn't reveal the relative genetic composition of the nuclear genome and frequently can not detect introgression along the margins of hybrid zones due to repeated back crossing with parental taxa.

The objectives of the present study are to 1) determine the spatial population structure of pure and intergradient populations spanning the entire complex; 2) assess the relative genetic composition and diversity of intergradient populations and determine the extent of introgression into populations along its margins; 3) evaluate likely migration routes; 4) and correlate relative genetic composition with specific morphological characteristics of pure and hybrid populations. This study should facilitate delineation of pure and intergradient populations of the complex, inform a more uniform taxonomic treatment and enable further genetic and ecological study of this complex.

Methods

Molecular Analysis

The study area encompasses the entire *Abies grandis-Abies concolor* complex. Sampling localities span the hybrid zone of central Idaho, northwest California, northeast Oregon, southwest Oregon and include reference populations of phenotypically pure parental species throughout their collective range (Figure 1). The collection transects were designed to sample all major regional forms of pure and intergradient populations, which have been previously characterized through morphometric analysis, terpene analysis and common-garden studies (Daniels, 1969; Frederick, 1977; Hamrick and Libby, 1972; Lacaze and Tomassone, 1967) across the entire range of environmental conditions they inhabit. At each location, (Table 1), 4-6” of a foliage-bearing branch tip within 3 meters of the ground was collected from 4-15 individuals that were randomly selected. Approximately 100 mg of

fresh or 40 mg dry foliage was removed from each sample and ground with mortar and pestle in liquid nitrogen. Total genomic DNA was extracted and purified using Qiagen DNeasy Plant Minikits. Extracted DNA was then quantified using a Beckman DU 640 spectrophotometer (Beckman Coulter) and stored in elution buffer at -20° C. Two leaves from each sample of second year growth were removed for morphometric evaluation and each specimen was then pressed and deposited in the University of Idaho Stillinger Herbarium.

Twenty three microsatellite loci developed for other *Abies* (Table 2) were screened for amplification with a panel of two grand fir from the northern Rocky Mountains, two concolor fir samples from the Sierra Mountains and two hybrid samples from central Idaho following the published polymerase chain reaction (PCR) conditions (Chunlan *et al*., 2007; Cremer *et al*., 2005; Hansen *et al*., 2005; Rassmussen *et al*., 2008; and Saito *et al*., 2005). PCR product was visualized on 1.5% agarose gels stained with Sybr Safe and 1kb ladder for imaging. The forward primers for each locus that amplified within or near the range of reported allele sizes were labeled at the 5' end with VIC, NED, TET, HEX or FAM, (Applied Biosystems) and PCR was repeated with the same samples. To verify amplification of microsatellite loci and assess polymorphism 1ul of PCR product was added to 10ul of formamide containing LIZ 500 size standard (Applied Biosystems) and analyzed with an ABI 3130xl capillary sequencer and Genemapper software.

Six primer pairs reliably amplified polymorphic products, Table 2. Six other loci, As16, As07, SF g6, SF 78, SF 333 and NFH3, amplified product, but did not generate useable amplicons and were not included in further analysis. Two multiplexes were developed using

the Qiagen multiplex kit as follows; Mulitplex 1 included .07uM forward and reverse primers for Abfi18 (Saito *et al.,* 2005) and NFF3 (Hansen *et al*., 2005) in a 7ul total volume reaction with 1ul of total genomic DNA. Multiplex 2 included .056uM forward and reverse primers for As09, As13, As20 (Chulan *et al.,* 2007) and SF 50 (Cremer *et al*., 2005) in a total reaction volume of 7ul with 1ul of total genomic DNA. Thermal cycling was completed with a Tetrad2 Thermal Cycler (MJ Research) with the following profiles; Multiplex 1, denaturation for 15 minutes followed by 10 cycles with 30s at 94 °C, annealing temperature starting at 62 °C and dropping 0.4 °C per cycle, 1 minute extension at 60 °C, followed by 25 cycles of 30s at 94°C, 30s at 58°C, 1 minute extension at 60°C followed by 15 minutes at 60°C; For Multiplex 2, we used, denaturation for 15 minutes followed by 10 cycles with 30s at 94 °C, annealing temperature starting at 60 °C and dropping 0.4 °C per cycle, 1 minute extension at 60 °C, followed by 25 cycles of 30s at 94°C, 30s at 56°C, 1 minute at 60°C followed by 15 minutes at 60°C. Amplification products were then analyzed as above. The exact test of Guo and Thompson (1992) was used to estimate deviation from Hardy-Weinberg equilibrium and linkage disequilibrium for each study population and locus utilizing the program Genepop with Markov chain parameters, dememorization, batches and iterations per batch set at 10000 (Raymond and Rousset, 1995; Rousset, 2008). These parameter values are set much higher than the default values because they reduce the standard error in the P-value estimate. Within population fixation index coefficients, F_{is}; fractionated allelic richness, N_a; and pair-wise fixation index coefficients, F_{st}; (Weir and Cockerham, 1984) were determined for all study populations with the program F-stat (Goudet, 1995).

We used the Bayesian clustering program STRUCTURE 2.2 (Pritchard *et al*., 2000), to estimate the number of populations contributing to the observed genetic variation, using the ΔK criterion of Evanno *et al.* (2005). The algorithm of STRUCTURE describes the probability of the multi-locus genotypes given the number of source populations that have contributed to the variability of allele frequencies (ln P(D/K). The maximum number of source populations (K) was tested with ten replicates each for K =1 to 30 with a burn-in period of 100,000 replicates followed by 1,000,000 Markov Chain Monte Carlo (MCMC) replicates. The results indicate K=3 as the most probable number of source populations contributing to the genetic variation within the complex, which corresponds with the following three groups; 1) an intergradient group in the northern Sierra, Siskiyou and Klamath Mountains, 2) a group that includes *A. concolor* of the central and Southern Rocky Mountains,, and 3) a group that includes *A. grandis*. This analysis that determines maximum hierarchical population structure within the complex also permits assessment of admixture of individual genotypes in further analyses.

The ancestry of all individuals was then assessed with STRUCTURE (Pritchard *et al.,* 2000) using the admixture analysis with the same parameters as above and the number of populations (K) set at three. In this case the admixture model assumes the maximum number of source populations is three and determines the most probable proportion of ancestry from the three groups based on allele frequencies for each individual with an ancestry coefficient in the form of a q-value with 90% credibility intervals. Previous studies have evaluated the use of conservative and relaxed criteria to determine the significance of percentage ancestry estimates. The more conservative method determines percentage

ancestry is significant if the 90% credibility intervals of a q-value do not overlap 0, however in many cases with a limited number of loci, the 90% confidence intervals are quite wide. In these situations this conservative method can underestimate admixture and frequently researchers arbitrarily set a threshold q-value when determining admixture in hybrid studies with Bayesian clustering programs (Vaha and Primmer, 2006). While numerous studies have used a q-value threshold of 0.1, (Bohling *et al.,* 2011) we used a slightly more conservative q-value threshold of 0.15 in this study.

Morphometric Analysis

Previous studies (Daniels, 1969) discovered several quantitative foliage characters that had strong geographic patterns of variation across the complex, Table 3. Six characters were selected from the suite of characters available based on three criteria; 1) they exhibited strong geographic patterns of variation, 2) were easily evaluated, and 3) were unambiguous. All characters were measured at 30X magnification with a dissecting microscope. The angle of the apex of each leaf (LAA) was categorized as < 60°; 60°-90°; 90°-120°; or >120°. The depth of the apical notch (DAN) was categorized as absent; present but too small to measure; measurable but <0.1 mm; 0.1-0.2 mm; or >0.2mm. Adaxial leaf groove (ALG) was categorized as absent; present but basal only <0.25% of leaf length; present 0.25-0.5% of leaf length; or full length. Rows of adaxial stomates (RAS) were recorded at three reference points, 0.5 mm above the petiole (RAS-b); mid-leaf (RAS-m); and 0.5 mm below the apex (RAS-a,), Figure 3a-d. The characters bark periderm color and leaf angle were not used because many of our samples were represented by pressed foliage

only. Two second year leaves were randomly selected and removed from each specimen that was collected and cataloged for the molecular study. Principal component analysis (PCA) was performed on all six characters to assess character variation within and among intergradient and pure populations. The nonparametric Wilcoxen test for differences between means of all population pairs and nonparametric regression analysis to assess correlations between latitude and morphometric characters were also performed.

Results

Morphometric Analysis

The morphometric characters were measured on two randomly selected leaves from ten putative hybrid populations with 104 individuals, six populations of *A. grandis* with 54 individuals, six populations of *A. concolor* with 37 individuals, and two populations with 10 individuals of *A. concolor* variety *lowiana* of the Sierra Mountains and 10 individuals of the Transverse Range of southern California. Statistics are summarized for each of the characters within these groups, Table 4. The populations of *A. grandis* all had a full-length adaxial groove and an apical notch that varied from 0.10 to >0.25mm in depth, a variably rounded apex and no rows of adaxial stomata at mid-leaf or distal third. Two individuals in each of populations SN, LA and CL had 1-3 partial rows of adaxial stomata on the apical third of the leaf. One individual in population AL had a few adaxial stomates restricted to the apical notch, but the remainder of *A. grandis* populations didn't have any adaxial stomates. All samples of *A. concolor* of the southern and central Rocky Mountains had several full-length rows of adaxial stomates with an average across all populations of 10.6; variably

pointed apices that ranged from <60° to 90°; apical notch absent or minute and usually no adaxial groove, though there were several samples with a basal groove < 0.25 of leaf-length and a few samples in population SS with adaxial grooves up to 0.5 leaf-length. The form of *A. concolor* variety *lowiana* of the Sierra Mountains is represented by a single population, PL, which typically had fewer full-length rows of adaxial stomata than *A. concolor* of the Rocky Mountains with an average of 6.2; apices usually 90°-120° with a few <90° and a few >120°; an apical notch usually minute to 0.1mm deep, though a notch was absent on a few sampled leaves.

Population BB of the Transverse Range of Southern California was characterized by an average of 7.7 rows of adaxial stomates for the entire leaf, with apices most commonly 60°- 90° with a few 90°-120° and a basal adaxial groove absent to 0.5 leaf-length, though basal on most. Nearly all of the hybrid populations had a prominent full-length adaxial groove, except one individual in population SM (0.5 groove) and apices that ranged from 60°-120°; and a highly variable number of rows of adaxial stomates ranging from a few rows restricted to the apical third to several full-length rows with an average of 3.2 rows. Overall, hybrid populations ranged from nearly pure grand fir morphology, most commonly among populations closest to *A. grandis* populations, to phenotypes representative of nearly pure *A. concolor* of the Transverse Range, population BB. Regression analyses of morphometric characters along latitudinal transects through the Rocky Mountains revealed strong correlations between latitude and morphometric characters with *A. grandis* like character states being positively correlated with latitude and *A. concolor* like character states being

negatively correlated with latitude. The same pattern was detected in a Sierra and Cascade Mountain transect, but with generally lower R^2 values, Table 5.

Principal component analysis is particularly useful in morphometric studies because it converts numerous possibly correlated characters into uncorrelated vectors which describe the inner structure and variance in the data, usually with relatively few vectors accounting for most of the variability. The correlation matrix recovered from the principal component analysis shows that the relative abundance of adaxial stomates, RASa-b, are negatively correlated with a rounded and notched apex and an adaxial groove, Table 6. All six variables contribute to the eigenvector F1, though RASa-m contributes the most as shown in Table 7. The second eigenvector F2 is most influenced by notch depth, which accounts for 77.6% of the variation, though RASa-d also contribute significantly. The eigenvalues are depicted with a scree-plot in Figure 4. Eigenvector F1 accounts for 77.6% of the variability and F2 for 8.43%. Thus, these first two eigenvectors include 86% cumulative variability.

The bi-plot of the first two eigenvectors reveals two partially overlapping clusters, Figures 5 and 6. The first includes putative hybrids that range from -2.5 with *A. grandis* to 2 with *A. concolor* variety *lowiana* of the central Sierra Mountains of population PL along the eigenvector F1. The distribution of this cluster along F2 ranges from 2 to -2 and it is noteworthy that F2 is largely influenced by notch depth. The second group ranges from approximately 5 with *A. concolor* of the central and southern Rocky Mountains to slightly >2 with population BB of the Transverse Range of Southern California. The Wilcoxon test for differences between all population means for F1 was performed, Figure 7. This analysis suggests the first cluster is comprised of a gradient of partially overlapping phenotypes from

pure *A. grandis* in the north to nearly pure *A. grandis* to several groups of hybrids that culminates in an intergradient group that includes population WH of the Warner Mountains in southern Oregon, population WE of Trinity county California and population PL of Plumas County California. It is noteworthy that population BB of the Transverse Range of southern California appears to be more similar to *A. concolor* of the Southern Rocky Mountains than it is to population PL of the central Sierras.

Population Genetic Analysis

Six-locus genotypes were determined for eighteen putative hybrid populations with a total of 191 individuals, six *A. grandis* populations with a total of 54 individuals, five *A. concolor* populations of the central and southern Rocky Mountains with a total of 50 individuals, one population of *A. concolor* variety *lowiana* of the central Sierra Mountains with 10 individuals and a population of 10 individuals of the Transverse Range of southern California. F-statistics are summarized per population across all loci in Table 8. The apparent departure from Hardy-Weinberg equilibrium for loci SF50 and ABFi18 as well as a heterozygote deficiency for all loci are particularly noteworthy. Fractionated allelic richness, N_a, is highest among putative hybrid populations, 4.919-8.041, and *A. concolor* variety *lowiana* populations PL and BB of the Sierra Mountains, 7.064-7.101, while *A. grandis* and *A. concolor* of the southern and central Rocky Mountains range 3.596-5.767 and 4.467-5.82 respectively. One population each of *A. concolor* of the southern Rocky Mountains, FL, and *A. concolor* variety *lowiana* of the Transverse Range of southern California BB, as well as putative hybrid populations, PR, and ,WA, had significant departure from Hardy-Weinberg

equilibrium, <0.05. The subpopulation fixation index, F_{is}, was >0.1 for the geographically and ecologically isolated populations of *A. concolor* variety *lowiana* of the Transverse Range, BB, and *A. concolor* of the southern Rocky Mountains, FL, as well as the ecologically isolated hybrid population WA of Southern Oregon. A chart of Fst values between population pairs is in appendix II.

Utilizing the ΔK criterion of (Evanno, 2005) it was determined the complex has the highest probability of consisting of three groups; 1) a stabilized hybrid group consisting of morphologically intergradient individuals distributed primarily from the Siskiyou, Klamath and central Sierra Mountains, 2) *A. concolor* of the southern and central Rocky Mountains, and 3) *A. grandis*. A tree depicting the relationships between these three clusters based on net nucleotide distance was computed with the Neighbor Joining algorithm (Saito and Nei, 1987), PHYLIP (Felsenstein, 2005), Figure 8. Based on net nucleotide distances group two, *A. concolor* and group three, *A. grandis* are separated by the greatest distance, 0.0762, and group one of intergradient individuals is closer to both group two, 0.0155, and group three, 0.0513.

The Bayesian clustering program STRUCTURE (Pritchard, 2000) was used with the admixture model and a burn-in period of 100,000 reps followed by 1,000,000 MCMC reps to estimate the ancestry of each individual with a q-value that includes 90% confidence intervals. Theoretically these estimates of admixture can vary between replicates and many researchers have averaged percentages over several runs. However, in the present study percentage ancestry values varied by <.001 between replicates and the results from a single run only are reported here.

The geographic pattern of percentage ancestry estimated with STRUCTURE (Pritchard, 2000) utilizing the admixture model for each individual within study populations is depicted with bar charts in three geographic transects, Figures 9-11. The Rocky Mountain transect depicts a clinal transition from pure *A. grandis* (group 3) with all individuals >0.95 in the north into populations that are highly variably admixed south of 46° N with a general decrease in percentage ancestry of *A. grandis* (group 3) and an increase in significant but highly variable ancestry, >0.15, of groups one and two to the south through central Idaho and eastern Oregon with the notable absence of any pure *A. grandis* or *A. concolor* from the central and southern Rocky Mountains. The southern end of the Rocky Mountain transect from 40º 36' 48" N to the south is relatively uniform in pure or nearly pure *A. concolor* (group 2), Figure 9. Population means of ancestry coefficients for *A. concolor* (group 2)*, A. grandis* (group 3) and stabilized hybrid (group 1) are depicted in Figure 12 and listed in Table 10. Lowess regression analysis confirms percent ancestry for all three groups is strongly correlated with latitude in the Rocky Mountains, Table 11.

The Pacific slope transect is quite similar to the Rocky Mountain transect, as it also depicts a clinal transition from pure *A grandis* (group three) in the northernmost populations >0.90 into populations that are highly variably admixed south of 47° N with a general decrease in percentage ancestry of *A. grandis* (group 3) and an increase in significant but highly variable ancestry, >0.15, of groups one and two to the south through southern Oregon. There are some individuals with >0 .95 ancestry to group one and the notable absence of any pure individuals of *A. grandis* (group 3) or *A. concolor* of the southern and central Rocky Mountains (group 2).

There is a transition from the highly admixed populations of southern Oregon which include individuals with significant ancestry from all three groups to populations with an increase in percentage ancestry of *A. concolor* (group 2), and a major decrease in the influence of *A. grandis* (group 3) to the south through the southern Sierra Mountains, Figure 10 and Table 10. Lowess regression analysis confirms the correlation between latitude and the relative genetic influence of *A. grandis* (group 3) and *A. concolor* (group 2) on this transect, Table 11, though the relationships are not as strong as on the Rocky Mountain transect. A third longitudinal transect transitions from an intergradient population in the Cascade Mountains of south-central Oregon with individuals that have highly variably admixed ancestry from all three groups through disjunct populations in the Warner and Steens Mountains further east. Along this transect there is a decrease in the influence of *A. grandis* (group three) and an increase in the influence of group one and *A. concolor* of the central and southern Rocky Mountains (group two), Figure 11 and table 10.

A third transect was constructed to sample populations occupying sites with maximum contrasting ecological conditions in a minimum of geographic distance. Accordingly, populations were sampled from the north slope, study population N, and south slope, study population S, of Mount Ashland in the Siskiyou Mountains of southern Oregon. Individuals were sampled randomly from population S between 4620 to 4800 feet elevation on aspects from 160°-200°. Common associates on this site include *Pinus ponderosa*, *Calocedrus decurrens* and *Arctostaphylos patula*. Individuals from population N were sampled randomly from 5300 to 5435 feet elevation on aspects from 330°-40°. Common associates include *Abies shastensis*, *Taxus brevifolia*, *Clintonia uniflora* and *Asarum*

caudatum. Percentage ancestry differed between these populations based on q-values including 90% confidence intervals, with a greater influence or percentage ancestry of *A. grandis* (group two) in population N and a higher percentage ancestry of group one in population S, Figure 10 and Table 11.

Discussion

The overall geographic patterns of genetic and morphological variation reveal pure populations of *A. grandis* in the northern Rocky Mountains and northern coastal region and pure populations of *A. concolor* in the southern and central Rocky Mountains, with populations of highly variable admixed ancestry spanning the region between. Pure populations of *A. grandis*, RL and TC, are characterized genetically by ancestry coefficients > 0.85 to their own group and relatively low levels of allelic richness, < 4, in comparison to populations with significant admixed ancestry. Pure *A. grandis* populations are typified morphologically by; 1) a full-length adaxial groove, 2) an apical notch > 0.15mm deep, 3) virtually no adaxial stomates and, 4) a rounded apex >90°. Though bark periderm color was not used in the present study, Daniels (1969), completed extensive bark tallies in this region and discovered these populations have virtually 100% red periderm. Nearly pure populations of *A. grandis*, LA, CL and SN, further inland and to the south of pure populations show subtle signs of introgression from completely intergradient hybrid populations further south. While these populations are predominantly made up of individuals with pure phenotypes and >0.85 ancestry to *A. grandis*, they also include a few individuals with < 0.85

ancestry to *A. grandis,* a few individuals with 1-3 partial rows of adaxial stomates and apical notches <0.15mm in depth. These nearly pure populations have slightly higher allelic richness than pure populations, 4.523-5.767. Further evidence of introgression in *A. grandis* from this region come from previous bark tallies that discovered up to 5% of trees sampled had yellow periderm (Daniels, 1969).

Pure populations of *A. concolor* are comprised of many disjunct populations in the central and southern Rocky Mountains with noted differences in morphology (Hunt, 1993) and chemically (Zavarin, 1975). Further evidence of variation between regional populations comes from a molecular study (see chapter 1) that discovered regional differences in mitochondrial haplotypes and some chloroplast haplotypes between central and southern Rocky Mountain populations. However, in the present study we find that nearly all of these *A. concolor* populations are characterized genetically by >0.85 ancestry to their own group. Noteworthy exceptions to this general pattern occur in populations OW and WF, which each include individuals with < 0.85 ancestry to *A. concolor*. Pure *A. concolor* is characterized morphologically as follows: 1) average > 10 rows of adaxial stomates; 2) basal to absent adaxial groove (occasionally has a basal groove < 0.5 leaf length in population SS); 3) a pointed to acute apex <90°, and 4) a minute to absent notch (Table 4). As noted, minor differences exist between SS of the southern Rocky Mountains, which included a few individuals with nearly 0.5 leaf length adaxial groove and populations FL, WF and OW did not.

Although the present study did not include analyses of bark periderm color, a previous study (Daniels, 1969) discovered *A. concolor* of this region included populations

with predominantly yellow periderm, but found some populations with a large percentage of individuals with red periderm. There is general agreement on the morphological characteristics and geographic distribution of these pure taxa between our findings and the contemporary taxonomic treatments of *Abies* (Hunt, 1993; Liu, 1971; Farjon, 1990) as well as the comprehensive morphometric analysis of Daniels (1969).

Admixed populations resemble a morphological and genetic gradient from nearly pure *A. grandis* based on morphometric analysis and ancestry coefficients at approximately 46° in the Rocky Mountains and Cascade Range to nearly pure *A. concolor* of the Transverse Range in Southern California, population BB, with the relative influence of these taxa being positively and negatively correlated with latitude in a clinal manner, Table 7 and Table 11. This general pattern is complicated by the genetic influence of a third source population, (group 1), that appears to have the greatest influence in populations of the southern Cascade, Siskiyou and northern Sierra Mountains, Table 10 and Figure 12. We didn't find any pure individuals with ancestry coefficients >0.85 of *A. concolor* or *A. grandis,* within this region, however approximately 20% of the individuals did have ancestry coefficients > 0.90 for group 1. Based on net nucleotide distance, group 1 is somewhat intermediate to *A. concolor* and *A. grandis* but more similar to *A. concolor*, Figure 8. Morphologically group 1 has much higher levels of variability than the pure taxa with no apparent difference between individuals with > 0.90 ancestry coefficients for group one and variably admixed individuals in populations from this region.

It is probable that group one is a regional form of *A. concolor* with significant introgression from *A. grandis* that occurred at some time in the past, which is consistent

with intermediate genetic distance to the pure taxa (Figure 8) and highly variable intergradient morphological characteristics of the individuals with high ancestry coefficients to group one. Populations of this region where group one has the highest genetic influence were characterized by relatively high haplotype diversity and the incongruent combination of a chloroplast haplotype of *A. grandis* and a mitochondrial haplotype of the Sierra Mountain segregate of *A. concolor* (see chapter 1). It is also important to note that populations within this region typically had wide 90% credibility intervals around ancestry coefficients, which indicates relatively weak statistical support for these estimates for individuals with highly admixed ancestry typical of this region. Another important characteristic of populations within this region is that they include individuals of admixed ancestry from all three groups, with a positive correlation between latitude and the relative influence of *A. grandis.*

A major finding of this study is that populations of the Central Sierra Mountains, population PL, resemble hybrid populations to the north morphologically with high levels of variability (Figures 5-7) and have admixed ancestry from *A. concolor* of the central and southern Rocky Mountains and group one with 40% of the population having > 0.90 ancestry to group one (Figure 12 and Table 10). The population from the Transverse Range in southern California, population BB, intergrades morphologically between *A. concolor* of the central and southern Rocky Mountains and population PL of the central Sierra Mountains (Figures 5-7). One individual in this population has > 0.85 ancestry from *A. concolor*, but the remainder of the individuals have admixed ancestry predominantly from *A. concolor* with several individuals with > 0.15 ancestry coefficients from group one (Figure 12 and Table 10).

Both populations within this region have relatively high allelic richness, >7, well within the range exhibited by hybrid populations to the north. Morphologically BB has population mean F1 values similar to population SS of the southern Rocky Mountains although population BB includes highly variable individuals well within the range of F1 values exhibited by population PL. DNA sequence data showed that this population from the Transverse Range of southern California shared a mitochondrial haplotype with *A. concolor* of the central and southern Rocky Mountains and a chloroplast haplotype of *A. concolor* variety *lowiana* (see chapter 1). Hammrick and Libby (1972) found populations of this region more closely resembled *A. concolor* of the central Rocky Mountains than populations further north in the Sierra Mountains based on growth characteristics. Based on these data, we surmize there has been extensive introgression into populations of concolor fir of the central Sierra Mountains from the hybrid zone to the north and introgression from the populations of the central Sierra Mountains into populations of *A. concolor* of the Transverse Range in southern California.

Thus, we conclude the populations of the *A. grandis-A. concolor* complex between 47º in the Cascade Mountains and 46º in the northern Rocky Mountains and 34º N in the Transverse Range of southern California span an extensive gradient of admixed populations from nearly pure *A. grandis* in the north to nearly pure *A. concolor* in the south with a greater influence of group 1 in between. Our conclusions concur with Liu (1971) who regarded populations within the complex from the Southern Cascade to the mountains of Southern California as *A. grandis-A. concolor* hybrids and treated them taxonomically as *A. concolor* variety *lowiana* and acknowledged hybridization between these populations and *A.*

grandis to the north. However, they are inconsistent with the treatment of Farjon (1990) who described *A. concolor* of the Sierra Mountains as morphologically within the range of phenotypes found in *A. concolor* of the central and southern Rocky Mountains and regarded these regional forms as the same taxon, *A. concolor*. They are also inconsistent with the treatment of Hunt (1993) who designated the Sierra Mountain segregate *A. lowiana* and the central and southern Rocky Mountain segregate *A. concolor* with apparent disjunct populations in the southern Cascade and Siskiyou Mountains and the Transverse Range of southern California (Figure 2). The inconsistencies between our findings are likely due to differences in sampling and the different characters evaluated of these highly variable populations in these two taxonomic treatments.

There is a clear pattern of associations between the low elevation, mild maritime climates and *A. grandis,* which essentially has the same distribution as the Coastal disjunct distributions of *Thuja plicata* and *Tsuga heterophylla*. This association between *A. grandis* and relatively mesic, mild conditions is evident in the positive correlation between latitude and ancestry coefficients in both transects of the Pacific Slope (Figure 9) and Rocky Mountains (Figure 10).

A similar pattern is revealed in the transect from the Oregon Cascades through inland populations that are in the rain-shadow of the Cascades (Figure 11) which shows a decrease in the influence of *A. grandis* toward the inland populations. Further evidence for this apparent association was found in the ecological transect from the Siskiyou Mountains with population, N, on the more mesic north slope and population, S, on the relatively more xeric south slope; Population N had a significantly higher ancestry coefficients from *A. grandis,*

while population S had higher ancestry coefficients for group one. These populations had significantly different F1 values based on the Wilcoxon test, Figure 7. A similar pattern was found on a transect from the south and north slope of Black Butte in central Oregon based on a morphometric analysis which showed a significant increase in trees with the yellow periderm and an increased number of adaxial stomates on the south slope, which are characteristics associated with *A. concolor* variety *lowiana* (Zobel, 1973). *A. concolor* appears to be associated with more xeric conditions and colder winters at more southerly latitudes and much higher elevation in the central and southern Rocky Mountains.

Admixed populations occupy a range of environments; from relatively mild mesic conditions of nearly pure *A. grandis* populations at lower elevation as far north as 46° N in the Rocky Mountains and as far towards the Coast as the Willamette Valley of Oregon to much more xeric high elevation sites in the rain shadow of the Cacsade Mountains and higher elevation sites in the Sierra Mountains. A feature of this complex of major evolutionary importance is the connectivity of the Cascade, Klamath, Siskiyou and Sierra semi-continuous band of mountains. This band of mountains likely provided a route of dispersal, migration and secondary contact between divergent, geographically and ecologically isolated pure taxa in response to climate shifts that took place from the Pliocene to the Pleistocene with glacial and interglacial series and finally the transition to contemporary climates.

A similar route from the southern Cascade through the Klamath and Ochoco Mountains of central Oregon to the Wallowa Mountains of northeast Oregon and Mountains of central Idaho has been suggested as a significant migration route for a number of plant

taxa with Sierran affinities and disjunct populations in central Idaho (Lorain, 1989). Pair-wise Fst values (Weir and Cockerham, 1985) between populations from the southern Cascade, Klamath and Siskiyou Mountains and populations through the Sierras and inland Oregon along these routes are very low (appendix II), which indicates past or recent gene-flow between these populations. Further inland populations restricted to mountain tops isolated by many miles of shrub steppe desert, have much higher Fst values with significant Fis values, which indicates they have been isolated for a many generations. There likely was a continuous band of forest connecting these ranges to the Cascade and Sierra Mountains during the last glacial maxima when elevational zones may have been compressed by as much as 3000 feet (Barnosky, 1989).

Summary

The emerging picture of the intergrading *A. grandis-A. concolor* complex is a dynamic balance between climate shifts correlated with range contraction and divergence and climate shifts correlated with range expansion and reticulation. This system of Mountains, the Cascade, Coast, Siskiyou, Klamath, Ochoco, Wallowa and Rocky Mountains ranges currently support regionally isolated populations separated by many miles of shrub steppe desert, but under cooler macroclimates, elevational zones were likely compressed and there was likely contact between formerly divergent populations. Almost every regional segregate in this complex from the southern Sierra Mountains to the nearly pure grand fir of the Inland Northwest shows some evidence of secondary contact with it's nearest neighboring

population with some evidence of introgression into pure populations along the margins of these regions, through admixed ancestry and elevated allelic richness, cp-type/mt-type incongruence and or hyper-variable morphological intermediacy. *A. concolor* variety *lowiana* of the central Sierra Mountains looks intermediate to intergradient populations to the north in the hybrid zone and the population from the Transverse Range of southern California, which in turn appears to be intermediate between the hybrids to the north and *A. concolor* of the southern Rocky Mtns. Thus, the Cascade and Sierra Mountains appear very much like a bridge between divergent pure populations that are clearly ecologically, morphologically and genetically distinct for gene flow during climate shifts and at some time in the past there appears to have been a similar bridge between the Southern Sierra Mountains and southern Rocky Mountains. Detailed analysis of correlations between ecological site factors and pure and intergradient populations await further study.

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Figures

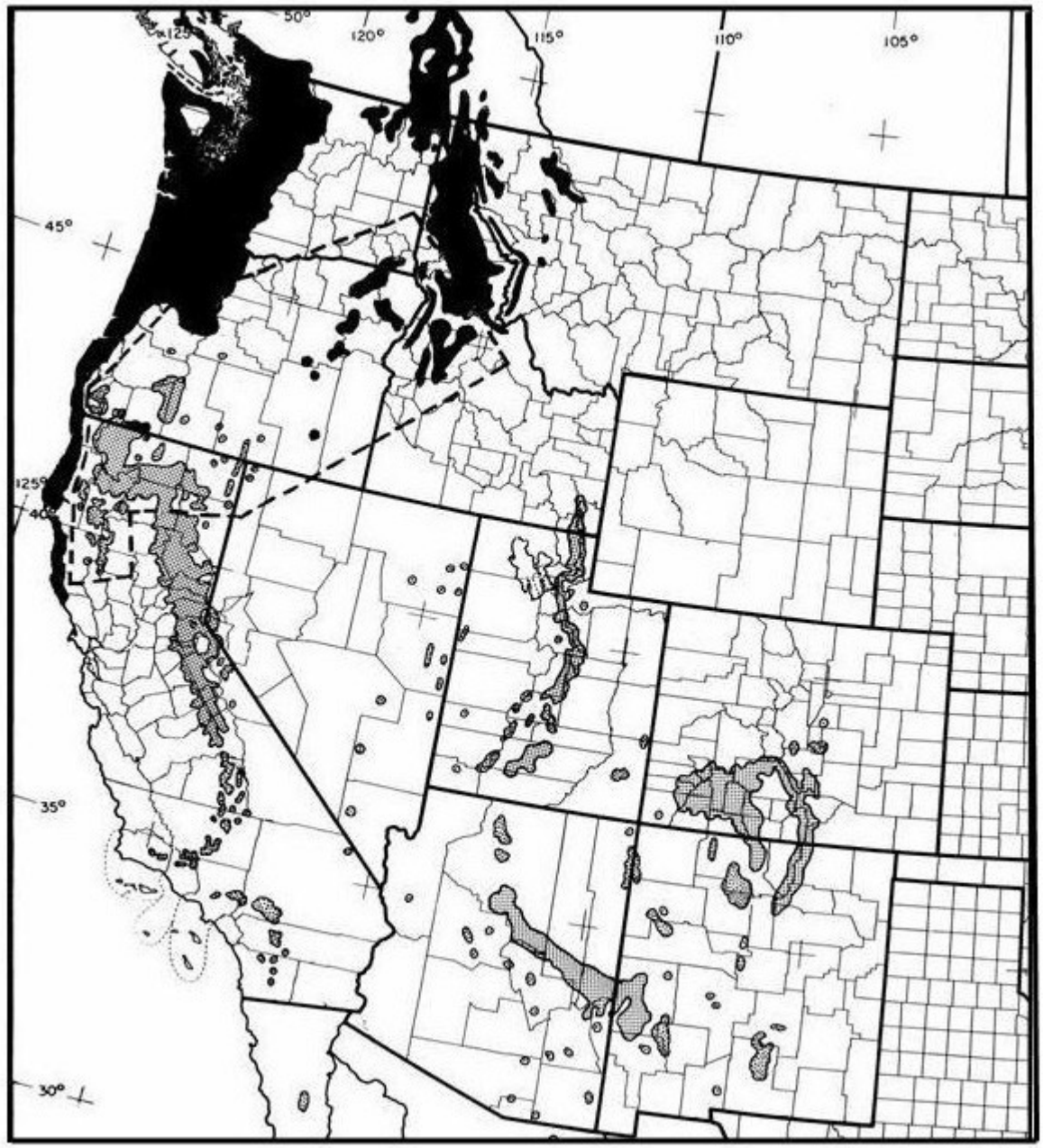

Figure 2.1 Map of the *A. grandis-A. concolor* complex; *A. concolor* populations represented by the stippled pattern and *A. grandis* shaded in black. The indeterminate boundary of intergradient populations is bordered by dashes. Adapted from E. L. Little 1971.

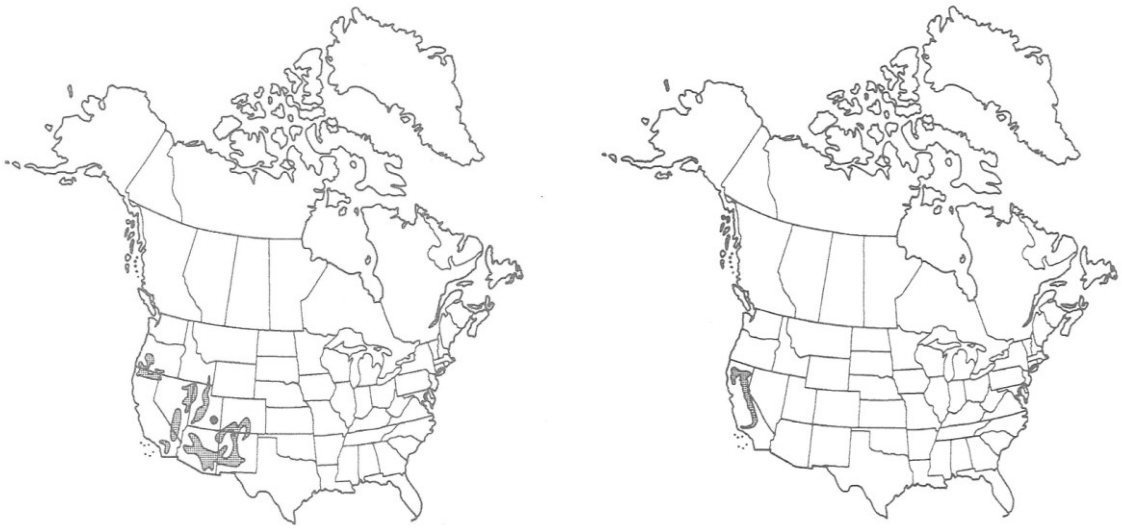

Figure 2.2 Distribution of *Abies concolor* and *Abies lowiana* from the Flora of North America (Hunt, 1993).

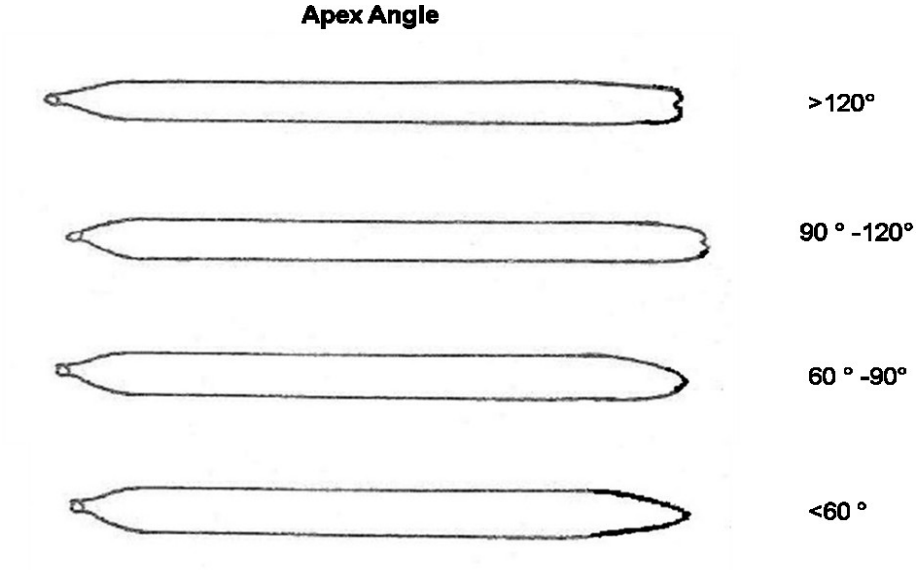

Figure 2.3a Individual leaves depicting the categories ( ) of the morphometric character leaf apex angle (LAA): <60° (1); 60 -90° (2); 90° -120° (3) and; >120°.

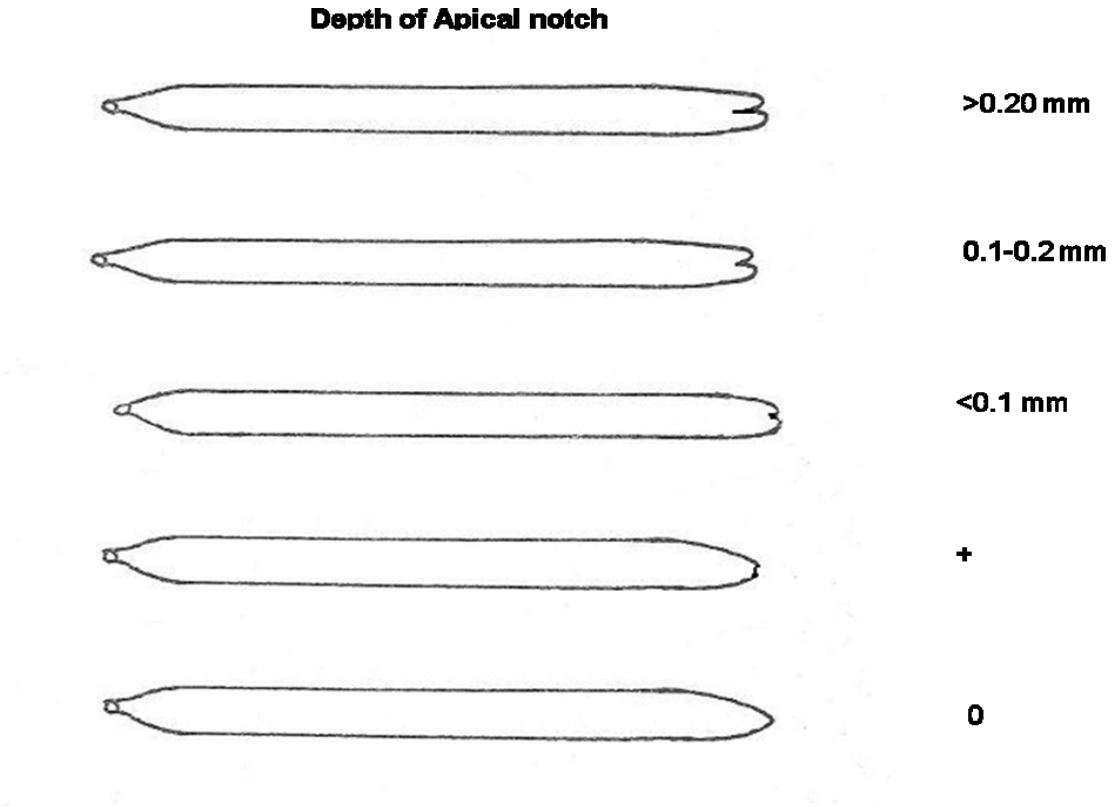

Figure 2.3b The four categories ( ) for the morphometric character depth of apical notch (DAN) are depicted with individual leaves: notch is absent (0); notch is present but too small to measure accurately (0.5); notch 0.05-0.1 mm (1); notch 0.1-0.2 mm (2); notch >0.2 mm (3)

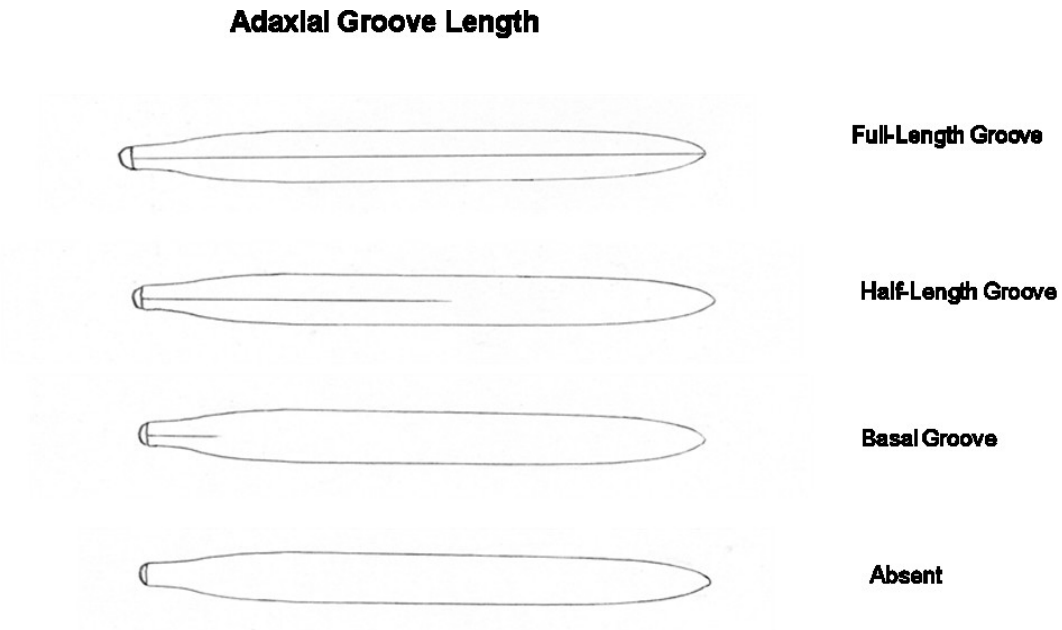

Figure 2.3c Individual leaves depicting the four categories () for the morphometric character adaxial leaf groove (ALG): absent (0); basal < 0.25 of leaf-length (0.25); 0.25-0.5 of leaf-length (0.5) and; full-length.

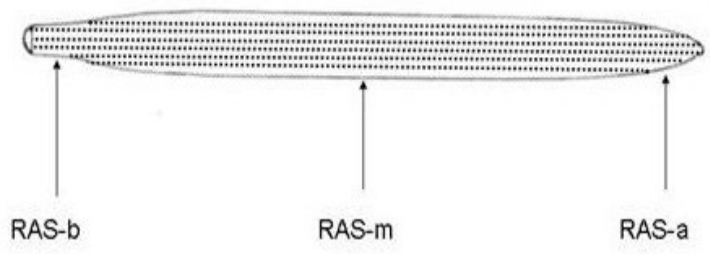

Figure 2.3d Depiction of the morphometric characters rows of adaxial stomates: RAS-b = the number of rows 5 mm from the end of the petiole; RAS-m = the number of rows at mid-leaf and; RAS-a = the number of rows 5 mm below the leaf apex.

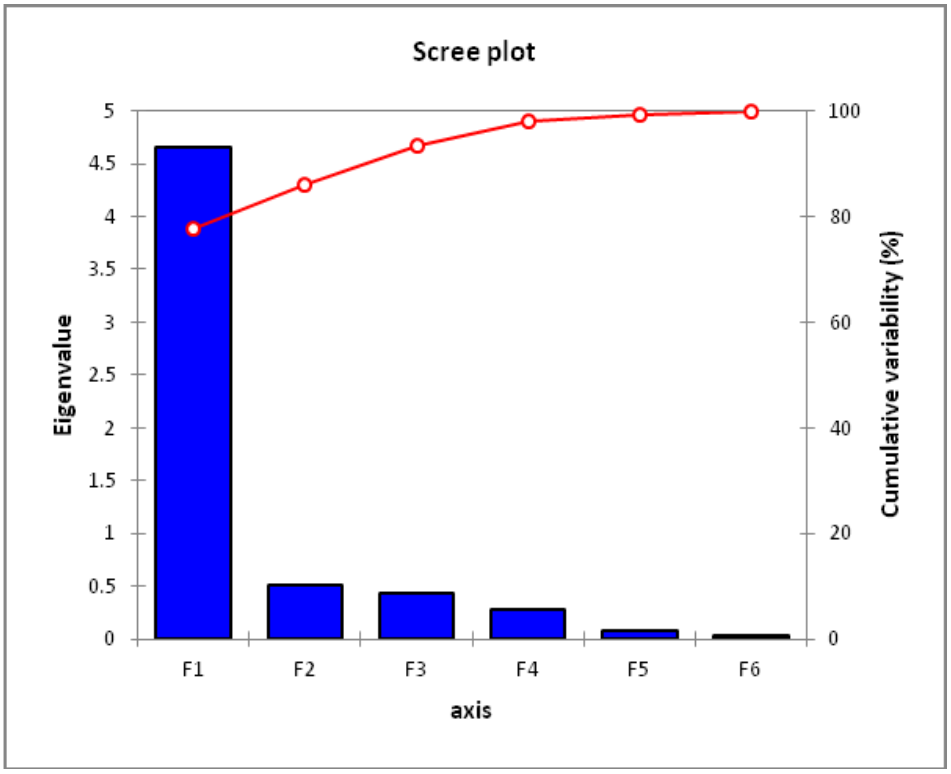

Figure 2.4 Scree plot depicting eigenvalues and cumulative variability of the eigenvectors F1-F6.

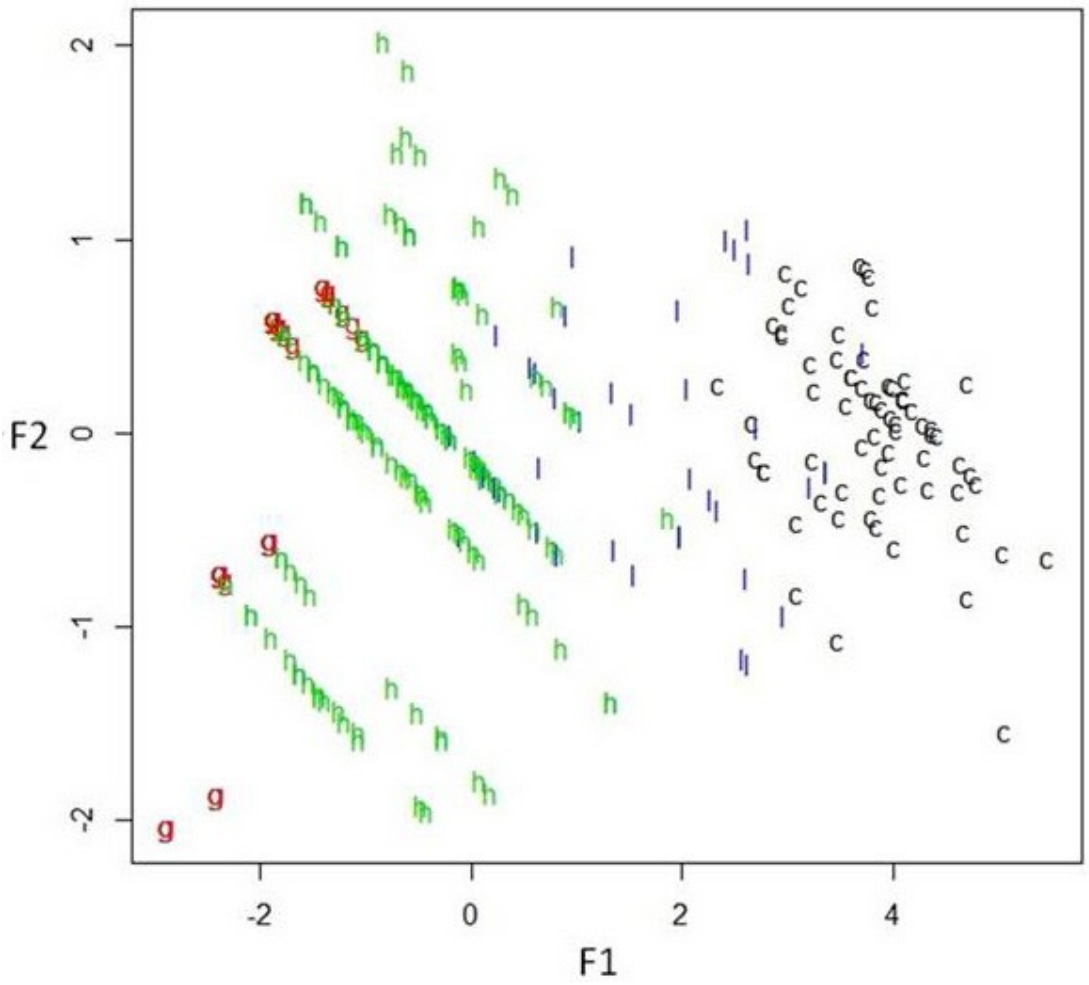

Figure 2.5 Biplot of individuals from study populations along Eigenvector F1 and F2 as follows: *Abies grandis*, **g**; hybrids, **h**; *Abies* concolor variety *lowiana*, **l;** and.*Abies concolor* of the central and southern Rocky Mountains.

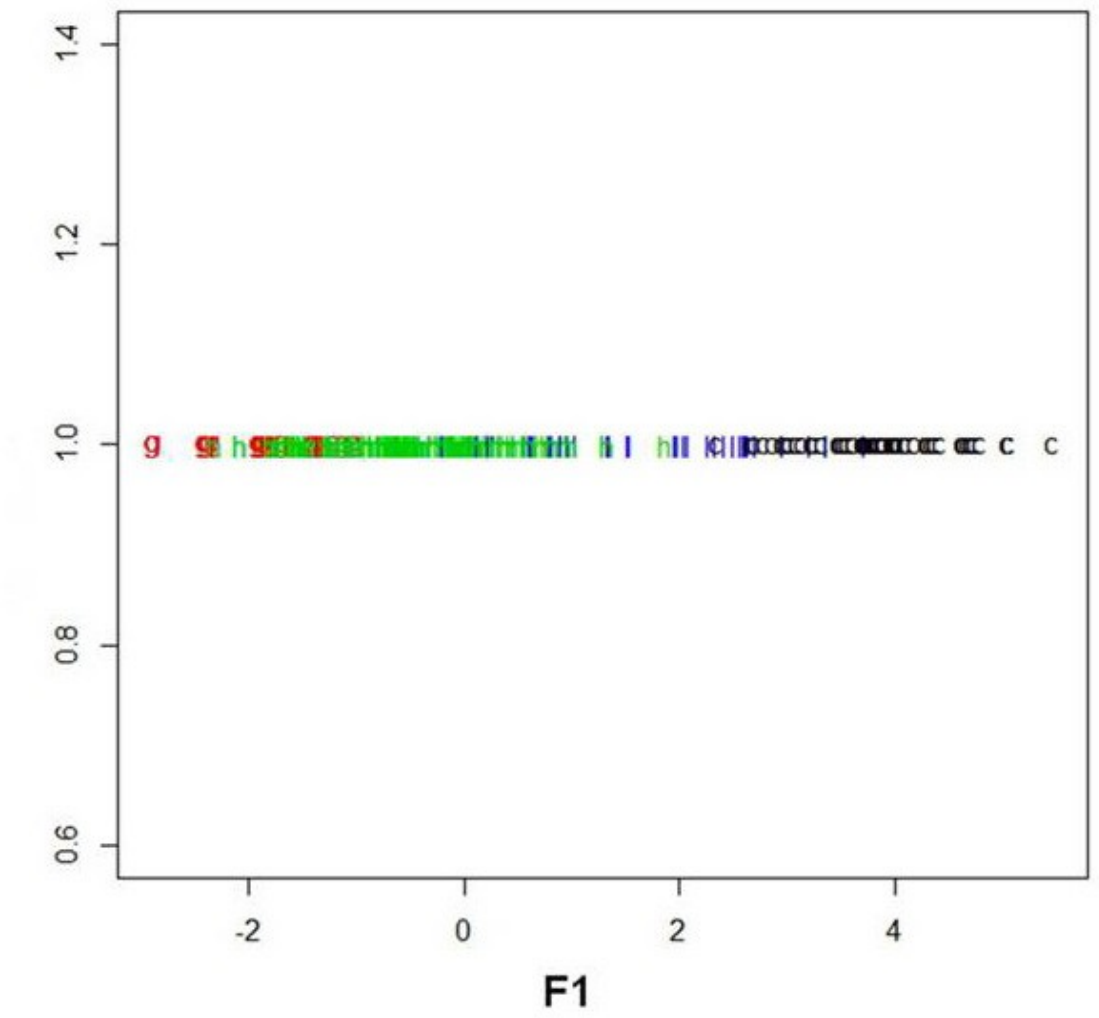

Figure 2.6 Graphic depiction of individuals from study populations along Eigenvector F1 as follows: *Abies grandis*, **g**; hybrids, **h**; *Abies concolor* variety *lowiana* of the Sierra Mountains and Transverse Range, **l;** and.*Abies concolor* of the central and southern Rocky Mountains.

F1	Population										
-2.358	**RL**	a									
-2.348	**AL***	a									
-2.057	**TC**		b								
-1.964	**SN***		b	c							
-1.919	**LA***		b	c							
-1.831	**CL***			c							
-1.438	**PR**				d						
-1.361	**N**				d	e					
-0.932	**KL**				d	e	f				
-0.841	**SD**				d	e	f	g			
-0.713	**BS**					e	f	g			
-0.686	**TB**						f	g			
-0.582	**IC**							g			
-0.406	**S**							g	h		
0.040	**SM**							g	h		
0.232	**WE**								h	i	
0.438	**WH**									i	
0.789	**PL**									i	
2.662	**BB**										
3.381	SS										
3.956	OW										j
4.186	FL										j
4.472	WF										j

Figure 2.7 Study populations listed with means of F1 values from principal component analysis. Populations sharing letters do not have significantly different means based on the Wilcoxon test between all population pairs p = 0.05. Populations in green font have predominantly admixed ancestry; populations in black font have > 0.85 ancestry to *A. concolor* of the central and southern Rocky Mountains, and populations in red have a majority of individuals with >0.85 ancestry to *A. grandis* with * indicating at least one individual with admixed ancestry.

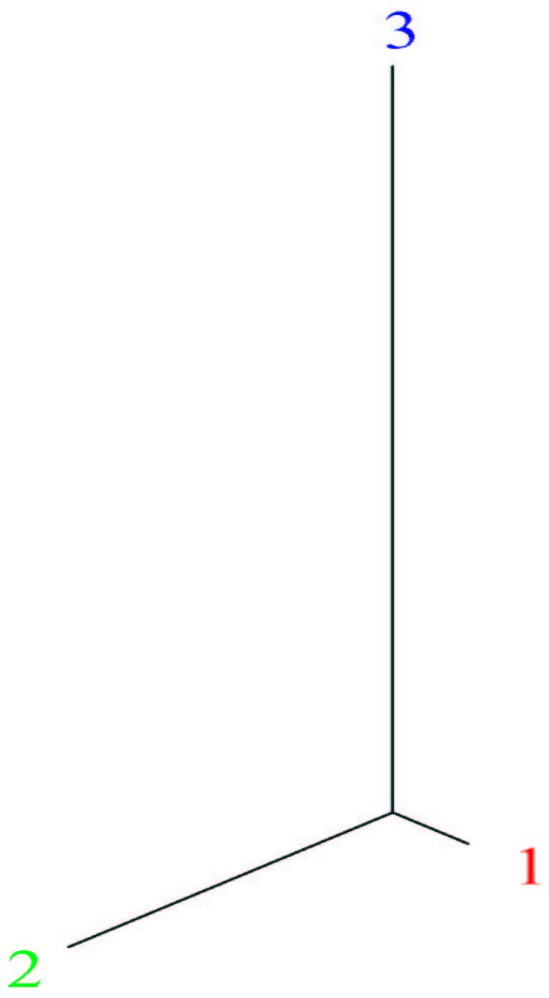

Figure 2.8 A tree depicting the relationships between the three population clusters based on net nucleotide distance was computed with the Neighbor Joining algorithm (Saito and Nei 1987), PHYLIP (Felsenstein 2005). Group **3**, *A. grandis*; Group 2, *A. concolor* of the central and southern Rocky Mountains; and Group 1, intergradient.

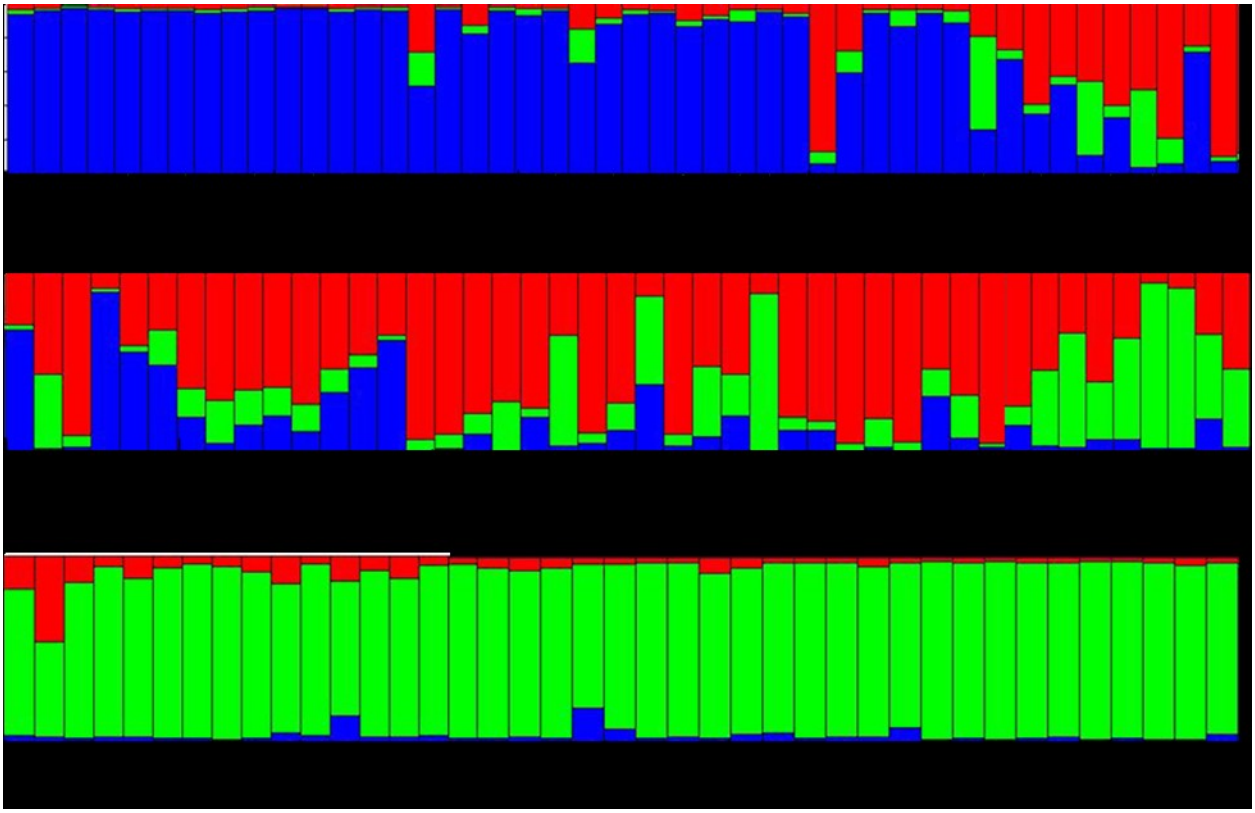

Figure 2.9 Percentage ancestry estimated with admixture analysis on STRUCTURE (Pritchard 2000) including all individuals in study populations along a transect from north to south in the Rocky Mountains as follows: **Group 3, *A. grandis*; Group 2, *A. concolor*** of the central and southern Rocky Mountains; and **Group 1, stabilized hybrid group.** Letters correspond to study populations in tables 1 and 4.

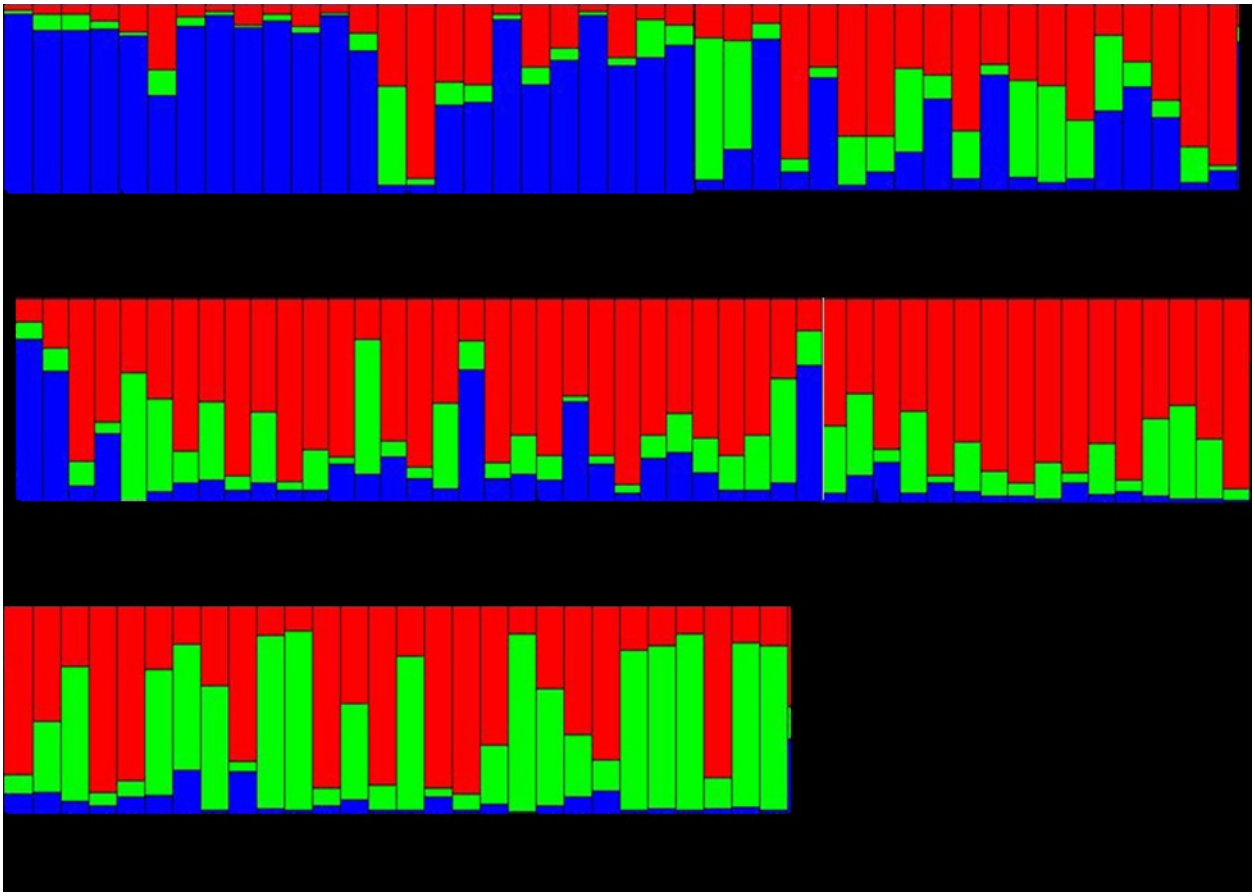

Figure 2.10 Percentage ancestry estimated with admixture analysis on STRUCTURE (Pritchard 2000) including all individuals in study populations along a transect from north to south along the Pacific Slope as follows: **Group 3, *A. grandis*; Group 2, *A. concolor*** of the central and southern Rocky Mountains; and **Group 1, stabilized hybrid group.** Letters correspond to study populations in tables 1 and 4.

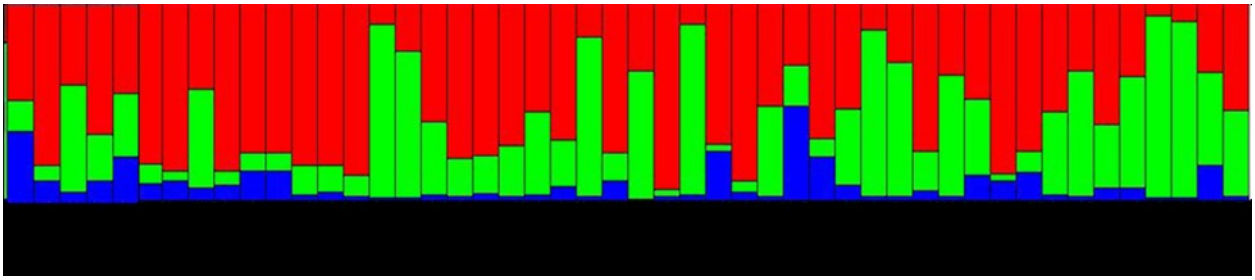

Figure 2.11 Percentage ancestry estimated with admixture analysis on STRUCTURE (Pritchard 2000) including all individuals in study populations along a transect from west to east in central Oregon as follows: **Group 3, *A. grandis*; Group 2, *A. concolor*** of the central and southern Rocky Mountains; and **Group 1, stabilized hybrid group.** Letters correspond to study populations in tables 1 and 4.

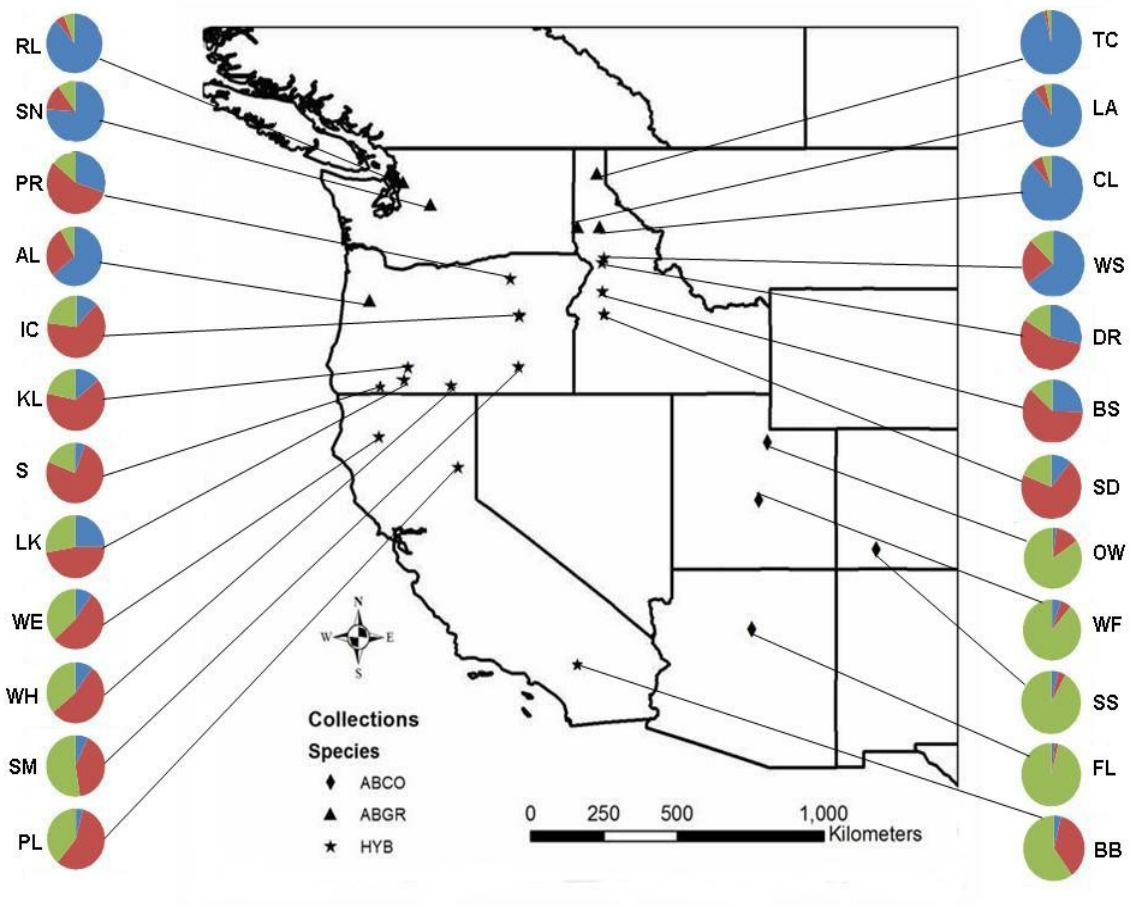

Figure 2.12 Depicts population mean ancestry coefficients to the stabilized hybrid (group 1), *A. concolor* (group 2) of the central and southern Rocky Mountains and *A. grandis* (group 3).

Tables

pop.	ID	Latitude	Longitude	Elevation (ft.)	State and County
TC	ABGR	48º 17' 28" N	116º 19' 42" W	2291	Bonner Co., ID
RL	ABGR	48º 02' 49" N	122º 13' 25" W	176	Snohomish Co., WA
SN	ABGRº	47º 23' 47" N	121º 23' 35" W	2575	Kittitas Co., WA
CL	ABGRº	46º 45' 47" N	116º 14' 49" W	2928	Clearwater Co., ID
LA	ABGRº	46º 45' 13" N	116º 54' 40" W	2712	Latah Co., ID
WS	HYB	45º 52' 37" N	116º 05' 47" W	4310	Idaho Co., ID
DR	HYB	45º 44' 58" N	116º 10' 05" W	4696	Idaho Co., ID
PR	HYB	45º 17' 11" N	118º 58' 04" W	2439	Umatilla Co., OR
BS	HYB	44º 55' 54" N	116º 09' 07" W	5388	Valley Co., Idaho
AL	ABGR	44º 39' 44" N	123º 14' 21" W	605	Benton Co., OR
IC	HYB	44º 15' 09" N	118º 41' 47" W	6800	Grant Co., OR
SID	HYB	44º 12' 56" N	116º 12' 42" W	5170	Valley Co., Idaho
SM	HYB	42º 46' 15" N	118º 43' 01" W	6362	Malheur Co., OR
KL	HYB	42º 45' 20" N	122º 04' 19" W	4371	Klamath Co., OR
LK	HYB	42º 23' 51" N	122º 11' 26" W	4961	Lake Co., OR
M	HYB	42º 23' 26" N	122º 25' 42" W	3461	Jackson Co., OR
GP	HYB	42º 16' 04" N	122º 36' 51" W	5800	Jackson Co., OR
WAH	HYB	42º 15' 39" N	120º 13' 59" W	6669	Lake Co., OR
WAL	HYB	42º 14' 02" N	120º 17' 35" W	5836	Lake Co., OR
KS	HYB	42º 13' 59" N	120º 46' 16" W	5456	Lake Co., OR
TB	HYB	42º 06' 57" N	122º 26' 31" W	4262	Jackson Co., OR
N	HYB	42º 04' 43" N	122º 47' 18" W	5435	Jackson Co., OR
S	HYB	42º 03' 05" N	122º 41' 35" W	4620	Jackson Co., OR
WE	HYB	40º 46' 49" N	122º 57' 39" W	4559	Trinity Co., CA
OW	ABCO	40º 36' 48" N	111º 07' 51" W	7543	Weber Co., UT
PL	ABLO	39º 54' 14" N	120º 32' 45" W	5827	Plumas Co., CA
WF	ABCO	38º 58' 43" N	111º 23' 50" W	8373	Sevier Co., UT
SS	ABCO	37º 33' 28" N	107º 49' 27" W	8421	La Plata Co., CO
FL	ABCO	35º 17' 08" N	111º 36' 52" W	7370	Coconino Co., AZ
BB	ABCO	34º 14' 29" N	116º 58' 28" W	6844	San Bernardino Co., CA

Table 2. 1. All study populations are listed with field identification; ABGR represents pure populations of *A. grandis*, ABGR° represents nearly pure *A. grandis*, ABCO represents *A. concolor*, ABLO represents *A. concolor* variety *lowiana* of the central and northern Sierra Mountains, and HYB represents populations that intergrade between *A. grandis* and *A. concolor* variety *lowiana*.

Locus	Accession no.	Reference	PCR Product (bp)
SF 1	DQ218453	Cremer *et al*. 2005	212
SF b4	DQ218454	Cremer *et al*. 2005	-
SF b5	DQ218455	Cremer *et al*. 2005	133
SF g6	DQ218456	Cremer *et al*. 2005	107-117
SF 50	DQ218457	Cremer *et al*. 2005	92-118
SF 78	DQ218458	Cremer *et al*. 2005	156-262
SF 83	DQ218459	Cremer *et al*. 2005	-
SF 239	DQ218460	Cremer *et al*. 2005	103
SF 324	DQ218461	Cremer *et al*. 2005	104
SF 331	DQ218462	Cremer *et al*. 2005	-
SF 333	DQ218463	Cremer *et al*. 2005	154-158
NFF2	AY966493	Hansen *et al*. 2005	-
NFF3	AY966494	Hansen *et al*. 2005	108-118
NFH3	AY966491	Hansen *et al*. 2005	97-127
ABfi13	AB194688	Saito *et al*. 2005	170
ABfi18	AB194692	Saito *et al.* 2005	282-308
As07	AB290131	Lian *et al.* 2007	112-134
As08	AB290132	Lian *et al.* 2007	180
As09	AB290133	Lian *et al.* 2007	233-261
As13	AB290134	Lian *et al.* 2007	223-245
As16	AB290135	Lian *et al.* 2007	212-224
As20	AB290136	Lian *et al.* 2007	176-204
As21	AB290137	Lian *et al.* 2007	-

Table 2.2. Lists all loci screened, including references and alleles identified in preliminary analysis.

Character	***Abies grandis***	***Abies lowiana***	***Abies concolor***
Adaxial Stomates	0, (+)	9 rows*	10 rows
Adaxial Groove	full-length	absent to full-length	absent to basal
Leaf Apex	rounded	rounded to acute	rounded to acute
Apical Notch	.1-.3mm	absent to .15mm	absent to .05mm
Leaf Angle	pectinate*	obscurely upswept*	strongly upswept*
Bark Periderm Texture	hard	corky	corky
Bark Periderm Color	Red	Yellow	Yellow,(red)

Table 2.3. Distinguishing morphological characters (Daniesls, 1969) between *A. grandis*, *A. concolor* variety *lowiana* and *A. concolor*. Characters with * exhibit environment dependent phenotypic plasticity. Character states with () indicate exceptions to the pattern: *A. grandis* frequently has one to a few individual adaxial stomates restricted to the apex and *A. concolor* of the central and southern Rocky Mountains has some populations with a significant number with red periderm.

Taxon	Groove	Notch	Apex	RAS-a	RAS-m	RAS-b
A. grandis	1(0)	1.77(0.63)	3.73(0.45)	0.02(0.10)	0	0
*A. grandis**	1 (0)	1.47(.69)	3.84(0.37)	0.37(0.85)	0	0
Hybrid	0 .99(.06)	0.98(.43)	3.23(0.61)	5.31(3.00)	3.03(2.95)	1.18(1.68)
A. concolor var. *lowiana*						
-PL	0.9(0.21)	0 .68(.34)	3.05(0.39)	8.3(1.38)	7.0(1.52)	3.3(1.72)
-BB	0.26(0.19)	0.58(0.41)	2.25(0.44)	9.6(1.39)	8.7(2.36)	5.75(1.59)
A. concolor	0.17(.20)	0 .16(.27)	2(.49)	11.65(2.10)	12.41(2.03)	7.62(1.86)

Table 2.4. Mean and (standard deviation) of all six morphometric characters across recognized taxonomic groups and hybrids: RASa-m are the number of rows of adaxial stomates of the apical third of the leaf (a);mid-leaf (m); basal third of the leaf (b). *A. grandis** includes nearly pure populations CL, SN and LA that are located along the northern margin of the hybrid zone. *A.concolor* variety *lowiana* includes only two populations, PL of the Sierras and, BB of the Transverse Range that are distinct enough to be listed individually.

Variables	Groove	Notch	RAS-a	RAS-m	RAS-b	Apex
Groove	1	0.617	-0.667	-0.789	-0.843	0.744
Notch	0.617	1	-0.603	-0.644	-0.616	0.584
RAS-a	-0.667	-0.603	1	0.928	0.853	-0.655
RAS-m	-0.789	-0.644	0.928	1	0.940	-0.712
RAS-b	-0.843	-0.616	0.853	0.940	1	-0.706
Apex	0.744	0.584	-0.655	-0.712	-0.706	1

Table 2.5. Correlation matrix from principal component analysis of the Six morphometric characters; 1.) length of adaxial groove, 2) Depth of apical notch, 3) ras-a, rows of adaxial stomates 5mm below the leaf apex, 4) ras-m, rows of adaxial stomates at mid-leaf, 5) ras-b, rows of adaxial stomates 5mm above the petiole and 6) apex angle.

Character	F1	F2	F3	F4
Groove	16.781	0.408	17.9605	42.605
Notch	12.303	66.696	20.580	0.125
RAS-a	17.296	12.561	14.975	12.966
RAS-m	19.623	8.270	3.941	0.070
RAS-b	19.216	7.496	0.133	7.120
Apex	14.780	4.569	42.406	37.135

Table 2.6. Contribution of the morphometric characters to the Eigenvectors, F1-F4, in the principal component analysis. Morphometric characters follow; 1.) length of adaxial groove, 2) Depth of apical notch, 3) RAS-a, rows of adaxial stomates 5mm below the leaf apex, 4) RAS-m, rows of adaxial stomates at mid-leaf, 5) RAS-b, rows of adaxial stomates 5mm above the petiole and 6) apex angle.

Morphometric Character	Rocky Mtn. Transect		Cascade & Sierra Mtn. Transect	
Adaxial Groove Length	0.853	+	0.846	+
Notch Depth	0.601	+	0.333	+
Apex Angle	0.717	+	0.406	+
Rows of Adaxial Stomates-a	0.830	-	0.692	-
Rows of Adaxial Stomates-m	0.867	-	0.595	-
Rows of Adaxial Stomates-b	0.831	-	0.524	-
Principal Component F1	0.919	-	0.773	-

Table 2.7. R^2 values obtained from a Lowess Method regression analysis of the morphometric characters and Eigenvector F1 values as a function of latitude for all individuals along a Rocky Mountain transect and Cascade and Sierra Mountain transect. + and – indicate if values are positively or negatively correlated with latitude. Rows of adaxial stomates are referenced as follows: 5mm from the apex (a), mid-leaf (m), and 5mm above the petiole (b).

Locus	No. of alleles	Size range (bp)	P_{HW}	H_o	H_s
SF 50	15	92-122	0.0000	.416	.484
NFF3	11	108-124	0 .4962	.648	.644
ABfi18	25	282-340	0 .0061	.719	.770
As09	24	233-295	0 .1559	.811	.827
As13	19	231-249	0 .0698	.820	.836
As20	22	176-218	0 .0860	.849	.872

Table 2. 8. Summary statistics per locus, Includes the size range of alleles, number of alleles, statistical significance of departure from Hardy-Weinberg equilibrium (P_{HW}), observed heterozygosity (H_o) and expected heterzygosity (H_s), for all six loci.

Population	ID	*n*	Na	Phw	Fis
TC	ABGR	10	3.596 (0)	.8107	-.074
SN	ABGRº	10	5.767 (1)	.0667	.051
CL	ABGRº	10	4.616 (0)	.5270	.027
LA	ABGRº	10	4.523 (0)	.1517	.004
WS	HYB	8	6 (0)	.8892	-.049
DR	HYB	8	5 (0)	.1391	.079
PR	HYB	10	5.692 (0)	.0023	.204
BS	HYB	9	5.219 (0)	.3059	-.021
AL	ABGR°	9	5.5 (0)	.8532	-.132
IC	HYB	10	5.954 (0)	.1757	.045
SID	HYB	10	6.243 (1)	.0637	.062
SM	HYB	9	4.919 (0)	.7550	-.069
KL	HYB	10	6.081 (0)	.0877	.087
LK	HYB	10	7.825 (1)	.0321	.063
M	HYB	11	6.654 (0)	.1572	.038
GP	HYB	12	8.041 (1)	.7566	-.029
WAH	HYB	11	7.301 (0)	.1182	.035
WAL	HYB	9	8 (2)	.0170	.113
KS	HYB	10	6.116 (0)	.6529	-.057
TB	HYB	8	8 (0)	.1292	.081
N	HYB	13	7.66 (0)	.2939	.027
S	HYB	14	6.986 (1)	.0974	.064
WE	HYB	10	7.196 (1)	.8148	-.040
OW	ABCO	10	5.82 (1)	.4466	.003
PL	HYB	9	7.064 (1)	.0208	.070
WF	ABCO	9	5 (0)	.6573	-.085
SS	ABCO	10	4.467 (1)	.7853	-.065
FL	ABCO	10	5.201 (0)	.0148	.119
BB	ABCO	10	7.101 (3)	.0001	.187

Table 2.9. Includes fractionated allelic richness averaged over all loci, N_a, with the number of private alleles in ( ); significance of exact test for departure from Hardy-Weinberg equilibrium, $P_{HW,}$ and the inbreeding coefficient, F_{is}, for all study populations.

Population	ID	*n*	Group I	ABCO	ABGR	Assignment %
TC	ABGR	10	.024 (.006)	.018 (.005)	.958 (.010)	**100**
RL	ABGR	4	.050 (.023)	.058 (.033)	.892 (.042)	**100**
SN	ABGRº	10	.144 (.132)	.095 (.156)	.761 (.278)	**60**
CL	ABGRº	10	.057 (.087)	.040 (.058)	.903 (.144)	**80**
LA	ABGRº	10	.060 (.038)	.048 (.057)	.893 (.090)	**70**
WS	HYB	8	.218 (.284)	.126 (.174)	.656 (.334)	**50**
DR	HYB	8	.564 (.206)	.160 (.181)	.276 (.250)	0
PR	HYB	10	.559 (.281)	.142 (.136)	.299 (.329)	**20**, **10**
BS	HYB	9	.614 (.166)	.131 (.068)	.255 (.197)	**10**
AL	ABGR°	9	.281 (.263)	.081 (.054)	.637 (.260)	**10**, **10**
IC	HYB	10	.654 (.254)	.226 (.201)	.120 (.110)	**30**
SID	HYB	10	.704 (.255)	.187 (.250)	.109 (.099)	**30**, **10**
SM	HYB	9	.406 (.229)	.528 (.259)	.066 (.060)	**22**
KL	HYB	10	.648 (.188)	.215 (.182)	.137 (.093)	0
LK	HYB	10	.470 (.300)	.284 (.257)	.246 (.279)	**10**
M	HYB	11	.493 (.213)	.242 (.178)	.265 (.233)	**9.1**
GP	HYB	12	.575 (.250)	.223 (.188)	.203 (.260)	**16.7**
WAH	HYB	11	.543 (.284)	.357 (.306)	.100 (.144)	**18.2**, **9.1**
WAL	HYB	8	.512 (.246)	.405 (.289)	.084 (.084)	**12.5**
KS	HYB	10	.647 (.263)	.312 (.275)	.042 (.040)	**10**
TB	HYB	8	.584 (.257)	.210 (.223)	.207 (.187)	0
N	HYB	13	.608 (.200)	.197 (.146)	.196 (.188)	**7.7**
S	HYB	14	.751 (.135)	.189 (.148)	.060 (.048)	**35.7**
WE	HYB	10	.532 (.270)	.369 (.272)	.099 (.064)	**10**
OW	ABCO°	10	.133 (.123)	.845 (.128)	.023 (.010)	**70**
PL	ABLO°	9	.572 (.335)	.391 (.343)	.037 (.025)	**44.4**
WF	ABCO°	9	.064 (.036)	.884 (.072)	.052 (.059)	**80**
SS	ABCO	10	.036 (.020)	.929 (.0256)	.035 (.021)	**100**
FL	ABCO	10	.025 (.007)	.958 (.012)	.018 (.008)	**100**
BB	ABLO°	10	.360 (.268)	.602 (.291)	.040 (.033)	20

Table 2.10. Ancestry statistics from STRUCTURE (Pritchard, 2000) analysis with the admixture model, including population means of the ancestry coefficients for the three source populations; 1) Group 1, 2) ABCO and 3) ABGR with standard deviation in ( ). Percent assignment includes the percentage of the populations with ancestry coefficients >.85 for Group 1, Group 2 (ABCO) and Group 3 (ABGR). Percent assignment =0 indicates none of the samples in the population had > 85% ancestry to any of the groups, thus all individuals have admixed ancestry.

Chapter 3

Ecological Associations of the *Abies grandis-Abies concolor* Complex

Abstract

The relative fitness of hybrid and parental genotypes determines the evolutionary outcome of natural homoploid hybridization and in many cases fitness is determined by environmental conditions. In practice, these relationships can be very difficult to evaluate, especially in taxa with long generation times. One approach in these situations is to assess correlations between environmental conditions and the occurrence and abundance of hybrid and parental genotypes. In this study we assess correlations between pure and hybrid genotypes of the *Abies grandis-Abies concolor* complex through the comparison of twenty climatic variables (Worldclim) with discriminant analysis for *A. concolor, A. grandis* and hybrid populations. We find that hybrid populations have adapted to a wider range of environmental conditions than either of the pure taxa. Hybrid populations inhabit environments that range from pure *A. grandis* in the northern Rocky and Cascade Mountains to habitats that are variably intermediate between *A. grandis* and *A. concolor* for a majority of environmental variables, but with lower precipitation in the summertime in the southern Cascade, Siskiyou and Sierra Nevada Mountains.

Introduction

Historically, many researchers considered interspecific homoploid hybridization as an evolutionary dead end, resulting only in the reinforcement of reproductive boundaries. Accordingly, the most commonly applied model of hybrid zone dynamics was the tension zone model (Arnold, 1997; Harrison, 1993). This model suggests that two forces maintain hybrid zones; 1) selection against hybrids regardless of the environment and 2) dispersal of parental species into regions of overlap. This perspective is based on the premise that divergent taxa that waste gametes on inferior hybrid crosses are selected against, thus reinforcing reproductive boundaries between diverging taxa. Major predictions of this model are that hybrid zones should resemble narrow clines with concordance and congruence of all characters; and that hybrids should have lower levels of fitness relative to the parental species, regardless of the environment. However, numerous detailed genetic analyses of natural hybrid zones have made it clear that hybrid zones aren't always characterized by such narrow contact zones as predicted by the tension zone model and that it is not universally applicable. Rather, hybridization has increasingly been recognized not only for its role in reinforcing reproductive boundaries between divergent species, but also as an important source of genetic variation to support adaptation to changing environmental conditions, exploitation of novel ecological niches and ultimately the evolution of new species (Anderson, 1949; Arnold, 1997; Lewontin and Birch, 1966; Rieseberg, 1995; Stebbins, 1959).

A second model of hybrid zone dynamics, the bounded hybrid superiority model, maintains that hybrids may have a higher level of fitness relative to the parental species in

novel environments. Thus, the major prediction of this model is that hybrid zones occur in areas characterized by different ecological conditions than those that occur where pure populations reside and that both pure and hybrid populations have a higher level of relative fitness in their own environments (Arnold, 1997)

The *Abies grandis-Abies concolor* hybrid complex is of great interest to evolutionary biologists because it spans a wide range of ecological conditions with pure and hybrid populations apparently occupying different conditions. Several historic studies based on morphometric analysis, terpene analysis and common-gardens (Daniels, 1969; Hamrick and Libby, 1972; Lacaze and Tommasone, 1967; Houkal, 1977) suggest the complex is comprised of three zones: 1) Type populations of *A. grandis* in the mesic forests of the northern Rocky Mountains and Pacific Coast with minor differences between these regional populations, 2) Type populations of *A. concolor* discontinuously distributed in the xeric forests of the central and southern Rocky Mountains and Mountains of northern Mexico with notable but minor differences between these regional populations, and 3) a zone from the Transverse Range of southern California through the Sierra, Siskiyou, and southern Cascade ranges to the Mountains of northeastern Oregon and adjacent Idaho that intergrade clinally from nearly pure *A. concolor* in the southern portions to nearly pure *A. grandis* in the north with the notable absence of pure individuals from central Idaho to the Central Sierra Mountains. There is general agreement among these researchers that this region is comprised of an extensive hybrid zone, however there are numerous contradictions regarding the identification, description, delineation and taxonomic treatment of these hybrid populations (Liu, 1971; Farjon, 1990; Hunt, 1993).

A recent molecular genetic study augmented with morphometric analyses of the intergrading complex determined these hybrid populations consist of individuals with variably admixed ancestry from three source populations, *A. grandis, A. concolor* of the central and southern Rocky Mountains, and a stabilized hybrid type from the northern Sierra and Siskiyou Mountains. The relative influence of *A. grandis* and *A. concolor* within this region is positively and negatively correlated with latitude respectively and the greatest influence of the stabilized hybrid source population is in the middle of the hybrid zone in the Siskiyou and southern Cascade Mountains, Figure 2 (see chapter 2). Along a transect from the coast to central Oregon the relative influence of *A. grndis* is negatively correlated with longitude and *A. concolor* is positively correlated with longitude. Within the hybrid zone the relative influence of *A. concolor* and *A. grandis* also vary with topographic and site factors. More xeric sites typical of exposed southern aspects support intergradient populations that more closely resemble *A. concolor* and more mesic sites with northern aspects support intergradient populations that more closely resemble *A. grandis* (Zobel, 1973).

Ecologically, hybrid populations span a range of environments from the mesic sites that support nearly pure *A. grandis* in the northern Cascade and Rocky Mountains to the nearly pure *A. concolor* of the Transverse Range in southern California. A large portion of the hybrid zone, from the mountains of central Idaho to the mountains of southern Oregon, is comprised of disjunct montane populations in the rain-shadow of the Cascade Mountains. Two sites supporting populations within this region were characterized by shorter growing seasons, cooler temperatures and higher vapor pressure deficits than sites supporting pure *A. grandis* in a previous study (Zobel, 1974).

Sites supporting hybrid populations in the Siskiyou Mountains and Sierra Mountains have been characterized by lower levels of precipitation during the growing season than sites supporting pure *A. grandis*. Thus, this expansive hybrid zone that spans an area larger than the area occupied by pure *A. grandis* with correlations between at least some hybrid populations and distinct environmental conditions relative to the pure taxa, contradicts every major prediction of the tension zone model.

Historically, common-garden and reciprocal transplant studies were the primary methods to assess the relative fitness of hybrid and pure genotypes in representative environments (Arnold, 1997). The advent of geographic information system (GIS) environmental data layers have enabled researchers to assess relationships between presence data of pure and hybrid populations and multiple thermal, moisture, soil and insolation variables to determine if they occupy distinct niches and identify candidate environmental selection pressures (Phillips *et al.*, 2006; Swenson, 2008). These methods have some advantages over traditional common-garden and reciprocal transplant studies of perennial woody plants that have long generation times, broad geographic ranges and large ecological amplitudes. Even over relatively short time periods subtle environmental changes can alter the patterns of relative fitness of parental and hybrid genotypes (Miglia *et al.*, 2005). The GIS based approach allows a more comprehensive range of environmental conditions to be assessed that relate directly to the landscape (Godsoe, 2010) and also integrates all stages of development that can contribute to relative fitness (Wu and Campbell, 2005).

In this study we analyze thermal, moisture and solar regimes from GIS-based data layers and presence data of pure *A. grandis, A. concolor* and hybrid populations to determine if hybrids occupy a novel ecological niche relative to the parental taxa and identify candidate ecological selection variables.

Methods

Study Populations

The study area encompasses the entire *Abies grandis-Abies concolor* complex across the range of ecological conditions pure and hybrid populations inhabit (Figure 1). Study population presence data include five populations of type *A. concolor*, six populations of type *A. grandis* and eighteen hybrid populations that have been genotyped with six microsatellites and morphometrically characterized in a previous study, Table 1 (see chapter 2). A prominent feature of the hybrid zone is that it represents a clinal gradient of admixed genotypes with the relative influence of *A. grandis* and *A. concolor* being positively and negatively correlated with latitude respectively and the greatest influence of a stabilized hybrid group centered in between in the southern Cascade and Siskiyou Mountains, Figure 2.

The first major task of this study was to delimit discrete hybrid classes which have presumably adapted to local selection pressures when the hybrid zone has been characterized as somewhat of a continuous gradient. Two classes of hybrids were identified based on subtle discontinua in morphological characters and variably admixed ancestry. Both classes are made up entirely of individuals with admixed ancestry and intergradient

morphology. The more northerly of the two classes consists of populations of central Idaho and south-central Oregon, with variably admixed ancestry from *A. grandis, A. concolor* and the stabilized hybrid group of the Siskiyou Mountains that will be designated HYB. The second class of hybrids occupy the Warner and Sierra Mountains and have admixed ancestry from the stabilized hybrid group of the Siskiyou and southern Cascade Mountains and from *A. concolor* with negligible ancestry from *A. grandis* that will be designated *A. concolor* variety *lowiana* (Liu, 1971). These presence data of genotyped and morphometrically characterized populations were augmented with samples from The Consortium of Pacific Northwest Herbaria (www.pnwherbaria.org), The Consortium of California Herbaria (http://ucjeps.berkeley.edu/consortium/) and the Intermountain Region Herbarium Network (http://intermountainbiota.org) as well as population locations from previous studies (Daniels, 1969; Scholz *et al.*, 1982; see chapter 2) for a total of 83 populations of *A. concolor,* 53 *A. grandis* and 59 hybrid class I populations from central Idaho and Oregon and 38 populations of *A. concolor* variety *lowiana*, Figure 2. Selection criteria for the augmented populations include verification of identification based on a suite of morphometric characters when possible and close proximity to populations that were analyzed genetically and morphologically in the study mentioned above so that all geographic regions and ecological conditions were sampled.

GIS Environmental Variables and Statistical Analysis

The ecological modeling data consists of 19 thermal and moisture variables that were obtained from the Worldclim dataset (http://www.worldclim.org/) and solar insolation (Fu and Rich, 1999) at a resolution of 30", or about 1 km^2. Due to extensive topographic relief of the study area the there were cases where the 1 km^2 resolution did not match actual topography of a popoulation site, ie elevation and aspect, which leads to erroneous environmental data for these sites. These sites were eliminated from the data set prior to statistical analyses. The nonparametric Steel-Dwass-Critchlow-Fligner procedure 2-tailed test between all pairs of hybrid classes and pure taxa and quadratic discriminant analysis were performed to determine if they occupy distinct environmental niches and identify candidate ecological selection pressures that contribute to their relative fitness. Nonparametric Lowess regression was performed along Pacific Slope and Rocky Mountain transects to assess correlations between key environmental variables and latitude and elevation.

Results

Environmental Differentiation of Hybrid and Pure Populations

Preliminary summary statistical analysis revealed that all but one of the environmental variables analyzed, bio-10 (mean temperature of the wettest quarter) had non-normal distributions with $P<0.01$ for the Shapiro-Wilk test. Consequently, the nonparametric Steel-Dwass-Critchlow-Fligner procedure two-tailed test was performed for all twenty environmental variables between population pairs to determine if they differed

significantly, Table 2. Three of the variables were different between all groups and are listed in descending order; Solar insolation was highest in *A. concolor*, then *A. concolor* variety *lowiana*, then HYB and then *A. grandis* 2) Bio-14, precipitation of the warmest month was highest in *A. grandis,* then *A. concolor*, then HYB and then *A. concolor* variety *lowiana* and 3) Bio-18 precipitation of the driest quarter was highest in *A. grandis,* then *A. concolor*, then HYB and then *A. concolor* variety *lowiana*. Population means and significance of differences between each population pair are summarized in Table 2.

Since its introduction (Fisher, 1936), discriminant analysis has been used extensively to group plant taxa based on multiple morphometric characters and to describe the environmental-niche a taxon occupies based on numerous environmental variables, despite the fact that these data typically have non-normal distributions. These methods with both linear and quadratic models have been tested extensively and they have been characterized as having nonparametric attributes provided prior probabilities are equal for each group and the number of samples are relatively large (Santiago and Hernandez, 2005). Fisher's asymptotic approximation test (box test) revealed that the assumption of equal within class covariance of the linear model was not satisfied with our data, so we utilized the quadratic model.

Factor correlations (Table 3 and Figure 4) show that solar insolation, bio-8 (mean temperature of the wettest quarter), bio-9 (mean temperature of the driest quarter), bio-2 (mean diurnal temperature range), bio-18 (mean precipitation of the driest quarter), bio-6 (minimum temperature of the coldest quarter) and bio-19 (mean precipitation of the wettest quarter) in descending order contribute the greatest to the factor F1. Bio-17

(precipitation of the driest quarter) and bio-18 (precipitation of the warmest quarter) contribute the greatest to factor F2. Together F1 and F2 factors account for 94.83 % of the variability between samples. The analysis revealed four well resolved clusters corresponding to each of the pure taxa and hybrid classes with very little overlap, Figure 5. The model was evaluated by classifying all presence locations based solely on the environmental variables and comparison of the resulting classification to the prior. The model correctly classified 100% of *A. concolor*, *A. grandis* and HYB class hybrids, however 4 of 38 sites with prior identification as *A. lowiana* class hybrids were assigned to the HYB class, which results in 89.43% accuracy for this class.

Nonparametric Lowess regression was performed on the environmental variables along transects in the Rocky Mountains and Cascade Mountains to assess correlations with latitude and elevation, Table 4. Bio-2 (mean diurnal range of temperature), bio-17 (mean precipitation of driest quarter) and bio-18 (mean precipitation of the warmest quarter) are correlated with latitude on both the Cascade and Rocky Mountain transects from the northern most *A. grandis* populations to the southern most class HYB hybrid populations. Bio-19 is positively correlated with latitude along the Rocky Mountain transect, but support for this relationship on the Cascade transect is not as strong. Bio-6 (minimum temperature of the coldest month), bio-8 (mean temperature of the wettest quarter) and bio-9 (mean temperature of the driest quarter) and solar insolation are correlated with elevation along both transects. Along both transects elevation is negatively correlated with latitude. Thus, both transects form ecoclines of the environmental variables that contribute the most to the F1 and F2 factors from the discriminant analysis.

Discussion

Overview of Ecological Patterns

Overall, the ecological patterns of the *A. grandis-A. concolor* complex coincide very well with the phenotypic and genetic patterns described in previous studies (Daniels, 1969; Zobel, 1973). Pure *A. grandis* is generally a seral component of mesic low to mid elevation forests of the Pacific Northwest and northern Rocky Mountains with a relatively mild maritime climate. Relative to the rest of the complex this region is characterized by the lowest levels of solar insolation, the lowest diurnal range of temperature (bio-2), the highest annual precipitation (bio-12), highest precipitation of the driest quarter (bio-17), the highest precipitation of the warmest quarter (bio-18) and highest precipitation of the coldest quarter (bio-19). In contrast, pure *A. concolor* is generally a dominant component of much more xeric, mid to high elevation forests with a pronounced continental climate in the central and southern Rocky Mountains with a few small populations in the mountains of northern Mexico. Relative to the rest of the complex, this region is characterized by the largest diurnal range of temperature (bio-2), lowest annual precipitation (bio-12), lowest minimum temperature of the coldest month (bio-6), and the lowest precipitation of the coldest quarter (bio-19). To summarize the climatic differences between these regions, they differ significantly for nineteen of the twenty environmental variables, all except (bio-5) the maximum temperature of the warmest month, Table 2.

Between these geographically, ecologically and genetically distinct pure regions of the complex is an expensive region from the Cascade Mountains and northern Rocky Mountains at approximately 46° to the Transverse Range of southern California occupied by

hybrid populations with variably admixed ancestry from nearly pure *A. grandis* in the north to nearly pure *A. concolor* in the south, Figure 3. Mean diurnal range of temperature (bio-2) decreases, precipitation of the driest quarter (bio-17) and precipitation of the warmest quarter (bio-18) increase clinally with latitude through the hybrid zone. This trend holds true through our two classes of hybrid populations, 1) HYB from central Idaho to south-central Oregon and 2) *A. concolor* variety *lowiana* of the Warner Mountains of southernmost Oregon and northern California and the Sierra Mountains. Both classes of hybrids receive lower precipitation during the warmest quarter (bio-18) and driest quarter (bio-17), than either region supporting pure populations and higher solar insolation and higher diurnal range of temperature (bio-2) than the region supporting pure *A. grandis*.

The region that supports *A. concolor* variety *lowiana* receives less precipitation in the warmest quarter (bio-18) and driest quarter (bio-17), higher levels of solar insolation, higher precipitation in the coldest quarter (bio-19), lower diurnal range of temperature (bio-2), higher minimum temperature of the coldest month (bio-6) and higher temperatures of the wettest month (bio-8) and driest month (bio-9) than the region that supports HYB populations, which is primarily in the rain-shadow of the Cascade Mountains and has a stronger continental influence than the region that supports *A. concolor* variety *lowiana.*

Thus, hybrids in this complex have adapted to regionally variable environmental selection pressures along this ecocline that in some respects are intermediate to the environments associated with pure populations, but novel with regard to moisture stress during the warmest and driest part of the season with higher levels of insolation than associated with pure *A. grandis*. This description emphasizes clinal variation in

environmental variables, however it is clear that topography also plays a large role as elevation is negatively correlated with latitude and is positively correlated with (bio-6) diurnal range of temperature and, mean temperature of the coldest quarter (bio-8), mean temperature of the driest quarter (bio-9), and solar insolation. Thus, there is a mosaic quality to what has been described as clinally variable macroclimate that varies throughout the hybrid zone with subtle changes in aspect, slope position, elevation and the redistribution of precipitation. To that end, there is strong evidence of selection fine spatial scales as significant phenotypic and genotypic (see chapter 2; Zobel, 1973) differences have been detected on transects that sampled slopes of northern and southern or southeastern aspects of the same formation in this complex.

Adaptation to Novel Environments

Clinal patterns of genetic variation have been attributed to secondary contact and introgressive hybridization (Anderson, 1949; Barton and Hewitt, 1985) and adaptation to selection pressures along environmental gradients (Berry and Kreitman, 1993). Within this complex, morphological characteristics of leaves vary with intensity of solar insolation and precipitation of the summer time. Shade leaves of pure *A. grandis* are 2-ranked with upper leaf angles nearly 135-160° (Daniels, 1969) and are thinner than those of *A. concolor* (Houkal, 1976) which translates to higher specific leaf area. Both of these characteristics are adaptations to maximize photosynthesis in low light intensity environments (Lusk and Warton, 2008; Niinemets, 2010).

A. concolor has prominent upswept leaves with upper leaf angles of shade leaves between 17-40°, reduced specific leaf area and a majority of all surfaces of the leaf covered in white epicuticular wax. These are characteristics of plants adapted to moisture stress with high levels of insolation (Taiz and Ziegler, 1999). Hybrid leaves intergrade with highly variable intermediacy of these traits clinally with the relative influence of *A. grandis* and *A. concolor* being positively and negatively correlated with latitude respectively. In the central Cascade Mountains there is a progressive decrease in the influence of *A. grandis* and an increase in the relative influence of *A. concolor* from low elevation populations of the west-slope to high elevation populations near the crest of the Cascades to populations further inland, in the rain-shadow of the Cascades (Zobel, 1973). This pattern is correlated with a transition from west to east with a progressively shorter growing season, higher vapor pressure deficit, cooler and more variable temperatures and lower annual precipitation. These same leaf characteristics vary with increasing moisture stress from microclimates on northerly to southern aspects of the same geologic formation (see chapter 2; Zobel, 1973; Zobel, 1975).

Different patterns of phenotypic plasticity of two of these leaf characters in response to light intensity between pure and hybrid populations were discovered by Daniels (1969). While there is evidence to support plasticity of leaf angles in response to intensity of insolation in hybrid and pure populations, hybrid populations span a wider range of angles from *A. grandis* angles in the shade to *A. concolor* angles under higher levels of light (Daniels, 1969). Similar patterns of plasticity in the relative abundance of adaxial stomates was only detected in hybrid populations and *A. grandis* populations with clear signs of introgression in

which adaxial stomatal abundance was positively correlated with light levels (Daniels, 1969). Plants which are capable of altering light harvesting through phenotypic plasticity have a large potential to adjust to light availability (Niinemets, 2010).

The correlation of these morphological characteristics with higher levels of moisture stress during the direst and warmest portions of the year, higher levels of insolation and greater diurnal range of temperature that distinguish hybrid classes from the pure taxa along regional gradients and in local topographic microclimates suggests these selection pressures have played key roles in the formation and maintenance of this hybrid complex. Further evidence that some hybrids have adapted to higher levels of moisture stress during the summertime comes from a study that assessed growth and response to frost and drought of 43 *A. grandis* provenances. The only populations that did not experience any mortality following a drought were from central Oregon in the HYB class hybrid region (Scholz and Stephan, 1982). Although the above explanation emphasizes morphological adaptations to thermal, solar and moisture regimes, there is evidence from gas-exchange based studies for key physiological adaptations of hybrid populations to moisture stress.

These studies show that hybrid populations near the crest of the Cascades and populations east of the crest, have shorter growing seasons than low elevation west slope populations and have greater stomatal conductance throughout the early portions of the growing season when moisture is not limiting (Zobel, 1975). A similar study showed that hybrid populations from populations near the crest of the Cascade Mountains and east of the crest had lower infiltration pressures at a given xylem water potential than populations

of low elevation west slope, which indicates these hybrid populations are experiencing lower moisture stress at a given xylem water potential (Zobel, 1974).

Conclusions

A. grandis and *A. concolor* are genetically, geographically and ecologically distinct with clear adaptations to northerly mesic forests with generally mild environmental conditions, abundant precipitation and lower levels of solar insolation and adaptations for much more xeric high elevation forests with colder winters, lower precipitation and higher levels of solar insolation, respectively. Hybrid populations occupy an expansive area between these pure regions from nearly pure *A. grandis* at approximately 46° N in the Cascade Mountains to nearly pure *A. concolor* in the Transverse Range in southern California. Key environmental variables vary clinally throughout this region; summertime precipitation is positively correlated with latitude and diurnal temperature range is negatively correlated with latitude. The two classes of hybrids, *A. concolor* variety *lowiana* of the Sierra and Warner Mountains and HYB hybrids of central Idaho and central Oregon experience environmental conditions that are intermediate to the regions supporting pure populations for some environmental variables, but with reduced summertime precipitation than both pure regions and higher levels of solar insolation and greater diurnal range of temperature than *A. grandis*. Extensive sampling of populations within the area supporting the two hybrid classes found 100% of the individuals to have admixed ancestry and intergradient phenotypes. Percentage ancestry, morphological features and physiological characteristics of hybrids vary with key environmental variables, moisture stress and solar

insolation clinally and with topographic features, aspect, slope position and elevation. This apparent association between genotypes, morphological characteristics, physiological characteristics and environmental conditions in type *A. grandis*, *A. concolor* and hybrid populations is consistent with the bounded hybrid superiority model with the added modification of significant heterogeneity in microclimates associated with extreme topographic relief of the region. It is likely that the system of mountain ranges and valleys that comprise this complex with contrasting microclimates that support different genotypes in close geographic proximity have contributed to and maintained the high levels of genetic and phenotypic diversity that characterize it. This heightened genetic diversity and phenotypic plasticity in traits effecting photosynthesis in response to environmental conditions of populations in the hybrid zone suggests they may be more capable of adapting to environmental perturbations such as a dynamic macroclimate than the pure taxa. The result is perhaps greater than the sum of the parts; providing intergradient populations with the necessary genetic diversity to adapt to an ever changing environment and occupy novel ecological niches.

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Figures

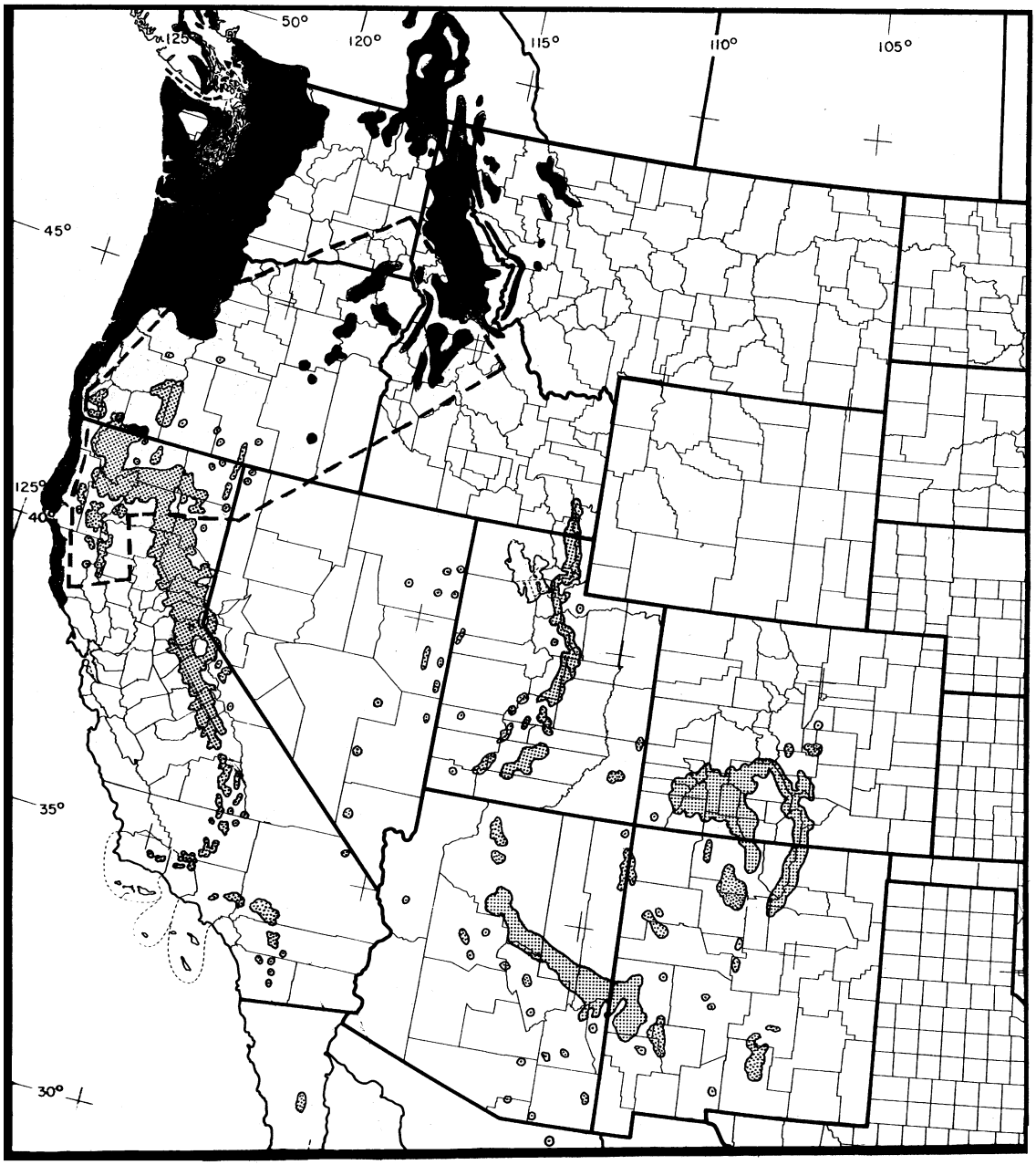

Figure 3.1. Map of the *A. grandis-A. concolor* complex; *A. concolor* populations represented by the stippled pattern and *A. grandis* shaded in black. The indeterminate boundary of intergradient populations is bordered by dashes. Adapted from E. L. Little 1971.

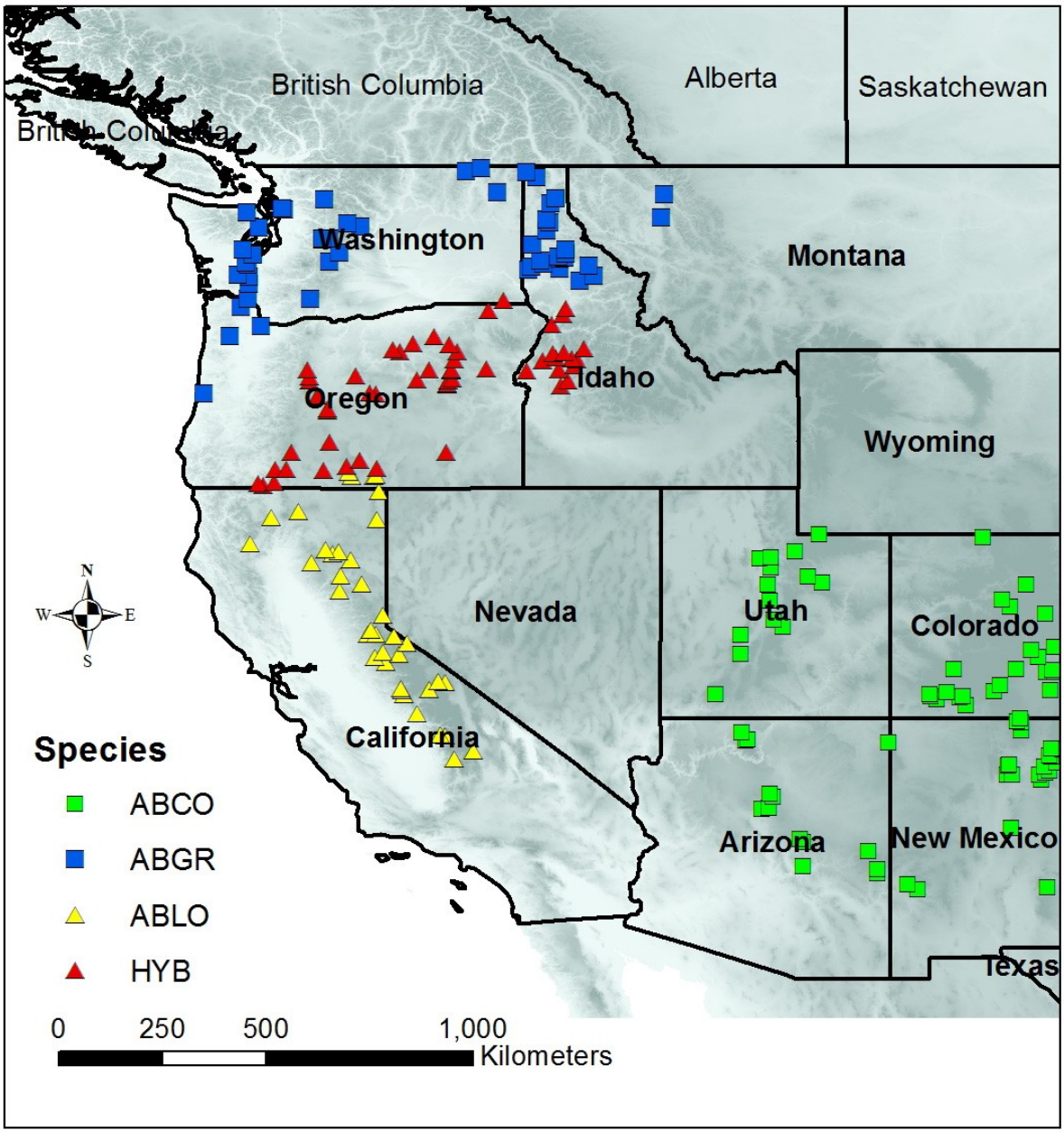

Figure 3.2. Locations of augmented populations of *A. grandis*, ABGR; *A. concolor* variety concolor, ABCO; hybrid class I, HYB; and hybrid class II, ABLO.

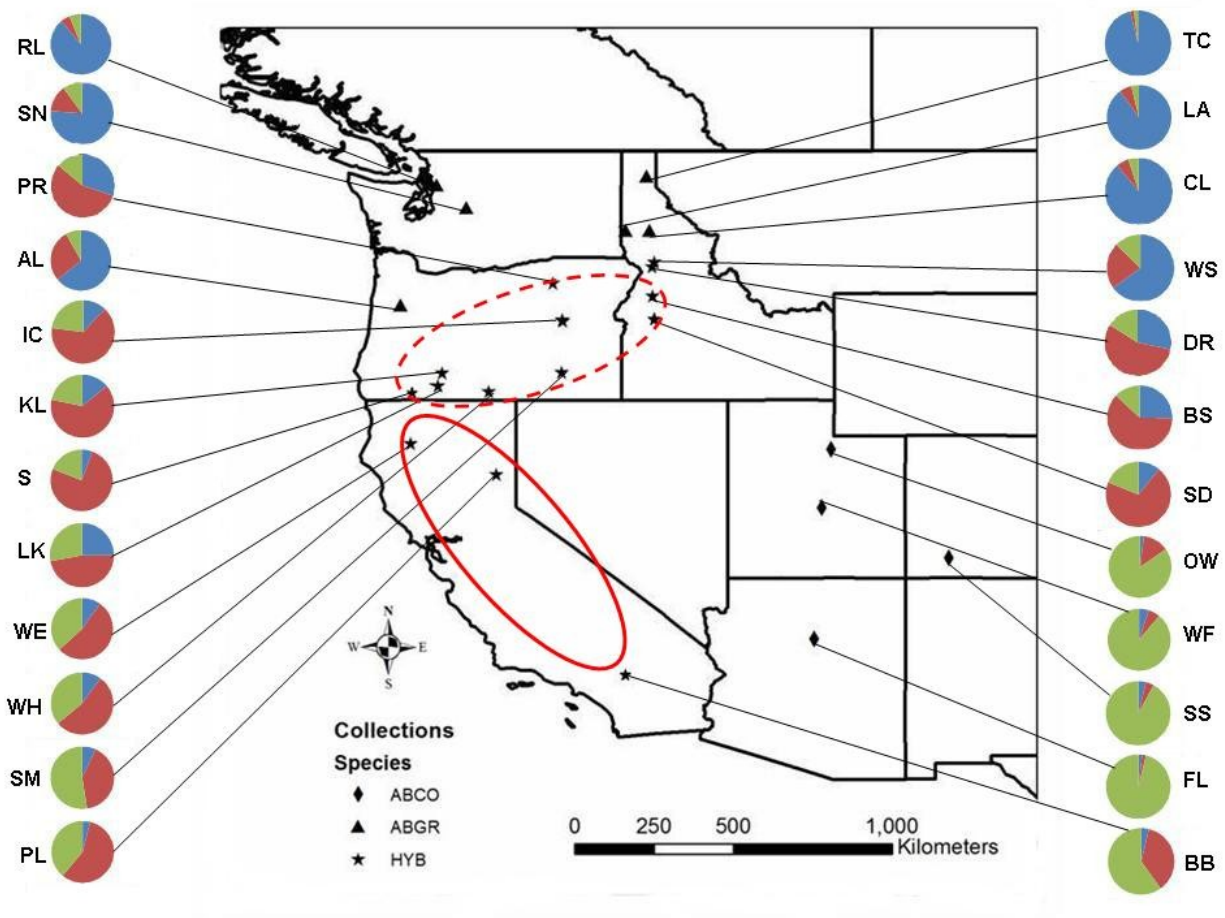

Figure 3.3. Depiction of population mean ancestry coefficients to the stabilized hybrid , *A. concolor* of the central and southern Rocky Mountains and *A. grandis*. Dashed line delineates hybrid class HYB and solid line delineates hybrid class *A. concolor* variety *lowiana*.

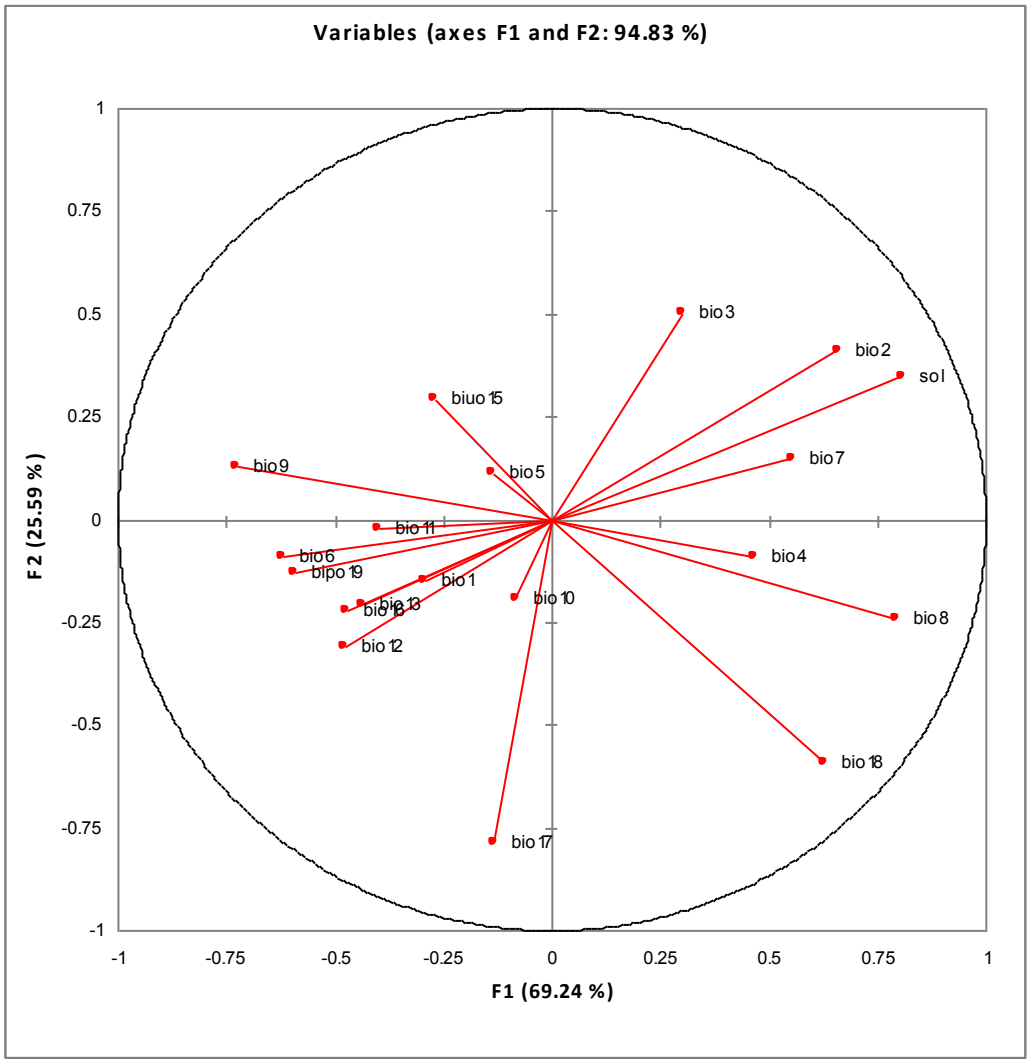

Figure 3.4. Depiction of the contribution of environmental variables to the first two factors F1 and F2 of the discriminant analysis.

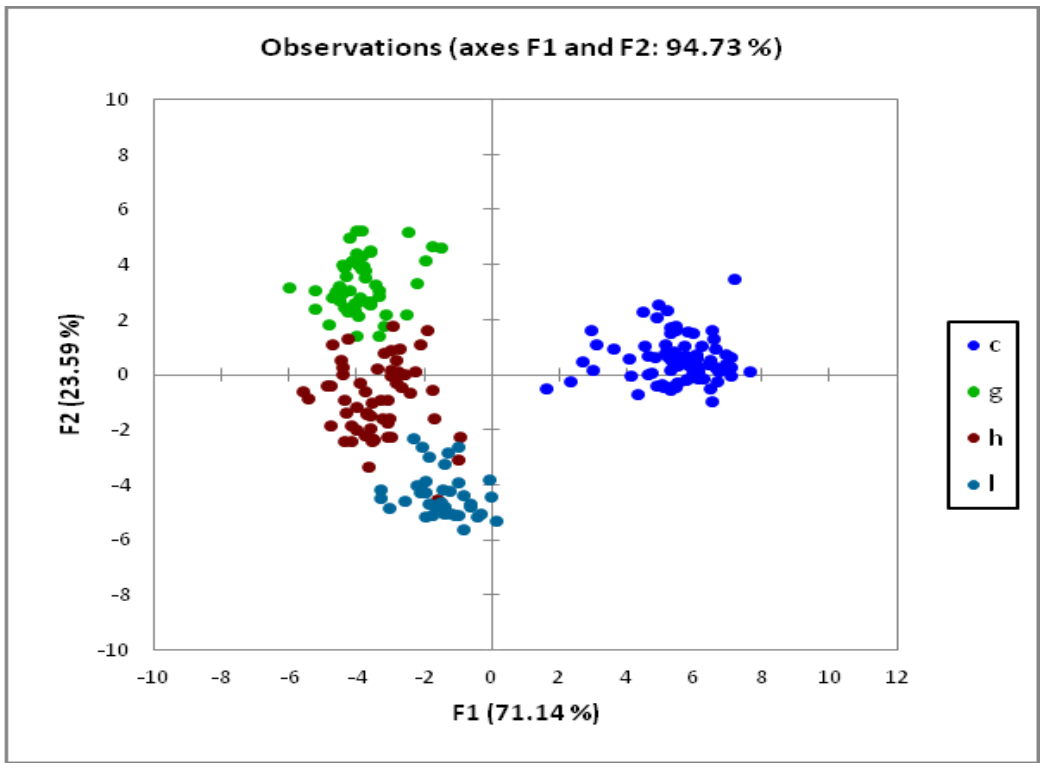

Figure 3.5. Biplot of F1 and F2 axes from discriminant analysis with 83 populations Of *A. concolor* (c) of the central and southern Rocky Mountains, 53 populations of *A. grandis* (g), 59 hybrid class 1 HYB (h) populations and 38 hybrid class II *A, concolor* variety *lowiana* (l) populations.

Tables

pop.	ID	Latitude	Longitude	Elevation (ft.)	State and County
TC	ABGR	48º 17' 28" N	116º 19' 42" W	2291	Bonner Co., ID
RL	ABGR	48º 02' 49" N	122º 13' 25" W	176	Snohomish Co., WA
SN	ABGRº	47º 23' 47" N	121º 23' 35" W	2575	Kittitas Co., WA
CL	ABGRº	46º 45' 47" N	116º 14' 49" W	2928	Clearwater Co., ID
LA	ABGRº	46º 45' 13" N	116º 54' 40" W	2712	Latah Co., ID
WS	HYB	45º 52' 37" N	116º 05' 47" W	4310	Idaho Co., ID
DR	HYB	45º 44' 58" N	116º 10' 05" W	4696	Idaho Co., ID
PR	HYB	45º 17' 11" N	118º 58' 04" W	2439	Umatilla Co., OR
BS	HYB	44º 55' 54" N	116º 09' 07" W	5388	Valley Co., Idaho
AL	ABGR	44º 39' 44" N	123º 14' 21" W	605	Benton Co., OR
IC	HYB	44º 15' 09" N	118º 41' 47" W	6800	Grant Co., OR
SID	HYB	44º 12' 56" N	116º 12' 42" W	5170	Valley Co., Idaho
SM	HYB	42º 46' 15" N	118º 43' 01" W	6362	Malheur Co., OR
KL	HYB	42º 45' 20" N	122º 04' 19" W	4371	Klamath Co., OR
LK	HYB	42º 23' 51" N	122º 11' 26" W	4961	Lake Co., OR
M	HYB	42º 23' 26" N	122º 25' 42" W	3461	Jackson Co., OR
GP	HYB	42º 16' 04" N	122º 36' 51" W	5800	Jackson Co., OR
WAH	HYB	42º 15' 39" N	120º 13' 59" W	6669	Lake Co., OR
WAL	HYB	42º 14' 02" N	120º 17' 35" W	5836	Lake Co., OR
KS	HYB	42º 13' 59" N	120º 46' 16" W	5456	Lake Co., OR
TB	HYB	42º 06' 57" N	122º 26' 31" W	4262	Jackson Co., OR
N	HYB	42º 04' 43" N	122º 47' 18" W	5435	Jackson Co., OR
S	HYB	42º 03' 05" N	122º 41' 35" W	4620	Jackson Co., OR
WE	HYB	40º 46' 49" N	122º 57' 39" W	4559	Trinity Co., CA
OW	ABCO	40º 36' 48" N	111º 07' 51" W	7543	Weber Co., UT
PL	ABLO	39º 54' 14" N	120º 32' 45" W	5827	Plumas Co., CA
WF	ABCO	38º 58' 43" N	111º 23' 50" W	8373	Sevier Co., UT
SS	ABCO	37º 33' 28" N	107º 49' 27" W	8421	La Plata Co., CO
FL	ABCO	35º 17' 08" N	111º 36' 52" W	7370	Coconino Co., AZ
BB	ABLO	34º 14' 29" N	116º 58' 28" W	6844	San Bernardino Co., CA

Table 3.1. All study populations that have been genotyped (Ott, 2014b) are listed with field identification; ABGR represents pure populations of *A. grandis*, ABGRº represents nearly pure *A. grandis*, ABCO represents *A. concolor*, ABLO represents *A. concolor* variety *lowiana* (Liu, 1971) of the central and northern Sierra Mountains, and HYB represents populations that intergrade between *A. grandis* and *A. concolor variety lowiana*.

Bio-Variables	Coding
Bio-1	Annual Mean Temperature
Bio-2	Mean Diurnal Range of Temperature
Bio-3	Isothermality (Bio-2/Bio-7) (*100)
Bio-4	Temperature Seasonality (Standard Deviation * 100)
Bio-5	Maximum Temperature the of Warmest Month
Bio-6	Minimum Temperature of the Coldest Month
Bio-7	Temperature Annual Range (Bio-5-Bio-6)
Bio-8	Mean Temperature of the Wettest Quarter
Bio-9	Mean Temperature of the Driest Quarter
Bio-10	Mean Temperature of the Warmest Quarter
Bio-11	Mean Temperature of the Coldest Quarter
Bio-12	Annual Precipitation
Bio-13	Precipitation of the Wettest Month
Bio-14	Precipitation of the Driest Month
Bio-15	Precipitation Seasonality (Coefficient of Variation)
Bio-16	Precipitation of the Wettest Quarter
Bio-17	Precipitation of the Driest Quarter
Bio-18	Precipitation of the warmest Quarter
Bio-19	Precipitation of the Coldest Quarter

Table 3.2. Coding of the WorldClim variables Bio 1-19.

Variable	ABGR	*P*	HYB	*P*	ABLO	*P*	ABCO
Bio-1	7.34 (2.26)	c,h	4.58 (1.35)	g,l	6.63 (1.96)	c,h	4.67 (2.36)
Bio-2	11.504 (1.59)	c,h,l	14.73 (1.48)	c,g	14.40 (1.16)	c,g	16.22 (1.16)
Bio-3	0.38 (.04)	c,h,l	0.42 (0.02)	g,l	0.44 (0.01)	c,g,h	0.42 (.03)
Bio-4	64.00 (12.58)	c	68.27 (7.07)	c,l	62.55 (3.17)	c,h	75.18 (6.50)
Bio-5	24.99 (2.45)	-	25.05 (1.88)	-	25.48 (2.37)	c	24.55 (2.48)
Bio-6	-5.09 (4.09)	c,h	-10.02 (2.65)	c,g,l	-6.08 (2.23)	c,h	-13.31 (3.15)
Bio-7	30.08 (5.28)	c,h	35.07 (3.48)	c,g,l	32.26 (2.30)	c,h	37.86 (2.46)
Bio-8	0.21 (3.83)	c,h	-2.86 (2.39)	c,g,l	0.07 (1.89)	c,h	11.09 (6.31)
Bio-9	15.14 (2.26)	c,h	13.30 (1.30)	c,g,l	14.89 (2.20)	c,h	3.79 (7.45)
Bio10	15.71 (1.49)	c,h	13.56 (1.33)	g,l	15.02 (2.00)	h	14.46 (2.36)
Bio-11	-0.58 (3.65)	c,h	-3.84 (2.05)	g,l	-0.55 (2.02)	c,h	-4.77 (2.74)
Bio-12	1144 (471.40)	c,h,l	737.69 (427.01)	c,g	811.95 (295.07)	c,g	532.13 (111.21)
Bio-13	175.11 (88.99)	c,h	110.81 (77.64)	g,l	136.50 (57.18)	c,h	76.32 (19.71)
Bio-14	27.70 (7.81)	c,h,l	18.58 (4.96)	c,g,l	9.5 (3.38)	c,g,h	22.90 (8.17)
Bio-15	47.811 (13.69)	c,l	43.37 (2.59)	l	63.92 (14.82)	c,g,h	37.00 (15.75)
Bio-16	502.38 (256.04)	c,h	311.41 (219.93)	c,g,l	377.9 (160.21)	c,h	197.68 (47.06)
Bio-17	112.34 (19.02)	c,h,l	77.51 (23.84)	g,l	43.82 (12.52)	c,g,h	81.49 (25.36)
Bio-18	123.79 (22.04)	c,h,l	87.95 (25.02)	c,g,l	50.03 (11.21)	c,g,h	175.47 (48.40)
Bio-19	465.02 (238.23)	c,h	287.81 (206.04)	c,g,l	369.66 (160.15)	c,h	114.74 (48.13)
Solar	477557 (2659)	c,h,l	526134 (21993)	c.g.l	545876 (21149)	c,g,h	582174 (2515)

Table 3.3. Means for the Worldclim variables Bio 1-19 and solar insolation for 53 *Abies grandis* (ABGR), 83 *A. concolor* (ABCO) and 97 Hybrid (HYB) populations are listed with temperatures in Celsius, precipitation in mm and solar insolation in watt hours per square meter. The Steel-Dwass-Critchlow-Fligner procedure two-tailed test was performed to determine if population means are significantly different between HYB (h), ABCO (c), ABLO (l) and ABGR (g) at $p<0.05$. - indicates no differences.

Variable	F1	F2	F3
Bio-1 Annual Mean Temperature	-0.293	-0.147	0.524
Bio-2 Mean Diurnal Range Temp.	0.661	0.410	-0.482
Bio-3 Isothermality	0.300	0.503	-0.005
Bio-4 Temperature Seasonality	0.464	-0.089	-0.399
Bio-5 Max. Temp, of Warmest Month	-0.137	0.112	0.005
Bio-6 Min. Temp. of Coldest Month	-0.620	-0.091	0.547
Bio-7 Temperature Annual Range	0.551	0.146	-0.543
Bio-8 Mean Temp. of Wettest Quarter	0.791	-0.243	0.082
Bio-9 Mean Temp. of Driest Quarter	-0.729	0.131	0.222
Bio-10 Mean Temp. of Warmest Quarter	-0.084	-0.191	0.402
Bio-11 Mean Temp of Coldest Quarter	-0.399	-0.020	0.576
Bio-12 Annual Precipitation	-0.481	-0.307	0.487
Bio-13 Precipitation of Wettest Month	-0.437	-0.209	0.516
Bio-15 Precipitation Seasonality	-0.272	0.220	0.539
Bio-16 Precipitation of Wettest Quarter	-0.476	-0.220	0.509
Bio-17 Precipitation of Driest Quarter	-0.134	-0.784	-0.060
Bio-18 Precipitation of warmest Quarter	0.624	-0.589	-0.162
Bio-19 Precipitation of Coldest Quarter	-0.385	-0.130	0.516
Solar Insolation	0.806	0.350	-0.173

Table 3.4. List of correlations between the environmental variables and factors F1, F2 and F3 from the discriminant analysis.

	Cascades & Siskiyou Mountains		**Rocky Mountains**	
Variable	Latitude	Elevation	Latitude	Elevation
Bio-2 Mean Diurnal Range Temp.	-0.802	0.544	-0.864	0.434
Bio-6 Min. Temp. of Coldest Month	0.306	-0.928	0.617	-0.832
Bio-8 Mean Temp. of Wettest Quarter	0.191	-0.849	0.133	-0.733
Bio-9 Mean Temp. of Driest Quarter	0.128	-0.577	0.154	-0.710
Bio-17 Precipitation of Driest Quarter	0.628	0.423	0.704	0.420
Bio-18 Precipitation of warmest Quarter	0.573	0.380	0.691	0.388
Bio-19 Precipitation of Coldest Quarter	0.385	0.446	0.613	0.182
Solar Insolation	-0.556	0.921	-0.398	0.788
Elevation	-0.585	NA	-0.665	NA

Table 3.5. Lists R^2 values from Lowess regression analysis testing the relationship between the environmental variables that contributed the greatest to discriminant analysis factors F1 and F2 and latitude and elevation along a Rocky Mountain transect and a Pacific Slope transect from the northern most *A. grandis* population to the southern most Class HYB hybrid population.

Appendix I

Genotypes Table

ID	AS09		AS13		AS20		NFF3		SF50		ABf18	
al1	233	255	241	245	186	186	112	114	92	92	286	294
al10	255	255	229	243	186	194	114	114	92	94	282	284
al2	239	255	231	243	182	186	114	114	92	94	282	284
al3	233	235	233	243	188	194	114	124	92	94	286	286
al4	231	233	243	245	204	206	114	114	92	94	284	286
al5	239	239	231	243	180	182	114	114	92	120	282	284
al6	233	235	237	243	180	214	114	114	92	92	282	284
al7	233	263	231	235	176	194	114	114	92	92	282	284
al8	233	233	231	243	206	212	112	114	92	94	282	282
al9	233	239	243	243	188	214	114	114	92	92	284	296
bb1	253	265	239	245	182	182	110	114	92	96	286	290
bb10	245	253	239	241	186	194	110	116	98	98	284	306
bb2	261	261	237	249	182	188	112	114	96	96	298	336
bb4	247	253	233	233	182	190	110	110	92	98	298	298
bb5	255	261	233	239	186	190	114	116	92	92	302	302
bb6	265	265	231	243	176	198	110	118	92	92	286	290
bb7	251	253	225	237	188	218	110	114	92	92	340	340
bb8	253	253	233	237	182	192	112	114	92	92	288	290
bb9	253	261	239	239	176	180	110	118	96	100	302	320
bs1	249	253	233	243	188	204	114	114	92	92	282	286
bs10	249	253	233	239	176	188	114	114	92	96	282	286
bs2	249	253	233	243	190	204	114	114	92	92	282	294
bs4	239	255	241	247	188	188	114	114	92	102	296	312
bs5	253	255	223	247	188	190	108	114	92	92	286	286
bs6	253	255	223	247	188	190	108	114	92	92	286	286
bs7	233	259	237	239	190	204	114	114	92	92	282	294
bs8	255	257	237	237	204	208	118	118	92	92	282	284
bs9	249	255	237	239	180	180	114	114	92	100	286	294
cl1	233	233	223	241	188	194	108	114	94	94	282	282
cl10	233	233	223	235	180	204	114	114	94	94	282	300
cl2	233	233	233	243	182	188	108	114	92	92	282	300
cl3	233	233	235	243	188	204	108	116	94	94	282	284
cl4	233	239	223	223	188	194	114	114	92	92	282	282
cl5	233	239	235	241	192	204	116	118	92	112	282	312
cl6	233	233	223	235	182	194	114	114	94	94	300	300
cl7	231	233	233	235	176	180	114	114	94	96	282	286
cl8	233	255	223	235	182	204	108	114	92	94	282	282
cl9	233	233	233	233	180	194	108	116	92	94	282	302
dr1	233	251	225	245	176	176	112	120	92	92	282	282
dr2	233	251	225	237	176	194	112	114	92	92	282	294
dr3	253	253	229	239	176	176	114	120	92	92	282	294
dr4	255	261	237	241	176	196	114	116	92	92	282	294
dr5	261	267	241	243	206	206	120	114	92	92	286	286
dr6	253	267	241	243	176	194	114	114	92	92	286	286
dr7	235	259	237	237	194	194	114	120	92	92	282	286
dr8	237	267	233	241	194	194	112	114	92	92	284	286
fl11	247	253	239	241	192	200	112	116	92	96	290	316
fl12	247	255	227	227	180	182	114	118	92	96	290	304
fl13	247	253	227	227	180	198	114	116	92	96	290	290
fl14	253	255	229	229	182	182	112	114	92	96	290	290
fl15	253	257	227	243	190	192	116	116	98	98	314	314
fl16	247	251	227	227	192	198	128	128	92	98	304	314
fl17	243	247	227	239	180	192	116	116	92	92	290	316
fl18	251	251	227	227	190	192	116	116	92	96	304	304

fl19	243	273	227	229	190	198	116	116	92	92	286	290
fl20	243	255	227	227	180	214	116	118	96	96	304	310
gp1	233	235	235	243	176	182	114	116	92	94	284	288
gp10	243	257	235	237	190	194	114	122	92	94	282	282
gp11	251	261	225	235	184	194	114	118	92	92	284	294
gp12	239	255	233	233	182	202	108	116	92	114	282	284
gp2	249	265	239	241	190	192	112	112	92	122	286	288
gp3	253	267	233	239	188	206	114	116	92	96	282	286
gp4	257	265	219	245	176	194	112	114	92	96	282	296
gp5	253	255	237	241	180	192	114	114	92	92	282	298
gp6	249	257	219	231	198	202	116	120	92	92	282	286
gp7	249	251	235	243	180	198	112	118	94	112	282	286
gp8	251	255	237	243	188	202	114	114	92	92	286	292
gp9	231	245	239	239	188	194	114	116	92	104	278	298
ic1	255	267	237	241	176	190	114	122	92	92	286	286
ic10	233	253	233	241	176	176	110	114	92	92	284	286
ic2	253	259	239	241	196	196	114	114	92	92	286	286
ic3	233	267	231	237	180	194	116	116	92	92	286	288
ic4	255	255	239	243	180	212	114	116	92	92	286	288
ic5	241	259	233	235	178	194	112	114	92	92	282	286
ic6	253	253	233	237	176	182	118	122	92	92	282	284
ic7	253	257	223	227	180	182	114	116	92	92	282	286
ic8	233	267	233	233	182	202	110	114	92	114	286	288
ic9	255	255	233	235	182	192	114	114	92	94	286	286
kl10	249	255	233	243	190	202	108	116	92	94	286	288
kl2	255	255	227	231	178	190	114	114	92	98	282	288
kl3	253	253	237	239	176	188	114	114	92	92	282	286
kl4	257	265	239	239	182	188	112	114	92	92	282	282
kl5	233	257	245	245	180	202	112	118	92	92	284	290
kl6	237	255	233	237	176	190	114	116	92	92	282	286
kl7	239	253	233	239	176	176	112	118	92	94	294	298
kl8	251	261	237	237	176	176	110	114	100	112	282	286
kl9	239	257	237	237	182	182	110	114	92	100	282	286
ks1	249	257	225	237	178	182	112	116	92	92	282	282
ks2	249	255	225	231	182	190	114	116	92	92	286	294
ks3	255	261	233	241	188	190	110	112	92	92	282	286
ks4	241	251	225	233	176	180	112	112	92	92	286	294
ks5	243	263	225	229	176	176	116	118	92	92	288	290
ks6	239	263	227	239	186	206	112	118	92	92	286	294
ks7	255	263	229	231	182	182	112	118	92	92	284	286
ks8	251	263	225	239	180	188	112	114	92	92	286	288
ks9	249	253	225	235	178	180	112	116	92	94	286	294
ks10	253	255	233	233	180	182	112	112	92	92	286	288
la1	233	257	223	237	176	196	114	114	92	118	-9	-9
la10	251	255	223	233	180	204	114	120	92	92	282	290
la2	251	255	223	249	194	194	114	114	92	112	282	300
la3	233	255	237	239	194	204	116	116	92	94	282	282
la4	233	233	231	231	194	204	114	116	92	94	282	282
la5	233	255	223	233	194	196	114	114	92	92	282	286
la6	233	255	223	237	176	194	114	114	92	92	282	286
la7	233	233	223	239	176	204	114	114	92	98	282	300
la8	233	255	237	237	176	188	116	116	92	94	282	282
la9	233	255	235	235	194	196	116	120	92	92	282	282
lk1	251	255	233	233	188	190	118	126	94	102	284	306
lk10	231	253	225	239	180	190	114	116	112	112	290	290

lk2	245	253	235	239	198	214	114	114	92	92	282	282
lk3	233	233	225	235	188	188	114	116	92	92	284	302
lk4	239	249	225	231	186	194	114	114	92	94	286	294
lk5	233	257	225	237	190	204	112	116	92	94	282	292
lk7	253	265	237	243	190	210	110	112	92	94	286	286
lk8	233	251	233	247	190	196	110	114	92	96	284	298
lk9	251	261	225	233	182	204	112	114	92	94	284	302
m10	255	261	241	243	188	192	114	114	92	92	286	294
m2	245	261	237	239	182	204	116	116	92	92	288	306
m3	251	261	235	239	190	206	114	114	92	92	282	286
m4	239	261	239	245	182	204	114	114	92	92	286	288
m5	233	247	237	243	176	194	114	114	92	92	290	296
m6	233	257	233	239	192	194	112	114	92	92	282	284
m7	233	235	229	235	202	202	114	118	100	118	282	286
m8	231	259	233	239	180	192	112	114	92	92	284	286
m9	233	239	245	245	210	210	114	114	92	120	284	286
ow1	249	255	237	239	182	192	114	116	96	96	296	296
ow10	253	253	221	239	176	182	114	114	92	92	288	294
ow2	255	267	229	239	176	206	110	114	110	114	290	298
ow3	255	255	239	243	176	200	110	114	92	96	296	314
ow4	245	261	243	243	176	190	110	114	92	92	310	314
ow5	255	267	239	239	176	200	116	128	92	92	290	298
ow6	253	267	239	243	182	200	112	114	92	92	290	314
ow7	253	263	239	241	190	200	110	114	92	92	296	314
ow8	253	253	239	247	190	200	110	114	92	92	290	352
ow9	245	257	243	243	176	200	114	114	92	98	298	298
pl1	253	259	235	243	176	192	110	114	92	96	302	306
pl10	253	261	227	245	176	182	114	116	92	110	286	308
pl2	263	307	235	241	176	218	110	114	92	100	282	294
pl3	253	255	247	247	180	196	110	114	92	94	290	292
pl4	251	251	241	245	182	202	114	114	106	106	292	294
pl5	251	253	241	241	182	192	114	118	92	92	286	296
pl6	255	255	233	237	176	176	110	114	92	100	282	292
pl7	251	251	239	245	182	196	114	116	100	100	292	292
pl8	253	255	239	241	188	198	114	114	92	92	284	286
pr1	237	261	241	241	176	194	114	114	92	92	284	286
pr10	249	267	239	249	180	196	110	114	92	92	286	296
pr2	251	251	235	235	180	182	114	116	92	92	286	286
pr3	249	261	231	241	182	194	114	114	92	92	282	286
pr4	233	255	237	249	176	202	114	114	92	94	282	286
pr5	251	251	235	235	176	196	114	114	92	102	286	308
pr6	267	267	231	239	180	208	108	116	92	92	286	286
pr7	231	233	237	243	194	196	114	114	92	92	284	284
pr8	233	233	231	249	176	180	114	114	92	92	286	286
pr9	255	255	235	235	188	194	116	116	92	118	296	298
sid1	231	253	225	235	176	180	114	118	94	94	286	286
sid10	247	249	225	235	176	182	114	116	92	92	290	296
sid2	249	253	241	245	176	194	114	118	92	92	282	282
sid3	231	253	225	245	180	188	108	114	92	92	294	294
sid4	249	255	233	241	176	188	114	114	92	104	286	286
sid5	257	273	235	245	180	180	114	118	92	92	286	286
sid6	259	267	225	237	180	222	114	116	92	104	286	312
sid7	233	257	225	243	176	182	116	116	92	92	286	286
sid8	251	255	225	243	176	196	118	118	92	92	282	286
sid9	261	267	237	237	180	208	114	116	92	104	284	286

sm1	263	263	231	243	194	194	114	116	92	92	286	286
sm10	243	249	231	239	188	192	112	114	92	92	284	286
sm2	239	243	231	243	180	192	112	112	92	96	286	292
sm3	239	263	231	243	192	194	112	114	92	92	286	286
sm4	243	255	241	243	182	190	112	114	92	92	282	286
sm5	251	263	237	243	192	196	112	112	92	96	286	288
sm6	243	243	239	243	192	192	114	116	92	92	286	286
sm7	243	253	231	241	182	192	112	114	92	92	286	316
sm8	243	251	231	241	182	194	114	114	92	92	282	308
sn1	231	233	241	241	194	194	118	120	92	92	282	294
sn10	231	239	241	243	180	194	114	116	92	98	294	294
sn2	233	257	241	243	194	214	116	120	96	96	282	284
sn3	233	233	249	255	176	188	114	116	92	94	282	282
sn4	257	257	243	249	180	194	114	120	92	120	282	294
sn5	233	255	239	241	204	204	114	114	92	94	282	284
sn6	233	239	237	243	180	180	114	116	92	92	282	294
sn7	233	257	235	241	176	194	114	114	94	94	284	284
sn8	231	233	223	235	194	208	114	118	92	94	294	308
sn9	253	263	241	243	204	206	116	116	92	106	286	294
tb1	257	267	241	249	196	202	114	114	92	92	282	284
tb2	255	255	233	239	200	206	114	116	92	94	282	286
tb3	233	263	225	233	180	182	114	114	94	112	286	292
tb4	233	253	235	245	180	180	112	120	92	92	286	294
tb5	261	263	233	241	188	206	110	110	92	94	282	298
tb6	255	257	225	235	176	194	112	112	96	96	282	300
tb7	247	251	231	237	186	188	114	116	92	94	284	294
tb8	233	253	237	239	186	194	112	116	92	92	288	296
tc1	233	233	225	237	188	204	114	114	96	118	282	300
tc10	231	233	233	237	188	194	108	114	92	92	282	300
tc2	233	233	223	237	204	204	108	114	92	94	282	282
tc3	233	233	237	237	176	204	114	114	92	94	282	300
tc4	233	233	233	237	176	204	112	114	92	94	282	300
tc5	233	233	223	223	176	188	112	114	94	120	282	282
tc6	233	233	223	237	176	188	112	114	92	94	282	282
tc7	231	233	233	241	194	204	114	116	92	92	282	282
tc8	233	233	233	239	194	194	108	114	92	92	300	300
tc9	233	233	223	237	176	204	108	112	92	92	282	300
wah1	257	259	239	241	182	192	112	122	92	112	286	296
wah10	255	259	237	239	176	192	112	114	92	92	282	286
wah11	249	253	227	241	196	196	114	114	92	92	286	290
wah2	251	255	229	235	182	188	114	116	100	120	284	286
wah3	253	253	239	241	200	202	110	116	106	106	286	296
wah4	255	259	233	245	178	186	112	114	92	92	284	294
wah5	261	265	239	241	176	200	116	118	92	94	286	296
wah6	255	257	231	237	190	196	114	114	92	92	284	284
wah7	251	255	235	237	176	188	112	114	92	100	286	288
wah8	253	259	225	241	178	178	112	114	96	96	284	302
wah9	239	257	237	241	176	180	116	116	92	96	282	296
wal1	241	247	237	243	194	202	114	118	92	94	282	288
wal2	239	255	217	241	182	198	112	114	92	96	286	304
wal3	253	259	235	235	198	198	114	116	92	92	286	296
wal4	231	259	231	241	176	180	110	114	92	92	294	310
wal6	251	265	239	243	196	208	112	114	92	92	296	296
wal8	251	253	233	237	182	182	110	114	96	96	282	282
wal9	257	259	231	241	208	208	114	114	92	94	292	292

we1	239	253	225	243	188	204	116	118	92	102	286	290
we10	233	237	225	233	176	180	112	114	92	96	294	298
we2	247	251	225	231	182	190	114	116	92	92	282	282
we3	263	265	215	239	180	204	112	112	92	92	284	302
we4	253	257	241	249	180	190	112	114	92	100	284	288
we5	239	251	225	233	190	194	114	114	92	100	282	288
we6	247	247	239	241	188	206	110	112	92	92	282	282
we7	253	265	241	241	182	190	112	116	92	94	282	300
we9	249	257	237	241	180	196	112	114	92	92	282	284
wf10	235	253	235	239	176	182	112	114	92	92	308	308
wf1	253	253	233	235	180	182	114	114	92	92	300	308
wf2	235	249	235	239	182	192	112	114	92	92	288	316
wf4	235	253	233	239	180	192	112	114	92	92	290	308
wf5	253	261	239	239	182	192	114	114	92	92	290	316
wf6	253	253	235	239	180	190	112	114	92	92	308	316
wf7	251	253	233	243	180	200	114	116	92	92	308	308
wf8	253	253	239	239	176	182	114	114	92	92	308	308
wf9	235	253	235	239	182	192	112	124	92	92	300	316
ws1	259	267	239	239	180	186	108	114	92	92	282	292
ws2	233	239	239	247	180	190	114	118	92	92	282	282
ws3	233	257	243	247	176	180	108	116	92	94	282	282
ws4	233	239	233	241	182	204	114	116	92	118	300	302
ws5	233	257	243	247	176	180	108	116	92	94	282	282
ws6	233	235	239	239	176	204	114	118	94	94	282	282
ws7	239	253	239	243	190	196	114	118	92	92	282	300
ws8	233	239	241	249	188	196	114	122	92	92	288	300
n1	233	255	231	245	182	182	114	112	96	96	278	284
n2	233	233	233	237	176	180	118	116	94	102	292	294
n3	233	259	225	249	180	194	116	114	92	92	278	292
n4	249	267	225	237	180	210	120	114	92	94	286	288
n5	233	263	231	237	192	194	120	116	96	96	292	292
n8	239	263	233	237	182	196	116	114	92	94	284	298
n9	231	233	225	237	190	206	114	116	92	114	286	288
n10	265	295	231	237	176	180	118	116	92	92	284	296
n11	257	265	235	239	176	188	118	114	92	92	286	288
n12	255	273	235	243	184	204	116	112	92	92	294	308
n13	233	253	235	237	180	206	114	114	94	116	282	282
n14	253	259	239	239	176	180	114	114	92	92	282	286
n15	233	247	221	239	180	198	118	114	92	92	282	286
s1	233	259	241	241	176	190	116	116	92	92	284	294
s2	247	259	235	239	176	184	114	114	92	94	294	298
s3	249	255	235	237	176	188	114	112	94	104	284	288
s4	249	249	235	239	186	204	116	112	92	94	286	286
s5	253	253	239	245	180	210	116	114	92	92	282	284
s6	249	257	237	247	190	190	114	112	92	102	284	286
s7	261	269	231	239	186	190	114	114	92	102	284	296
s8	233	257	239	245	180	196	114	110	92	102	282	292
s9	239	253	239	239	186	186	114	114	92	92	284	284
s10	241	255	233	249	176	176	114	114	92	104	284	306
s11	247	253	235	239	190	196	114	116	92	92	284	292
s12	253	259	239	241	190	200	114	118	92	102	282	292
s13	259	265	233	243	180	190	114	114	92	92	278	298
s14	253	295	245	245	182	202	114	114	92	92	286	294

Appendix I. Genotypes table lists alleles for all individuals at loci AS09, AS13, AS20, SF50, NFF3 and ABF18.

Appendix II

Fst Table

	AL	BB	BS	CL	DR	IC	KL	KS	LA	LK	M	OW	PL	PR	SD	SM	SN	TC	WH	WE	N
BB	.148																				
BS	.092	.092																			
CL	.109	.181	.171																		
DR	.121	.100	.076	.195																	
IC	.115	.062	.048	.171	.029																
KL	.079	.030	.019	.130	.023	.007															
KS	.180	.072	.114	.225	.077	.143	.040														
LA	.103	.154	.106	.064	.086	.218	.057	.171													
LK	.064	.016	.047	.085	.073	.109	.008	.052	.083												
M	.055	.059	.048	.130	.038	.143	.016	.060	.085	.022											
OW	.141	.034	.091	.222	.103	.131	.049	.123	.169	.069	.071										
PL	.110	.024	.072	.159	.070	.117	.025	.095	.124	.032	.036	.049									
PR	.084	.096	.060	.156	.033	.177	.039	.089	.091	.048	.009	.099	.044								
SD	.121	.075	.054	.165	.037	.142	.020	.041	.120	.034	.039	.098	.046	.009							
SM	.146	.116	.130	.247	.097	.177	.086	.072	.194	.101	.064	.143	.110	.075	.096						
SN	.063	.102	.099	.066	.069	.154	.064	.129	.062	.040	.050	.121	.072	.074	.071	.143					
TC	.155	.198	.169	.039	.165	.290	.125	.232	.050	.119	.142	.235	.197	.187	.193	.266	.113				
WH	.105	.027	.058	.159	.050	.097	.004	.044	.110	.013	.020	.049	.003	.030	.023	.072	.060	.178			
WE	.101	.042	.065	.146	.052	.117	.015	.023	.108	.005	.030	.082	.050	.071	.049	.075	.053	.154	.019		
N	.070	.035	.060	.092	.053	.105	.008	.053	.064	.000	.015	.070	.034	.028	.014	.089	.026	.109	.014	.026	
S	.078	.049	.039	.152	.066	.134	.014	.078	.111	.007	.013	.045	.026	.036	.040	.108	.062	.174	.007	.026	.014

Appendix II Fst Table. Fst coefficients between population pairs.