GENETIC STRUCTURE, GENE FLOW AND LOCAL ADAPTATION IN THE INTERIOR SPRUCE (*Picea glauca* x *Picea engelmannii*) HYBRID ZONE

by

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Abstract

Natural hybrid zones provide a great opportunity to study the evolutionary relationships between closely related species. I have combined ten microsatellites (SSR) and 311 single nucleotide polymorphism (SNP) markers with quantitative data to investigate the genetic structure, interspecific gene flow and adaptation of the economically and ecologically important *Picea glauca* (white spruce) *x P. engelmannii* (Engelmann spruce) hybrid zone in western North America. Climate modelling and paleoclimate analysis was used to study the historical evolutionary relationships between hybridizing species; and to predict future patterns of genetic variation in the zone. This modelling suggests these species may have been in contact for as long as 21,000 years. Current levels of admixture and introgression are extensive, as suggested by both the SSR and SNP analyses, with populations showing elevational and latitudinal unimodal clines in admixture. Hybrids occupy intermediate environments in the zone and show a higher genetic contribution from Engelmann spruce than from white spruce on average. Despite a long history of interspecific gene flow, pure species and hybrids are adapted to different environments. Results of the quantitative analysis based on long-term data on growth and survival, as well as bud phenology and cold hardiness, indicate that the white x Engelmann spruce hybrid zone is maintained by adaptation to the length of growing seasons and the persistence of the snowpack (exogenous selection), in which hybrids are fitter than pure species in intermediate environments, fitting the “Bounded hybrid superiority” model of hybrid zone maintenance. I identified 12 outlier SNPs among the 311 SNPs; these were genes responsible for carbohydrate metabolism, signal transduction and transcription factors. These results have significant implications for forest management and breeding of spruce species in British Columbia, where this species complex is managed as one species without considering the complexity in population structure and adaptive differences between pure species and hybrids.

Preface

For all the chapters of this thesis, I was responsible for the experimental design, planning the methodology, collecting and analyzing the data, and writing the thesis. This work would not have been possible without the support of my supervisor, Sally Aitken. She was responsible for providing guidance in all the steps of the research, obtain and provide funding and review draft manuscripts, and will be a co-author on all publications resulting from this thesis.

Besides my supervisor, three people had collaborated with this work. Tongli Wang, who is a research associate at UBC and the associate director of the Centre for Forest Conservation Genetics, conducted the climate modelling used in Chapter 3 (Climate analysis section). Also, David Roberts, who is a PhD candidate at the University of Alberta has produced the maps for the past and post-glacial climate modelling used for Chapter 4. Finally, Barry Jaquish, who is a senior researcher and head of the Interior Spruce breeding program from the British Columbia Ministry of Forests, Lands and Natural Resource Operations have provided the growth data used in the fitness analysis (Chapter 3). Tongli and Barry will be included as co-authors of any publications resulting from chapter 3; and David will be included as co-author of any publications resulting from chapter 4 of this thesis.

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1. Literature review and research objectives

1.1 Introduction

The study of hybrid zones can provide us with insights about evolutionary processes and the effects of selection and gene flow in the spread of adaptation (Barton and Hewitt 1989; Arnold 1997). Hybrid zones may also hold important clues regarding the genetic architecture of adaptively important traits in trees, in particular in cases in which species barriers are due to genes conferring differential adaptation (Lexer et al. 2005). Introgression in hybrid zones is an important source of new variation, and resulting hybrids may have the ability to succeed in disturbed or changing environments.

Picea glauca (white spruce) and *Picea engelmannii* (Engelmann spruce) are closely related tree species that hybridize extensively in British Columbia and the western part of Alberta. The hybrid zone is mainly composed of hybrids with a clinal intergradation of morphological and physiological characteristics along elevational gradients (Roche 1969; Ledig et al. 2006), with *P. glauca* restricted to low elevations and *P. engelmannii* to high elevations. Introgression in the zone of sympatry is extensive, possibly reflecting an ancient origin (Ledig et al. 2006). The process of maintenance of this hybrid zone is still unknown. It has been suggested that hybrids are fitter that their parents in intermediate environments (Roche 1969; Daubenmire 1974; Ledig et al. 2006); however, the fitness of natural or artificial hybrids has never been measured directly nor indirectly.

Because of their similarity in morphology, white spruce and Engelmann spruce have been treated as a complex, known as interior spruce, for forestry and conservation in British Columbia. Since the 1960's the B.C Ministry of Forests, Lands and Natural Resources Operations has developed an extensive breeding and tree improvement program. Currently, interior spruce represents the second most planted type of seedling in British Columbia, with almost 59 million seedlings planted annually (BC Ministry of Forests 2006/2007).

1.2 Interior spruce

White spruce is a transcontinental, boreal species that occurs naturally in almost all forested regions in Canada, with the exception of the Pacific Coast (Andalo et al. 2005, Rajora et al. 2005). With the exception of Alaska, where white spruce ranges from lowlands to alpine treeline, white spruce is generally restricted to low elevations (Nienstaedt and Zasada 1990, Rajora and Dancik 2000). Engelmann spruce extends from British Columbia and Alberta in the north, to New Mexico and Arizona in the south. Engelmann spruce is common in British Columbia and the northern United States, but its distribution becomes fragmented in the southern ranges of the Central Cordillera (Ledig et al. 2006). Engelmann spruce is a characteristic species of the subalpine forest. It grows at high elevations and is restricted to cold, humid habitats because of its low tolerance to high temperature and drought (Alexander and Shepperd 1990).

1.2.1 Phylogeography and phylogenetics

Picea is a large genus in the Pinaceae, with species in Asia, Europe and North America. Phylogenetic studies based on chloroplast, mitochondrial and nuclear data suggest that the closest genus to *Picea* is *Cathaya* (a current endemic Asian genus) (Wang et al. 2000; Liston et al. 2003). *Pinus*, *Picea, Cathaya*, and *Pseudolarix* became established in the early and middle Cretaceous (145-65 million years ago) according to molecular phylogenetic studies and fossil records (Wang et al. 2000).

In comparison to *Pinus*, the genus *Picea* is relatively monophyletic (Bouille and Bousquet 2006). Low sequence divergence and incongruent chloroplast, mitochondrial and nuclear phylogenies suggest a recent speciation or reticulate evolution in *Picea* (Bousquet et al. 2007). Also, mtDNA phylogenies are geographically more structured than cpDNA phylogenies and incomplete lineage sorting is evident at nuclear loci (Bouille and Bousquet 2005; Campbell et al. 2005).

Two major hypotheses of the origin of *Picea* suggested dispersal from Asia to North America (Wright 1955; Nienstaedt and Tiech 1972). However, recent molecular studies suggest that *Picea* originated in North America and then migrated first to Asia (center of diversity) through the Beringian land bridge, and then to Europe (Ledig et al. 2004; Sigurgeirsson and Szmidt 1993; Ran et al. 2006).

White spruce appears to have recolonized British Columbia since the last glacial maximum (less than 14000 years ago). During glacial periods, populations were pushed south of the present distribution, with the exception of scattered relicts (Nienstaedt and Zasada 1990). Alaska and the unglaciated Yukon Valley north from the ice front might have been a refugium for white spruce populations (Anderson et al. 2006). After the last glaciation, white spruce re-established its transcontinental range migrating north from the Appalachian Mountains and possibly south from a putative northern refugium in the Yukon Valley. Descendants from Northern and Southern refugial populations could not have come into contact until about 4000 years ago when Lake Agassiz in Manitoba, Minnesota, and northwestern Ontario dried up (Spurr and Barnes 1980).

Engelmann spruce colonized British Columbia and Northern Idaho only after the recession of the Cordilleran Ice Sheet (Ledig et al. 2006). Populations of Engelmann spruce appear to have been more abundant and occupied lower elevations in the Rocky Mountains and the Great Basin during the Pleistocene (Hamrick et al. 1994). When the temperature rose during the Xerothermic Period (10,000-7,000 years B.P), populations at the southern extremes become more fragmented. Finally, during the Holocene, Engelmann populations were pushed to higher elevations than present (Ledig et al. 2006). White and Engelmann spruce are closely related species (Sigurgeirsson and Szmidt, 1993; Weng and Jackson, 2000; Ran et al. 2006), which came into secondary contact during the last glacial maximum or at the end of the Pleistocene (Daubenmire 1974; Ledig et al. 2006).

1.2.2 Molecular markers studies

Published marker studies on Engelmann and white spruce have addressed different objectives. They have evaluated genetic variability in relation to: impacts of artificial selection and silvicultural practices (King et al., 1984; Desponts et al., 1992; Stoehr and El-Kassaby 1997; Rajora 1999; Rajora et al., 2005); impacts of forest fragmentation (O'Connell et al., 2006; Tremblay & Simon 1989); and evolutionary history (Alden and Loopstra 1987; Ledig et al. 2006, Rehfeldt 2004, Rajora and Dancik 2000). These studies have revealed high levels of polymorphism and heterozygosity in both white spruce and Engelmann spruce populations (Ledig et al., 2006, Rajora 1999, Rajora et al., 2005, O'Connell et al., 2006, Alden and Loopstra 1987) (Table 1.1). Slight deficiencies in heterozygotes and some inbreeding were found in most populations of white spruce. Only a large stand in Ontario showed an excess of heterozygotes in both parental and filial populations (Cheliak et al. 1985). Genetic differences between populations (fixation index F_{st}) varied from 0.01 (low differentiation) to 0.14 (moderate differentiation) using allozymes markers.

Chloroplast DNA markers have been used as species diagnostic markers to differentiate white spruce and Engelmann spruce from other hybridizing spruces. Blue spruce (*Picea pungens*) was differentiated from Engelmann spruce using four RFLP fragments (Stine and Keathley 1990). Seedlots of Sitka spruce (*Picea sitchensis*) and white spruce were classified using four restriction endonucleases in coastal British Columbia (Szmidt et al. 1988). Mitochondrial markers were also used to differentiate *Picea* species. Twelve mitochondrial markers were used to differentiate *Picea* species (*P. glauca, P. engelmannii, P. mariana, P.pungens, P.rubens, P. sitchensis, P. abies*) from other conifers such as *Abies balsamea*, *Tsuga canadiensis*, and others (Jaramillo-Correa et al. 2003).

1.2.3 Mating system

Picea species are monoecious (separate female and male strobili are produced by the same individual), and predominantly outcrossing (Morgenstern 1996). Estimates of outcrossing rate ranged from 0.7 to 1 in white spruce populations and from 0.9 to 0.95 in Engelmann spruce populations. As is expected for a widespread, wind-pollinated conifer species, white spruce show large-scale pollen mediated gene flow dispersing as far as 3000 m away from the donor tree (O'Connell et al 2007). Although their levels of outcrossing (t_m) are high, some self-fertilization has also been found in white spruce populations. Denti and Schoen (1988) found self-fertilization ranged from 0 to 22% in clonal populations in Ontario.

Self-fertilization or small population sizes (Coles and Fowler 1976; Park et al.1984) seem to explain the levels of inbreeding found in natural populations of white spruce (Table 1.2). In populations of central New Brunswick, an inbreeding coefficient of 0.145 was calculated from two stands (Coles and Fowler 1976; Park et al.1984). Inbreeding depression is high, as evidenced by the severe effects of self-fertilization on seed set, early growth and survival. The total number of lethal equivalents per zygote for white spruce (from pollination to age 17) is estimated to 12.6, relatively high in comparison to other conifers and other life forms in general (Fowler and Park 1983). The number of lethal equivalents is estimated as four times the negative natural logarithm of the self-fertility (percent full seed from selfing divided by percent full seed from crossing).

1.2.4 Adaptive variation

In temperate forest tree species, adaptive differentiation usually evolves as a response to local climate, involving traits related to synchronization of growth and dormancy with local seasonal conditions (Howe et al. 2003, Savolainen et al. 2007). Populations from the mildest climates at low latitudes or low elevations have higher growth potential, long periods of active growth, and low tolerance to the cold; while those from colder environments at high elevations

or latitudes display high cold tolerance, short growing seasons, and low growth potential (Howe et al. 2003, Rehfeldt 2004).

Studies of genetic diversity in white spruce using quantitative traits have revealed significant population differentiation, especially for important traits such as growth (Furnier et al 1991; Jaramillo-Correa 2001; Li et al 1993; Andalo et al 2005; Green 2005); bud phenology (Li et al 1997; Jaramillo-Correa 2001); and wood density (Corriveau et al 1987, Jaramillo-Correa 2001). Clinal patterns of adaptive variation have been found in white spruce. Height at ages 8 (Qst=0.082) and 13 (Qst=0.069) were negatively correlated with latitude (r=-0.78 and r=-0.79, respectively) and longitude (r=0.85)(Jaramillo-Correa et al. 2001). Wood density (Qst=0.102) was positively correlated with longitude (Jaramillo-Correa 2001; Beaulieu and Corriveau 1985); and seedling timing of height-growth cessation was negatively correlated with elevation (Green 2005). Andalo et al. (2005), studying populations in Quebec, found that differentiation in growth was shaped not only by temperature (correlated with latitude) but also by precipitation. Rehfeldt (2004) also found clinal variation among populations associated with gradients in several climatic variables (summer-winter temperature differential, length of the frost free period and Julian date of the last spring frost, among others).

Quantitative genetic variation among Engelmann spruce populations is also arranged along geographic clines (Rehfeldt 1994). Populations along elevational or latitudinal gradients showed adaptations to growing seasons of different length and to a variable frost-free period (Rehfeldt 1994). Winter-summer temperature regimes are also determinants of genetic differentiation among populations for quantitative traits (Rehfeldt 2004). In the northern part of Engelmann spruce range (Kootenays mountains in British Columbia) populations separated by 420 m of elevation differ significantly for shoot elongation (Rehfeldt 1994). In the south, populations are more differentiated due to steeper clines. Populations from the southwestern United States (south of Utah and Colorado) differ from populations from the north to such an

extent that they may deserve subspecific recognition (Rehfeldt 1994; Ledig et al. 2004; Rehfeldt 2004; Ledig et al.2006).

1.2.5 Interior spruce breeding in British Columbia

White spruce and Engelmann spruce are important economic components of British Columbia's forests, managed collectively as "interior spruce". Today, interior spruce is the second most planted type of seedling (after lodgepole pine), representing 28% (59 million) of the seedlings planted annually in British Columbia (B.C Ministry of Forests 2007).

Interior spruce trees are highly valued for their wood quality relative to fast-grown plantation trees from elsewhere in the world. White spruce is one of the preferred species for pulp and paper production and it is also used in many ways by the lumber industry (Corriveau et al. 1987). Engelmann spruce lumber is used for construction when great strength is not required. Rotary cut spruce veneer is used in manufacturing plywood. Specialty items such as violins, pianos, and aircraft parts are produced from Engelmann spruce (B.C Ministry of Forests 2007).

Aiming to produce better trees for reforestation, B.C forest breeders began the interior spruce breeding research program in the late 1960s. The breeding focused on improving traits related to tree size, wood traits and white pine shoot tip weevil (*Pissodes strobi*) resistance through recurrent selection (FGC 2005-2006). Parent-tree selection and open-pollinated progeny testing began in Prince George, East Kootenay and Smithers regions to expand later in the 1970s to other seed planning zones. To determine the location of seed planning units and establish seed transfer limits, the B.C Ministry of Forests, Mines and Lands established several common garden experiments called provenance tests. Provenance tests help to reveal natural patterns of genetic differentiation among populations from different geographical regions.

Tree improvement in British Columbia is a complex and long process involving two main components: breeding and seed production. In the breeding component, the first step is to

select wild parent trees that exhibit desirable phenotypic traits. After that, seed is collected and seedlings are tested in progeny tests. Progeny testing requires 5 to 15 years before reliable results are obtained. Based on progeny performance, parent trees are reselected and intermated through controlled pollination. Using seed from these crosses, new families are used for second-generation progeny testing and further selection. In the production component, scions from selected parent trees from progeny tests are collected and grafted into seed orchards. Seed orchards, developed to produce seed from selected parent trees in breeding programs for reforestation, are established to provide seed for different geographic areas and elevational zones known as seed planning units (Table 1.3). Seedlings grown from orchard seed exhibit desirable traits and are planted throughout the province to replace forest stands that are harvested, destroyed by fire, or killed by insects or disease (B.C Ministry of Forests 2005).

1.3 Hybridization, hybrid zones and introgression

Natural hybridization and hybrid zones have long attracted the attention of evolutionary biologists (Harrison 1993). Hybridization, defined as the process by which new offspring result from the cross-fertilization between individuals of different taxa, is very common in plants. Similarly, introgression can be defined as the movement of genes between species, mediated by hybridization followed by backcrossing (Rieseberg and Carney 1998). Hybrid zones are regions in which genetically distinct populations meet, mate and produce hybrids (Barton and Hewitt 1985; Hewitt 1988).

1.3.1 Importance of the study of hybrid zones

Debates about the evolutionary significance of hybridization have a long history (Harrison 1993). Some researchers have argued that hybridization is a potent evolutionary force that facilitates adaptive evolution and can lead to new species (Anderson 1949, Arnold 1997). According to this, new gene combinations resulting from hybridization promote the development of novel adaptations (Rieseberg et al. 2003). In contrast, natural hybridization

between divergent populations has been considered an evolutionary dead end or as evolutionary noise by some researchers (Mayr 1942).

Although definitive support of either point of view is lacking, recent studies have supported the first hypothesis for plant species. Some sunflower (*Helianthus*) species found in the most extreme habitats have proved to be ancient hybrids, suggesting that new combinations generated by hybridization have contributed to ecological divergence (Rieseberg et al. 2003). Also, introgression (Wheeler and Guries 1987) and hybrid speciation are an important part of the evolutionary history of pines in North and Central America (Wang et al.1990; Wang and Szmidt 1994). Studies in *Quercus* (oaks, e.g., Whittemore and Schaal 1991; Kremer and Petit 1993) and *Eucalyptus* (e.g., Mc Kinnon et al. 2001) have suggested hybridization and introgression in natural populations. In both oaks and *Eucalyptus*, haplotype similarity of chloroplasts corresponds more closely with the geographic proximity of individuals than with their species designation.

1.3.2 Origin and maintenance of hybrid zones

There are two possible origins for hybrid zones. They can be formed as a result of secondary contact between populations that have differentiated in allopatry (Chapman 1892; Mayr 1942), or as a consequence of environmental gradients and consequent varying selection pressures in sympatry or parapatry (Endler 1977). The former hypothesis is consistent with the belief that geographic isolation is a prerequisite for differentiation and speciation. Primary intergradation and secondary contact can produce identical patterns of variation; therefore we should be cautious when inferring process from pattern (Harrison 1993).

It has been suggested that hybrid zones are mainly transient, and because of that they can face one of three scenarios 1) hybrid zones can lead to speciation via “reinforcement’ (evolution of pre-mating barriers to gene exchange in response to selection against hybrids). In plants, speciation can occur through polyploidy (a frequent mode of speciation in annual plants) or through diploid hybrid speciation (Turelli et al. 2001). 2) Hybrid zones can lead to the fusion

of the parental types. 3) Hybrid zones can lead to the extinction of one or the other parental form (Mayr 1942, 1963; Harrison 1993). In contrast to the previous viewpoint, empirical (Moore 1977) and theoretical (Barton and Hewitt 1985) analyses indicate that hybrid zones can remain stable over long periods of time (Arnold 1997). In stable hybrid zones, hybrids can persist without genetic swamping of either or both of the parental species.

The maintenance of hybrid zones has also been subject to considerable debate mainly because of different points of view about the power of natural selection and gene flow as homogenizing force (Harrison 1993). Environment-independent models suggest that hybrid zones are maintained by a balance between dispersal and selection against hybrids, with selection being independent of the environment (Mayr 1942; Barton 1979). These models assume that hybrids are less fit than their parents regardless of location (endogenous selection). Hybrid inferiority is thought to happen due to genetic incompatibilities between the two parental species (early generation hybrids as F1) or due to the break-up of co-adapted gene complexes that affect fitness traits (Barton and Hewitt 1985).

Environment-dependent models suggest that hybrid zones are maintained through selection gradients due to environmental heterogeneity (Endler 1973, 1977; Slatkin 1973; Moore 1977; Harrison 1986). In these models, hybrid fitness varies with the environment (exogenous selection). A combination of exogenous and endogenous selection is also possible for both environment-dependent and environment-independent models (Barton 2001, Arnold 1997).

The Bounded Hybrid Superiority model (Moore 1977) and the Mosaic model (Harrison 1986) are examples of environment-dependent models. In the Bounded Hybrid Superiority Model, hybrid individuals are fitter than either parental species in environments that are intermediate to the parental habitats, but are less fit than the parental species in their respective native habitats. Under this theory, hybridization and backcrossing would occur for many generations resulting in introgressed populations. The Mosaic Model is similar to the Bounded Hybrid Superiority Model but for situations when the parental species are distributed

in a spatial mosaic rather than a discrete separation of two environments or along a unidirectional environmental gradient.

The Tension zone (Barton and Hewitt 1985, 1989; Hewitt 1988) is an example of an environment-independent model. The Evolutionary Novelty model (Arnold 1997) combines endogenous and exogenous selection by suggesting endogenous selection against certain hybrid genotypes and exogenous selection acting for or against different hybrid genotypes leads to the invasion of parental or novel habitats by more fit individuals.

1.3.3 Background on *P.glauca x P.engelmannii* hybrid zone

The taxonomic, genetic and ecological relationships between white spruce and Engelmann spruce have been a continuous source of interest to botanists and ecologists of North America (LaRoi and Dugle 1967). High-elevation Engelmann spruce hybridizes extensively with low-elevation white spruce in areas where their ranges overlap, at the eastern periphery of its range in Alberta and at its lower elevational limits in British Columbia (Figure 1.1) (Roche 1969). Hybridization followed by advanced generations of hybridization or backcrossing has produced populations of intermediate morphological and physiological characteristics according to a clinal intergradation along elevational gradients (Roche 1969; Daubenmire 1974; Ledig et al. 2006). Hybrids have also been found in northern Colorado, southern Wyoming (Ledig et al. 2006) and Montana (La Roi and Dugle 1968). Introgression is thought to be extensive in the area of contact. Ledig et al. (2006) found that although Engelmann spruce's glacial refugium was in the south, the number of alleles increases from south to north, suggesting that introgressive hybridization with white spruce might have played an important role in the high levels of genetic diversity of Engelmann's populations in British Columbia and Alberta. Previous studies have led to similar conclusions (Ogilvie and Von Rudloff 1968; Roche 1969; LaRoi and Dugle 1968; Daubenmire 1974; Yeh and Arnott 1986; Sutton et al. 1994).

Earlier descriptions of the hybrid zone were based on morphological traits or elevation (Wright 1955; Garman 1957; Horton 1959; Taylor 1959; La Roi and Dugle 1968; Roche 1969). The most evident differences in morphology between these species are the seed cone scales (Taylor 1959, Daubenmire 1974, Taylor 1993, Strong and Hills 2006). White spruce cones are characterized by stiff, obovate-triangular scales with the apex being rounded or flattened, rather than erose, whereas Engelmann spruce has thin, papery, wedge-shaped scales, with the apex being erose to truncate (Taylor 1959). Horton (1959) proposed five categories to differentiate parental and hybrid populations in the Rocky Mountains and foothills of Alberta (La Roi and Dugle 1968). White spruce was predominant at elevations of less than 1220 m (4000 ft); Engelmann spruce was predominant at high elevations of more than 1825 m; and the intermediate hybrid swarm was located between these two elevations. Roche (1969), however, suggested that the elevations of the hybrid swarms in the Rocky Mountains (above 1220 m) were the highest in the province, and different from typical pure Engelmann stands at 1220-1524 m found elsewhere in British Columbia. Although elevation might be an important criterion to differentiate the species, elevation ranges for species and hybrids distributions vary with latitude.

Despite the increasing number of studies and the different methods used, there is still debate about whether white spruce and Engelmann spruce represent extreme phenotypes along a genetic continuum (Taylor 1959, Horton 1959, Rajora and Dancik 2000) or if they deserve specific recognition (Garman 1957, Wright 1955, Daubenmire 1974, Roche 1969). Taylor (1959) considered Engelmann spruce a subspecies of white spruce. Also Rajora and Dancik (2000) suggested that clinal variation in needle allozymes was a result of a single species evolving along an altitudinal gradient rather than species differentiation. Discriminant analysis based on quantitative and allozyme data showed that sympatric populations of white, Engelmann and their hybrids can be separated on average but not discretely (Rehfeldt 2004, Rajora and Dancik 2000). In places like British Columbia, where hybridization is extensive,

white, Engelmann spruce and their hybrids are treated as a complex called “interior spruce” (Ledig et al. 2004).

1.4 Research objectives

In the following chapters I investigate the genetic structure, gene flow and local adaptation in the interior spruce hybrid zone using microsatellites (SSR), single nucleotide polymorphisms (SNPs), and quantitative data (height, bud burst, bud set, cold hardiness and survival). In the first chapter, I review literature relevant to this work. In the second chapter, I characterize the population structure of the white spruce x Engelmann spruce hybrid zone for neutral genetic markers; compare the role of introgression in shaping the levels of genetic diversity in sympatric vs. allopatric populations; and test for allele frequency differentials between hybrids and pure species. In the third chapter, I identify the mechanism for hybrid zone maintenance; elucidate the effect of climate on the genetic variation in the hybrid zone; assess the effects of artificial selection on hybrid index; and predict future patterns of genetic variation in the zone using climatic models. In the fourth chapter, I assess the recent evolutionary history of white spruce and Engelmann spruce by using palaeoclimatic analysis; study the current patterns of interspecific gene flow; identify some of the genes that may be responsible for isolating barriers and adaptive differences between the species; and identify the main types of selection that have resulted in deviations from neutrality in outlier loci.

Table 1.1 Estimates of expected heterozygosity (H_e), observed heterozygosity (H_o), mean number of alleles per locus (A), fixation index (F_{st}) and percentage of polymorphic loci (%P) of *Picea glauca* and *P. engelmannii* based on molecular markers.

Species	Region	Marker type	Populations	Loci	H_e	H_o	A	F_{st} (G_{st})	P(%)	Reference
P.glauca	Saskatchewan	RAPD	13	51	0.34[1]		1.82			Rajora (1999)
P.glauca	Alberta	Microsatellites	16 stands		0.85[2]	0.65	16.38	0.02		Rajora et al. (2005)
P. glauca P.engelmannii	Alberta	allozymes	14	23	0.18	0.06	1.88	0.123	66.2	Rajora and Dancik(2000)
P.glauca	Ontario	allozymes	23 stands	6	0.34	0.34	3.03			O'Connell (2006)
P.glauca	Ontario	allozymes	1 stand	14	0.3[3] 0.18					Cheliak (1985)
P.glauca	Quebec	allozymes	6	27	0.32	0.25	2.15	0.11	76.2	Tremblay and Simon (1989)
P.glauca	Quebec	allozymes	2	12	0.20[4]			0.01	63.9	Desponts (1992)
P.glauca	Newfoundland	allozymes	2	4	0.28[5]	0.25	3			Innes and Ringius (1990)
P.glauca	Alaska	allozymes	4	13	0.27[6]	0.26	3	(0.015)	92	Alden and Loopstra(1987)
P.glauca, P.engelmannii	British Columbia	allozymes	9		0.2[7]	0.188	2.55	(0.035)	67.65	Stoehr and El-Kassaby(1997)
P.engelmanni	British Columbia	allozymes	16	24	0.25		2.4	0.147	80	Ledig et al. (2006)

[1] Overall mean values of old growth, natural regeneration and plantation populations.
[2] Overall mean for mixedwood and conifer dominated stands.
[3] First value for filial population and second value for adult population.
[4] Overall mean values for natural populations, provenances and selection trees.
[5] Overall mean values for coastal and inland popoulations.
[6] Overall mean values for floodplain, midslope, upper slope and tree limit populations.
[7] Overall mean for breeding zone (natural populations) and seed orchard populations.

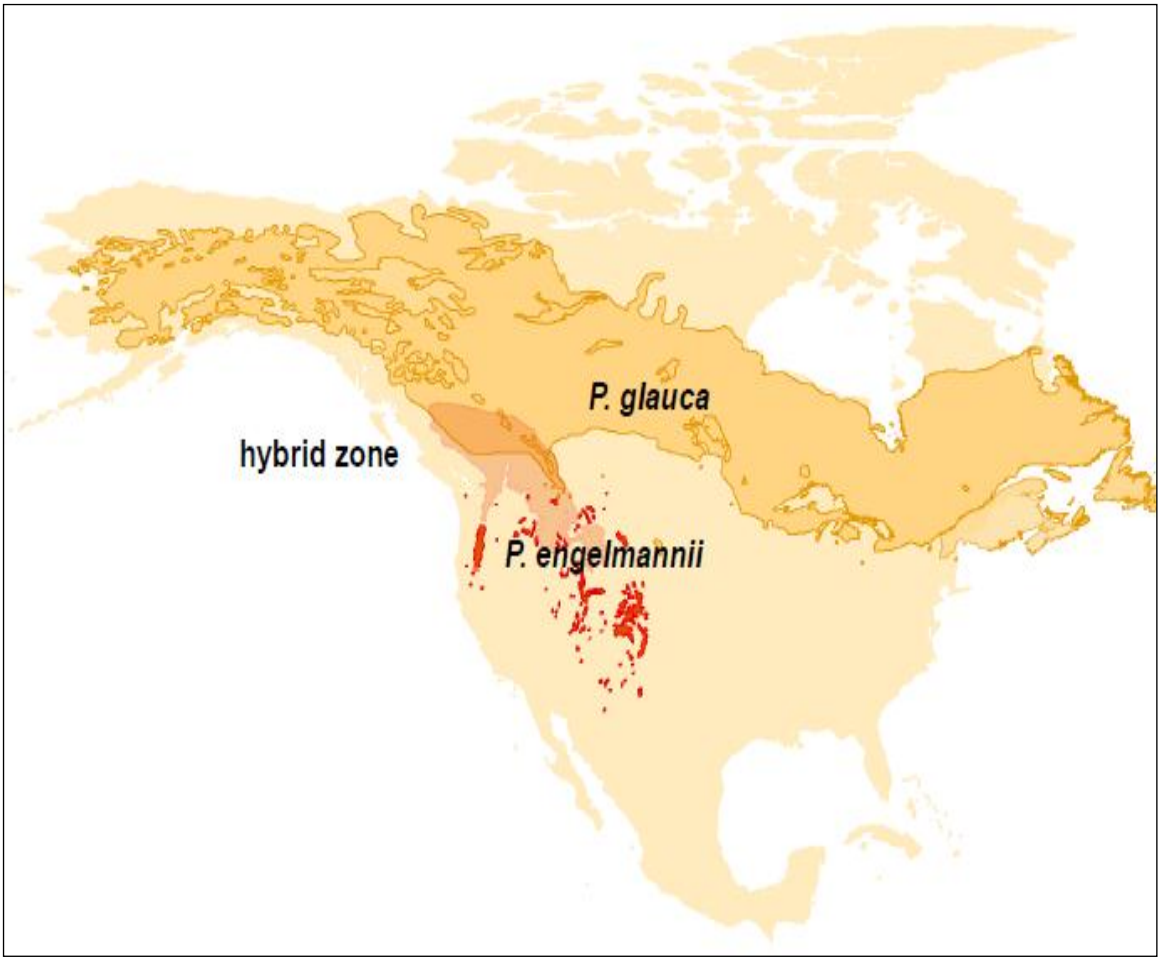
Table 1.2 Single (t_s) and multilocus (t_m) estimates of outcrossing rates and inbreeding coefficient (F_{is}) from published studies in *Picea glauca* and *P. engelmannii* in North America.

Species	t_s (mean)	t_m (mean)	F_{is} (mean)	Region	Reference
P. glauca	0.9-1.0 (0.98)	-	-0.002-0.048 (-0.066)	Ontario	Cheliak et al. (1985)
P. glauca	0.91	0.94	-	Ontario	O'Connell et al. (2006)
P. glauca	0.75-0.99 (0.9)	-	0.029-0.039 (0.033)	Alberta	King et al. (1984)
P. glauca	0.5-0.93 (0.71)	0.7-0.76	-0.013-0.279 (0.113)	Newfoundland	Innes and Ringius (1990)
P. engelmanni	0.87-0.9 (0.89)	0.89-0.95 (0.93)	-0.104-0.026	Western Canada & U.S	Ledig et al. (2006)

Table 1.3 Interior spruce Seed Planning Units created by the B.C Ministry of Forests, Lands and Natural Resource Operations for tree breeding and seed orchards in British Columbia. Data was taken from Forest Genetics Council 2007.

Seed planning unit	Region	Elevation (m)	Area (ha)	Total seedling production (million) from seed orchards
SPU 44	Nelson	1-1000	2,110,271	3.15
SPU 4	Nelson	1000-1500	2,110,271	7
SPU 5	Nelson	1500-1900	1,399,689	8.28
SPU 14	Prince George	600-1200	6,972,597	25.55
SPU 42	Prince George	1200-1550	2,380,019	6.9
SPU 25	East Kootenay	750-1700	1,612,927	1.27
SPU 30	Thompson Okanagan	700-1300	4,958,256	3.35
SPU 28	Thompson Okanagan	1300-1900	2,981,748	5.53
SPU 35	Bulkley Valley	500-1200	2,262,320	9.58
SPU 40	Peace River	650-1200	11,644,978	14.2

Figure 1.1 Distribution of *Picea glauca*, *P. engelmannii* and their hybrids in North America.



2. Population structure, admixture and diversity of a broad spruce hybrid zone (*Picea glauca* x *Picea engelmannii*) along a latitudinal and elevational gradient

2.1 Introduction

Hybrid zones can shed light on evolutionary processes involved in species divergence and on the effects of selection and gene flow in adaptation (Barton and Hewitt, 1989). As a result of hybridization, genetic variation is increased and new gene combinations are tested. These new combinations resulting from hybridization may promote the development of novel adaptations (Anderson 1949; Arnold, 1997; Rieseberg *et al.,* 2003).

Picea glauca (white spruce) is a transcontinental, boreal species that occurs naturally in almost all forested regions in Canada, with the exception of the Pacific Coast. White spruce generally occurs at low elevations, with the exception of in Alaska, where it ranges from low elevations to alpine treeline. *Picea engelmannii* (Engelmann spruce) extends from British Columbia and Alberta in the north, to New Mexico and Arizona in the south. Engelmann spruce grows at high elevations and is restricted to cold, humid habitats because of its low tolerance of high temperature and drought (Alexander and Shepperd, 1990).

Over the last century, the genetic and ecological relationships between white spruce and Engelmann spruce have attracted considerable scientific interest (La Roi and Dugle, 1968; Rajora and Dancik, 2000). Engelmann spruce and white spruce are closely related species (Sigurgeirsson and Szmidt, 1993; Ran et al. 2006) that hybridize extensively in areas where their ranges overlap, at the eastern periphery of Engelmann spruce's range in Alberta and at white spruce's lower elevational limits in British Columbia (Roche, 1969). Hybrids have also been found in northern Colorado, and southern Wyoming (Ledig et al. 2006), as well as in Montana (La Roi and Dugle, 1968).

Variation within the white x Engelmann complex has been studied for morphological traits (Wright 1955; Taylor, 1959; La Roi and Dugle, 1968; Roche, 1969), flavonoids and

phenolic compounds (La Roi and Dugle, 1968), and cytology (Nkongolo et al. 2005). Variation in all of these characters was found to be clinal, related to elevation and latitude, with hybrids showing an intergradation of morphological and physiological characteristics along elevational gradients. The most obvious differences in morphology between these species are the seed cone scales; white spruce cones are characterized by obovate-triangular scales with the apex being rounded, whereas Engelmann spruce has wedge-shaped scales, with the apex being erose to truncate (Taylor, 1959).

Population genetic studies using allozymes (Rajora and Dancik, 2000; Ledig et al. 2006; Stoehr and El-Kassaby, 1997) and RAPDs (Khasa and Dancik, 1996; Nkongolo et al. 2005) found higher levels of genetic diversity in sympatric than in allopatric populations of white and Engelmann spruce, suggesting that introgressive hybridization might have played an important role in the high levels of genetic diversity found in British Columbia and Alberta populations. No species-diagnostic codominant alleles have previously been found for the white x Engelmann complex, only allele frequency differentials (Rajora and Dancik, 2000) and species-specific dominant marker bands (Khasa and Dancik, 1996; Nkongolo et al. 2005).

In British Columbia, where hybridization is extensive, and the complex has high economic and ecological importance, white spruce, Engelmann spruce and their hybrids are treated as a single, economically important taxon called “interior spruce”. Interior spruce is the second most commonly planted tree in British Columbia, with tens of millions of trees planted annually in the interior of the province for reforestation. The similarity between the species and the apparent lack of reproductive genetic barriers have raised the question of whether white spruce and Engelmann spruce simply represent extreme phenotypes along a genetic continuum (Taylor, 1959; Rajora and Dancik, 2000) or if they deserve specific recognition (Wright, 1955; Roche, 1969). Taylor (1959) considered Engelmann spruce a subspecies of white spruce. Also Rajora and Dancik (2000) suggested that clinal variation in needle allozymes was the result of a single species evolving along an altitudinal gradient rather than species differentiation. Discriminant analysis based on quantitative, phenotypic and allozyme

data showed that sympatric populations of white spruce, Engelmann spruce, and their hybrids can be separated, on average, but not discretely (Rehfeldt, 2004; Rajora and Dancik 2000).

The goals of this chapter are as follows. 1) to characterize the population structure of the white spruce x Engelmann spruce hybrid zone, using neutral genetic markers for future purposes of finding adaptive genetic variants; 2) to compare the role of introgression in shaping the levels of genetic diversity in sympatric vs. allopatric populations; and 3) to test for allele frequency differentials between hybrids and pure species, and compare them to differences among populations along altitudinal and latitudinal gradients.

2.2 Materials and methods

2.2.1 Sample collection and DNA extraction

Fresh needle tissue from 805 individuals from thirteen populations (9 allopatric and 4 sympatric) of interior spruce was sampled for DNA extraction (Figure 2.1, Table 2.1). In order to use the species' available resources, needles were collected from grafted individuals (natural clones) in clone banks previously established by the British Columbia Ministry of Forests, Lands and Natural Resource Operations. Individuals included in these clone banks were grafted from selected mature, healthy trees in natural populations. Currently in British Columbia, seed is managed for reforestation within geographic areas called Seed Planning Zones (SPZ) and by elevation rather than by species composition. In this study, a population is defined as individuals that occur within the same SPZ. Individuals had a diffuse distribution across the SPZ in terms of latitude, longitude, elevation and climate, and geographic coordinates within SPZs are available for all of them. Diffuse sample distribution often leads to a more reliable identification of population structure than clustered sample distribution (Schwartz and McKelvey, 2009).

Needle tissue was stored at -80°C before isolation. DNA extraction was done using the CTAB protocol (Doyle and Doyle, 1987). DNA quality and concentration was assessed visually

using 0.8% agarose gels and quantified based on Nanodrop 2000C Spectrophotometer readings (Thermo Fisher Scientific Inc.).

2.2.2 DNA amplification

Polymerase Chain Reactions (PCR) were performed using a PTC-100 thermal cycler (MJ Research Inc, Waltham, US). Each reaction had a total volume of 10uL and contained 20 ng. of nuclear DNA, 1 uL of 2 mM dNTP, 1 uL 10X Paq5000 Reaction Buffer, 1U Paq5000 DNA Polymerase, 0.5 pmol of M13 Infrared Label Primer and 1 pmol each of forward and reverse tailed primers. Samples were amplified using a modified version of Rungis' (2004) protocol, in which the annealing temperature varied according to each primer (Table A.1 in Appendix). The PCR profile involved 2 min at 95C° initial denaturation step; followed by 30 cycles of 95°C for 20s, appropriate annealing temperature for 20s, 72°C for 30s and an extension cycle of 3 min at 72°C. Following amplification, 3 uL of loading dye was added to each reaction. Amplification products were electrophoresed on a LI-COR 4200 automated sequencer using 7% polyacrylamide gels (Long Ranger TM, BioWhittaker Molecular Applications, Rockland, Maine). Amplification bands were scored using Saga 3.3 automated microsatellite analysis software (LI-COR Inc., Lincoln NE, USA).

Twenty-five published nuclear microsatellite (SSR) loci (Rungis et al. 2004; Hodgetts et al. 2001) were tested for resolution and level of polymorphisms in 0.8% agarose gels. Of the 25 microsatellites tested, 17 were successfully amplified, but only 15 exhibited polymorphisms. Of these, 5 primers were eliminated because they produced amplifications with multiple stutter bands making scoring difficult. A total of 10 microsatellites were chosen that were polymorphic, and amplified and scored reliably (Table A.1 in Appendix). Allele size ranges corresponded to those predicted by the original sequence for which primers were designed.

To test for the presence of genotyping errors and null alleles in the data set, the program *MICRO-CHECKER* (Van Oosterhout et al. 2004) was used. Results of the *MICRO-*

CHECKER analysis indicated the presence of null alleles and possible stuttering in SSR09 and SSR10, and null alleles in SSR03. These loci were re-scored prior to genetic analyses.

2.2.3 Population structure and admixture

Admixture proportion (Q) was estimated using the Bayesian clustering approach implemented in *STRUCTURE* version 2.2 (Pritchard et al. 2000). Models with a putative number of clusters (K) from 1 to 10 were tested using 50,000 iterations for the pre and post-burn periods using the admixture model. The degree of admixture, α, was inferred from the data. Each run was repeated twenty times in order to estimate K using the method developed by Evanno et al. (2005) with the program *Structure Harvester* version 0.6.7 (Earl, 2011).

Geographical coordinates (longitude and latitude) and genetic data for each sample were used to estimate admixture coefficients and population structure with *TESS* version 2.3 (Chen et al. 2007). The admixture model was performed for values of K_{max} ranging from 2 to 14. Markov chain Monte Carlo (MCMC) algorithms were run for a length of 50,000 sweeps with burn-in periods of 30,000 sweeps. Each run was replicated thirty times. The twenty percent lowest DIC (Deviance Information Criterion, Spiegelhalter et al. 2002) runs were averaged and plotted against the number of clusters (K_{max}) to estimate which K_{max} provided the best fit with the genetic data. Based on the results of the Evanno test, alignments of clusters for K=2 for *STRUCTURE* and *TESS* were optimized using the program *CLUMPP* 1.1.2b (Jakobsson and Rosenberg, 2007) and graphed with the program *distruct* (Rosenberg, 2004).

Global and pairwise population F_{st} values were calculated using the Weir and Cockerham method (Weir and Cockerham, 1984) using *GenAlEx* (Peakall and Smouse, 2006). Differences between and within populations and between species were estimated with analysis of molecular variance (AMOVA) using *GenAlEx*.

Standard and unbiased population pairwise genetic distances and genetic identities were estimated following Nei (1972). Pairwise population F_{st} and Nei's genetic distances were

used as input for a Principal Coordinates Analysis (PCA) in which the distance matrix was converted into a covariance matrix and data were standardized (divided by the square root of n-1) following Orloci's (1978) algorithm in *GenAlEx.* Principal coordinates 1 and 2 were plotted using R 2.13.0 (R Development Core Team, 2011). To test for isolation by distance, correlations between genetic and geographical distances between populations were performed using Mantel tests based on Pearson's product-moment correlation with 1,000 permutations, using the *Vegan* package in R 2.13.0 (R Development Core Team, 2011). Genetic distance was expressed as $F_{st}/(1-F_{st})$ following Rousset (1997).

2.2.4 Hybrid index

Hybrid index was calculated using the *INTROGRESS* 1.1 (Gompert and Buerkle, 2010) package in R 2.13.1 (R Development Core Team, 2011). Estimated hybrid index ranged from 0 (all alleles inherited from white spruce) to 1 (all alleles inherited from Engelmann spruce). Only individuals with admixture proportions Q>0.8 from *STRUCTURE* results were used as reference for "pure species". Although the cut-off was set to Q>0.8, most of the individuals had Q>0.9. Geographic clines were created by plotting individual hybrid index against elevation and latitude to determine the extension and structure of the hybrid zone. Proportions of white spruce and Engelmann spruce (using mean hybrid index) for each population were calculated and displayed in a geographical map using *ArcMap10* (ESRI, 2010) (Figure 2.1). Hybrid index values were used to classify individuals into 11 hybrid classes with intervals of 0.1, where 0 was pure white spruce and 1 was pure Engelmann spruce. Hybrid index was regressed on latitude, longitude and elevation using simple and multivariate regressions using *SAS* 9.2 (SAS Institute Inc.).

2.2.5 Genetic diversity

To estimate genetic diversity and the variation of allele frequencies along elevation and latitudinal gradients, three separate approaches were used to stratify individuals into geographic groups. In the first one, individuals were assigned to populations according to their

provenance latitude. In the second one, individuals were grouped into ten elevational bands, in which each elevational band represents a population. Eight of the elevation bands covered an interval of 250 m, from 350 m to 2300 m. Two additional, higher elevation bands containing few individuals (2300-2800 m and 2800-3292 m) covered a wider range of elevation. In the last set of analyses, individuals were grouped into three groups: low elevation (<600 m), intermediate elevation (600-1800 m) and high elevation (>1800 m).

GenAlEx version 6.4 (Peakall and Smouse, 2006) was used to calculate allelic frequencies and patterns across populations (number of effective, local and private alleles) and genetic diversity (observed and expected heterozygosity). Chi-square goodness-of-fit tests were performed to measure deviations from Hardy-Weinberg equilibrium. Inbreeding coefficients for each population and each locus were estimated with the program *GENEPOP* version 4 (Rousset, 2008). Exact p-values were calculated using the Markov chain method with 10,000 iterations.

2.3 Results

2.3.1 Population structure, admixture and hybrid index

The hybrid zone exhibited clines along latitudinal and elevational gradients, in which hybrids occupy a broad area between 50-56 degrees latitude and between 600-1800 m in elevation. In the study area, hybrid index estimates suggest Engelmann spruce mainly occurs above 1800 m within this latitudinal range, and white spruce occurs below 600 m (Figure 2.2). Hybrid index showed significant correlations with elevation (R^2= 0.1, F=79.59, P=<0.0001), latitude (R^2=0.1, F=82.36, P=<0.0001) and longitude (R^2=0.03, F=27.14, P=<0.0001). Within the contact zone alone, elevation is the most important geographic variable shaping hybrid index (R^2=0.026, F=18.38, P=<0.0001); and when allopatric populations are included, latitude is the most important variable (R^2=0.1, F=82.36, P=<0.0001).

Both the *TESS* and *STRUCTURE* analysis and the F_{st} estimates showed clear differentiation between white and Engelmann spruce. Bar plots of posterior estimates of cluster

memberships for *STRUCTURE* and *TESS* indicated that admixture is extensive in the area of contact (all British Columbia populations sampled with the exception of Fort Nelson), and that hybrid populations cannot be distinguished genetically among SPZs (Figure 2.3). However, hybrids populations showed differences in their levels of admixture and genetic distances from pure species according to their latitude. Hybrid populations in the south of British Columbia (East Kootenay and West Kootenay), where elevations are higher, on average, had a substantially higher contribution from Engelmann spruce, whereas populations in the northern British Columbia (Finlay, Prince George), where elevations are lower, had more equal contributions from both pure species. Mantel tests revealed strong associations between geographical and genetic distances ($r=0.5712$, $p<0.001$), and between elevation and genetic distances ($r=0.5446$, $p<0.006$) (Figure 2.4).

The genetic structure of populations was also evidenced by the results of the Principal Coordinate Analysis (Figure 2.5). Principal Coordinate 1, which explained 52.4% of the variation in the data, divided Engelmann and white spruce. PC1 also separated the hybrid populations East Kootenay and West Kootenay, which are genetically closer to Engelmann spruce, from the rest of hybrid populations. Most of the hybrid populations (with the exception of West Kootenay, East Kootenay and Mount Robson) clustered together at the union of PC1 and PC2. PC2, which explained 22.58% of the variation in the data, divided Engelmann populations Salmon River and Teton Wasatch from Fishlake Lasal. All hybrid populations were clearly differentiated from the pure species populations. Salmon River and Teton Wasatch (Engelmann) populations were closer to the hybrid populations than Fishlake Lasal (Engelmann) and Fort Nelson (white spruce).

In the *STRUCTURE* analysis, some introgression was noticeable in the Salmon River (E1) Engelmann population. This introgression was also evident in the output for K=3 to K=6 in *TESS* (Figure A.10 in Appendix). E1 was genetically more similar (lower pairwise F_{st} and genetic distance) to hybrid populations than to Engelmann populations E2 and E3 (Table 2.2).

When plotting Delta K vs. the number of clusters for the Evanno test, there was a peak for K=2 indicating that there were two clusters in the data, each cluster representing one of the pure species (Figure A.11 in Appendix). This estimation of two clusters was not evident in the *TESS* results. The DIC curve decreases and then exhibits a plateau at Kmax=6 (Figure A.11 in Appendix). However, the clustering results remain relatively unchanged from K=2 to K=6 (Figure A.10 in Appendix), suggesting that the effective number of cluster that best fits the data could be estimated as K=2. The variability in estimated DICs can lead *TESS* to select models in which Kmax is greater than K (Durand et al. 2009).

2.3.2 Genetic diversity

While all populations were highly diverse for the microsatellite markers assayed, those from the introgression zone at intermediate elevations and intermediate latitudes had more alleles, on average, and tended to have higher heterozygosity (Table 2.4, Figure 2.6). For example, hybrid populations from the interior British Columbia had a total of alleles ranging from 62 (West Kootenay) to 74 alleles (Quesnel), with an average of 67.4± 4.18, whereas pure white and Engelmann populations had 40 and 41 respectively. When elevation was used to group populations, the number of alleles at intermediate elevations (850-1100 m and 1100-1350 m bands) was twice as high (87 and 84 respectively) as the number of alleles found at pure species habitats elevations (40 alleles at 350-600 m; and 41 alleles at >1800 m), although it should be noted that sample sizes were larger for intermediate bands.

Alleles within this hybrid zone could be sorted into three groups: alleles found in both pure species and hybrids; alleles only found in hybrid backgrounds, and alleles found in one pure species plus hybrids. All groups were similarly represented in number of alleles, however within the last category, more alleles were shared between Engelmann spruce and hybrids than between white spruce and hybrids. From all alleles summed across all loci, 75% were shared by pure species and hybrids; 17.8% were shared by Engelmann spruce and hybrid populations; 6.39% were only found in hybrid populations; and 1.74% were shared by white

spruce and hybrid populations (Table A.3 in Appendix). Similar patterns were found when elevation was used to stratify populations (Table A.4 in Appendix). Private alleles were only found in hybrid populations from 600 m to 1600 m of elevation.

Observed heterozygosities (H_o) were generally lower than expected heterozygosities (H_e) for most or all of the loci for the Central Plateau, Prince George, East Kootenay, Quesnel and Fort Nelson populations. Populations from Salmon River, Teton-Wasatch, Fishlake-Lasal, Bulkley valley, Mount Robson, McGregor, Finlay and West Kootenay, had a balance between the number of loci showing higher Ho and loci showing higher He (Table A.2 in Appendix). Mean expected heterozygosity (H_e) over all populations and across all loci was slightly higher than the observed heterozygosity (H_o), 0.55 vs. 0.464, respectively (Table 2.3). When elevational bands divided populations, mean H_e was 0.542 and mean H_o was 0.465 (Table A.5 in Appendix).

Deviations from Hardy-Weinberg equilibrium due to heterozygote deficiency were mainly found at locus SSR04 (5 populations), SSR09 (8 populations) and SSR10 (6 populations). Before their re-scoring, null alleles were found in loci SSR09 and SSR10. After the re-scoring of these loci, observed levels of heterozygosity were high in most of the populations and the estimated inbreeding coefficients were found not to be significantly different from zero in several populations, suggesting that heterozygote deficiency was a consequence of low interspecific gene flow at these loci or geographic substructure within the populations rather than null alleles. This pattern was also seen for locus SSR04. Also, two populations (Finlay and West Kootenay) showed deviations from neutrality due to heterozygote excess at locus SSR05.

The AMOVA found that most of the variation (93%) occurred within populations with only 3% of the variation among populations and 4% between species. Correspondingly, F_{st} values between species were relatively low, with average of all pairwise F_{st} of 0.092 ±0.003 SD between white and Engelmann spruce (Table 2.2). Nei's unbiased genetic distance was 0.21±0.047 across all pairwise values (Table 2.2). Despite their relatively low values, pairwise

interspecific F_{st} estimates were higher between allopatric pure species populations than between sympatric populations. Within sympatric populations, lower pairwise F_{st} estimates occurred among hybrid populations (intermediate elevations and intermediate latitudes) than between hybrids and pure species. Also, pairwise F_{st} estimates were higher between white and hybrid populations than between Engelmann and hybrid populations (Table 2.2). For example, the average F_{st} between white spruce and hybrids was 0.042±0.017, whereas F_{st} between Engelmann spruce and hybrids was 0.037±0.011.

2.4 Discussion

2.4.1 Population structure and introgression

Extensive introgression was observed between white and Engelmann spruce, where the majority of individuals in the zone have hybrid ancestry (Figures 2.2, 2.3 in this chapter; and A.10 in Appendix). Given the recent divergence between these two closely related species (Bousquet et al. 2007), this pattern could have also been caused by the retention of ancestral polymorphisms. However, ancestral polymorphisms alone could not produce the pattern observed, of gradual change in genotypes across latitude or elevation (Figure 2.2). Given the extension of introgression, and that sympatric populations showed less genetic divergence than allopatric populations, interspecific gene flow seems to be a more plausible explanation. Also, controlled crosses have confirmed that hybrids can be obtained easily and that F_1 hybrids are viable (Wright, 1955). These results are not limited to artificial crosses but also hold in natural conditions, suggesting ongoing interspecific gene flow.

The hybrid zone followed elevational and latitudinal clines in admixture, corresponding to climatic gradients in temperature and precipitation. Both of these clines suggest extensive hybridization and introgression between pure species, and the absence of strong selection against hybrids (as found in bimodal clines). Hybrids occupy a broad area between 50 and 56

degrees latitude and between 600 and 1800 m in elevation. In the study area, according to our hybrid index estimates, Engelmann spruce mainly occurs above 1800 m and white spruce occurs below 600 m of elevation. Other studies have reported white spruce at higher elevations (Rajora and Dancik, 2000; Roche, 1969).

This study suggests that the white-Engelmann hybrid zone is larger than previously reported (Roche, 1969). Prince George, which was considered a largely white spruce population for forest management purposes, is reported to have high levels of admixture. Also, Salmon River was thought to be a pure Engelmann population, however its genetic similarity (based on F_{st} and genetic distances) with hybrid populations and its undefined cluster membership in *STRUCTURE*, suggest that this population has some introgression from white spruce.

In general, hybrid populations are genetically closer to Engelmann spruce than white spruce. This asymmetric pattern was also found in Alberta populations using allozymes. Rajora and Dancik (2000) found that hybrid populations were closer to Engelmann populations based on a genetic distance analysis (based on allele frequency differences among populations) using 23 allozyme loci. However, when using multivariate discriminant analysis with the same allozyme data (based on differences in individual tree genotypes), hybrid populations were found to be more similar to white spruce. The authors favored results from the multivariate analysis over the genetic distance analysis.

Several lines of evidence coming from the results presented here favor the hypothesis of asymmetry towards Engelmann. First, average pairwise F_{st} values between Engelmann and hybrids were lower than between white and hybrids (0.037 vs.0.042 when populations were stratified by latitude and 0.035 vs. 0.05 when stratified by elevation). Second, the Principal Coordinate Analysis indicated that hybrid populations were genetically closer to Salmon River and Teton Wasatch (Engelmann spruce populations) rather than Fort Nelson (white spruce population). Third, the results of the hybrid index analysis also indicated that most of the hybrid

populations have a higher contribution from Engelmann spruce than from white spruce. Finally, the histogram showing hybrid classes showed a shift of the peak of the histogram towards Engelmann spruce, where pure Engelmann are more abundant than pure white and also where there is a lack of individuals in the white-backcross classes (hybrid index from 0.1 to 0.2) (Figure A.12 in Appendix).

The high number of individuals with a hybrid index similar to backcrosses suggests that the hybrid zone is not of recent origin and that postzygotic isolation is not strong. The most likely explanation for the lack of hybrid indices corresponding to white backcrossed individuals is simply the apparent difference in population density between white spruce and Engelmann spruce. However, the low number of white-backcrossed individuals may also indicate the presence of epistatic interactions, in which some hybrid combinations have reduced fitness. The absence of hybrid individuals in pure species habitats also suggests some form of extrinsic postzygotic isolation. Artificial crosses including F2s and backcrosses and reciprocal transplant experiments would be required to draw definitive conclusions about these topics.

2.4.2 Levels of genetic diversity

The results of this study indicate high levels of genetic diversity in populations in the contact zone between white spruce and Engelmann spruce. Mean expected heterozygosity and total number of alleles (Na) were higher at intermediate latitudes (interior British Columbia) and at intermediate elevations (600-1800 m) in places where the hybrids were present (Table 2.4, Figure 2.6). Considering that the number of alleles with a frequency higher than 5% was not very different among populations, the Na is mainly reflecting the presence of low-frequency alleles (both rare and private) in the hybrid populations. When latitude was used to stratify populations, a total of 41 low-frequency alleles were found only in the hybrids and when elevation was used to stratify populations, a total of 35 alleles were found. This phenomenon of increased genetic variability due to rare alleles has been observed in different taxa in several hybrid zones studies (Barton and Hewitt, 1985).

Questions about the origin and maintenance of these rare alleles within hybrid populations had led to several different hypotheses: i) selective advantage of alleles that would be otherwise deleterious or neutral in pure species; ii) increased mutation rates in hybrids; and iii) intragenic recombination; the first two hypotheses have the most support (Bradley et al. 1993; Schilthuizen et al. 1999). One explanation for the white spruce x Engelmann spruce hybrid zone is that mutations have arisen in hybrid individuals and then been linked to loci locally selected for within the contact area. Considering the extent of the hybrid zone, and the fact that these alleles were found in different environments than parental environments, it is possible that some of these alleles are favored through hitchhiking, as has been shown in other plant species (Rieseberg et al. 1996). However the number of rare alleles is too high for hitchhiking to explain all their presence unless linkage disequilibrium is very high in the hybrid zone. In chapter 3 I will explore signatures of selection on candidate SNPs across the hybrid zone.

In this study, the presence of species-specific alleles is reported for the first time for the white spruce x Engelmann spruce hybrid zone. Twenty-three species-specific alleles were found when latitude was used to stratify populations and 28, when populations were stratified by elevation. Previous studies in this hybrid zone using allozymes did not find species-specific alleles but only allele frequency differentials. However, some species-specific dominant marker bands were found using RAPDs (Khasa and Dancik, 1996; Nkongolo et al. 2005). The presence of diagnostic alleles found in this study can be explained by the higher level of polymorphisms in microsatellites compared to allozymes.

Deviations from Hardy-Weinberg equilibrium are common in hybrid zones (Jiggins and Mallet, 2000; Payseur, 2010). Higher levels of expected heterozygosity in comparison to observed heterozygosity have been previously observed in this hybrid zone and also in pure white spruce populations (Rajora and Dancik, 2000; Tremblay and Simon, 1989; Innes and Ringius, 1990). In this study, overall expected heterozygosity was higher than observed

heterozygosity (0.550 vs 0.464 when populations are stratified by latitude and 0.542 vs. 0.465, when stratified by elevation) and varied considerably among loci. These results are comparable with previous studies using microsatellites in white spruce populations (Rajora et al. 2005); but are higher than previous studies in the hybrid zone using allozymes (Stoehr and El-Kassaby, 1997; Rajora and Dancik, 2000). This difference between allozymes and microsatellites results is not surprising given the fact that variation is typically much higher at microsatellite loci due to their high mutation rates.

The results of this chapter indicate heterozygote deficiency in most of the hybrid populations of the zone. Heterozygote deficiency in this case could be caused by selection against hybrids, inbreeding, Wahlund effect or null alleles. Significant levels of inbreeding were found in white spruce populations of central New Brunswick (F_{is}=0.145) (Coles and Fowler 1976; Park et al. 1984) and also in a previous study in the white-Engelmann contact zone (Rajora and Dancik, 2000). In this study, however inbreeding coefficient was low, with an average of 0.07 across all loci (Table 2.3). The Wahlund effect can cause deficiency of heterozygotes as a result of random mating of subpopulations that differ in allele frequency. For example, evidence of Wahlund effect and increased linkage disequilibrium were found in the yellow-rumped warbler (*Dendroica coronata*) hybrid zone, when sample threshold distances were 30 km or greater (Brelsford and Irwin 2009). In Rajora and Dancik's (2000) study of the white-Engelmann hybrid zone in Alberta, genetic substructure within populations was caused by grouping individuals from slopes with different degrees, aspect and elevation in the same populations. In this study, individuals were lumped together from across wide areas; therefore some genetic substructure is likely. Rajora and Dancik (2000) also suggested that selection against hybrids might be a cause for the levels of heterozygote deficit they observed. It is fair to think that selection against hybrids may be occurring for some genes in the white-Engelmann spruce hybrid zone; however, given the level of heterozygote deficit found in the zone, this might not be the only explanation.

2.5 Conclusions

It is evident that interspecific gene flow occurs frequently and extensively between these two closely related species, even though species integrity is maintained. Chapter 3 provides information on the mechanism of maintenance of the hybrid zone and chapter 4 uses candidate-gene approaches to identify genes responsible for adaptation between species.

Given the economic importance of these species, there is growing concern about how the genetic resources are managed in the interior spruce hybrid zone. Currently, forest managers and breeders use elevation and cone morphology to differentiate the pure species. This information, although useful, is not always accurate. These results indicate a need for understanding the evolutionary relationships between white spruce and Engelmann spruce in order to manage the species.

Table 2.1 Geographical coordinates and climatic variables for 805 individuals of *Picea glauca, P. engelmannii* and their hybrids analyzed with 10 SSR loci. Two or three-letter codes are used to identify populations in subsequent tables and graphs.

Population	Province	Elevation range (m)	Latitude (degrees)	Longitude (degrees)	MAT * (°C)	MAP (mm)	Sample size
Picea glauca							
Fort Nelson (FN)	B.C	300-580	58.4-59.4	120.5-126.3	0.29	509	22
P.glauca x *P.engelmanni* hybrids							
Finlay (FNL)	B.C	677-1372	55-56.9	123.2-125.8	0.72	645	80
Central Plateau (CP)	B.C	671-1190	54.4-55.8	123-126	1.8	622	69
Bulkley Valley (BV)	B.C	647-1190	53.5-55.1	125.3-127.2	2.23	568	70
Prince George (PG)	B.C	610-1189	53.2-54.1	121.9-122.3	2.75	769.4	86
Mc Gregor (MG)	B.C	610-1372	53.9-55.3	120.9-122.9	2.3	1137	81
Mount Robson (MR)	B.C	701-1800	52.2-53.8	118.3-121.4	1.72	1166	50
Quesnel Lakes (QL)	B.C	670-1585	51.8-53.3	119.6-122.1	2.31	909	114
East Kootenay (EK)	B.C	1006-2012	49.2-50.8	115.1-116.6	1.89	962.7	86
West Kootenay(WK)	B.C	640-2225	49-50.4	115.3-119	2.45	1051.7	53
Picea engelmannii							
Salmon River (E1)	Idaho	1859-2530	43.8-46.2	113.7-115.9	2.76	994.9	13
Teton-Wasatch (E2)	Wyo.	1935-3292	40.4-43.8	109.5-111.6	2.51	843.4	35
Fishlake-Lasal (E3)	Col.	2606-3383	37.5-39.8	109.2-112.8	3.51	735.7	46

MAT= Average Mean Annual Temperature; MAP= Average Mean Annual Precipitation; B.C= British Columbia; Wyo.=Wyoming; Col.=Colorado

Table 2.2 Pairwise population F_{st} (below diagonal) and Nei genetic distance (above diagonal) for 13 populations of *Picea glauca x P. engelmannii*. Dark grey shading indicate F_{st} and genetic distances among *P. engelmannii* populations; medium gray, between *P. engelmannii* and introgressed populations; light gray, between *P. glauca* and introgressed populations; and white, among introgressed populations. Numbers in bold indicate F_{st} and genetic distances between *P. engelmannii* and *P. glauca*.

Pop	E1	E2	E3	CP	PG	EK	BV	MR	MG	QL	FNL	WK	FN
E1	0	0.132	0.220	0.135	0.120	0.136	0.124	0.153	0.141	0.143	0.120	0.113	**0.262**
E2	0.061	0	0.098	0.062	0.054	0.036	0.074	0.118	0.087	0.088	0.049	0.038	**0.271**
E3	0.079	0.043	0	0.111	0.083	0.060	0.085	0.088	0.124	0.123	0.111	0.091	**0.287**
CP	0.051	0.026	0.038	0	0.017	0.034	0.021	0.057	0.029	0.031	0.020	0.025	0.127
PG	0.043	0.028	0.029	0.007	0	0.020	0.023	0.054	0.038	0.037	0.029	0.024	0.141
EK	0.051	0.018	0.023	0.012	0.007	0	0.030	0.061	0.052	0.039	0.026	0.031	0.180
BV	0.048	0.031	0.029	0.008	0.009	0.010	0	0.044	0.029	0.031	0.030	0.033	0.116
MR	0.059	0.046	0.031	0.020	0.020	0.020	0.016	0	0.037	0.038	0.055	0.074	0.146
MG	0.051	0.033	0.039	0.009	0.014	0.016	0.010	0.013	0	0.020	0.031	0.050	0.105
QL	0.054	0.038	0.044	0.013	0.014	0.014	0.014	0.013	0.009	0	0.024	0.057	0.106
FNL	0.048	0.023	0.039	0.006	0.012	0.010	0.011	0.019	0.010	0.010	0	0.033	0.122
WK	0.043	0.020	0.033	0.010	0.010	0.012	0.013	0.027	0.020	0.024	0.015	0	0.181
FN	**0.092**	**0.088**	**0.095**	0.042	0.049	0.057	0.042	0.053	0.037	0.038	0.037	0.062	0

Table 2.3 Heterozygosities and inbreeding coefficient (F_{is}) over all 13 *Picea glauca* x *P. engelmannii* populations (SPZs) for 10 microsatellite loci. Mean expected heterozygosity (Mean H_e), mean observed heterozygosity (Mean H_o) and inbreeding coefficient (F_{is}) were averaged across all populations for each loci. Loci ID can be found in Table A.1 in Appendix. H_o, H_e and F_{is} estimates within population by locus can be found in Table A.2 in Appendix.

Locus	H_t	Mean H_e	Mean H_o	F_{is}
SSR01	0.503	0.489	0.459	-0.018
SSR02	0.828	0.807	0.754	0.060
SSR03	0.718	0.688	0.667	0.007
SSR04	0.506	0.454	0.307	0.206
SSR05	0.736	0.691	0.734	-0.015
SSR06	0.628	0.582	0.445	0.095
SSR07	0.156	0.145	0.137	0.030
SSR08	0.212	0.207	0.176	0.072
SSR09	0.726	0.647	0.354	0.173
SSR10	0.818	0.789	0.610	0.087
Mean	0.583	0.550	0.464	0.070
SE		0.021	0.023	

Table 2.4 Summary of genetic diversity statistics for each of the 13 populations of *Picea glauca x P..engelmannii* using 10 microsatellite loci. Number of different alleles (Na), number of different alleles with a frequency higher or equal than 5% (Na≥5%), private alleles and mean heterozygosity (Mean H_e) are shown. Population names corresponding to two or three-letter codes can be found in Table 2.1.

Population	Na	Na≥5%	Private	Mean H_e (SE)
E1	34	34	0	0.52 (0.06)
E2	49	38	0	0.49 (0.09)
E3	37	30	0	0.5 (0.07)
CP	68	35	2	0.56 (0.08)
PG	65	36	1	0.58 (0.07)
EK	70	35	0	0.56 (0.08)
BV	63	34	0	0.57 (0.08)
MR	64	33	1	0.55 (0.08)
MG	72	41	5	0.59 (0.08)
QL	74	40	0	0.58 (0.09)
FNL	69	35	1	0.57 (0.08)
WK	62	36	1	0.54 (0.07)
FN	40	36	0	0.53 (0.09)

Figure 2.1 Geographical distribution and degree of admixture for 13 populations of *Picea glauca*, *P. engelmannii* and their hybrids. Degree of admixture for each population was calculated averaging the individual hybrid indices estimated with the program *INTROGRESS*. Population names corresponding to two or three-letter codes can be found in Table 2.1. Black circles represent *P. engelmannii* and gray circles, *P. glauca* populations. Bi-color circles represent hybrid populations.

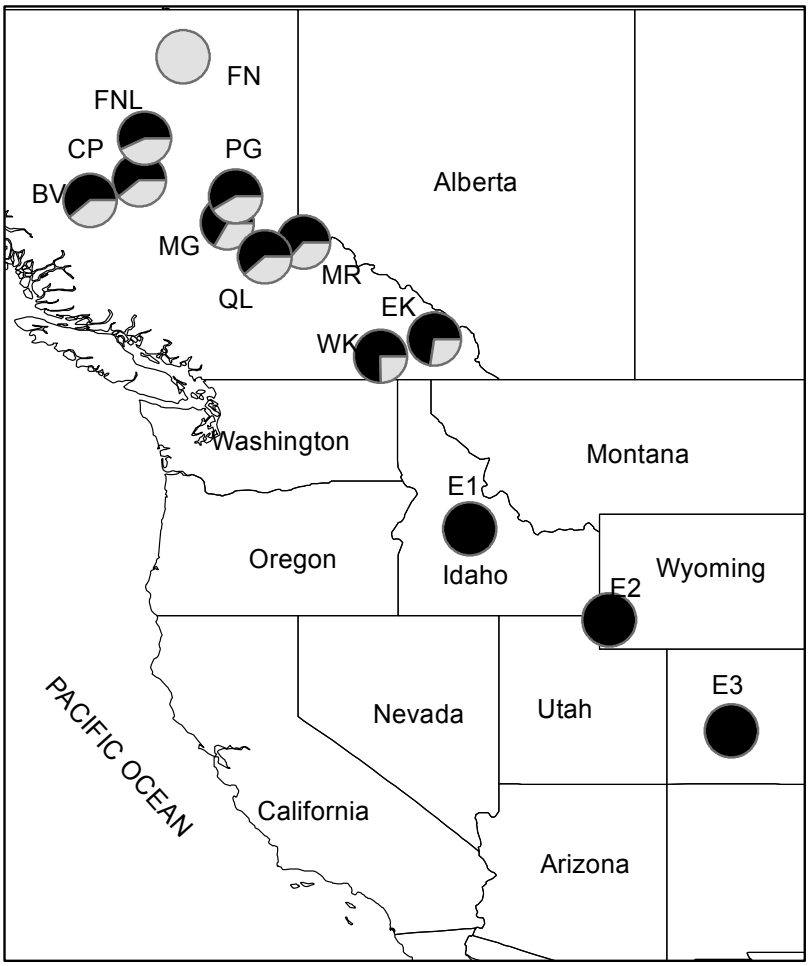


Figure 2.2 Elevational and geographic clines in the *Picea glauca* x *P. engelmannii* hybrid zone based on hybrid index. Hybrid index was calculated using the program *INTROGRESS* and ranges from 0 (pure *P. glauca*) to 1 (pure *P. engelmannii*). Each dot represents one or multiple individuals.

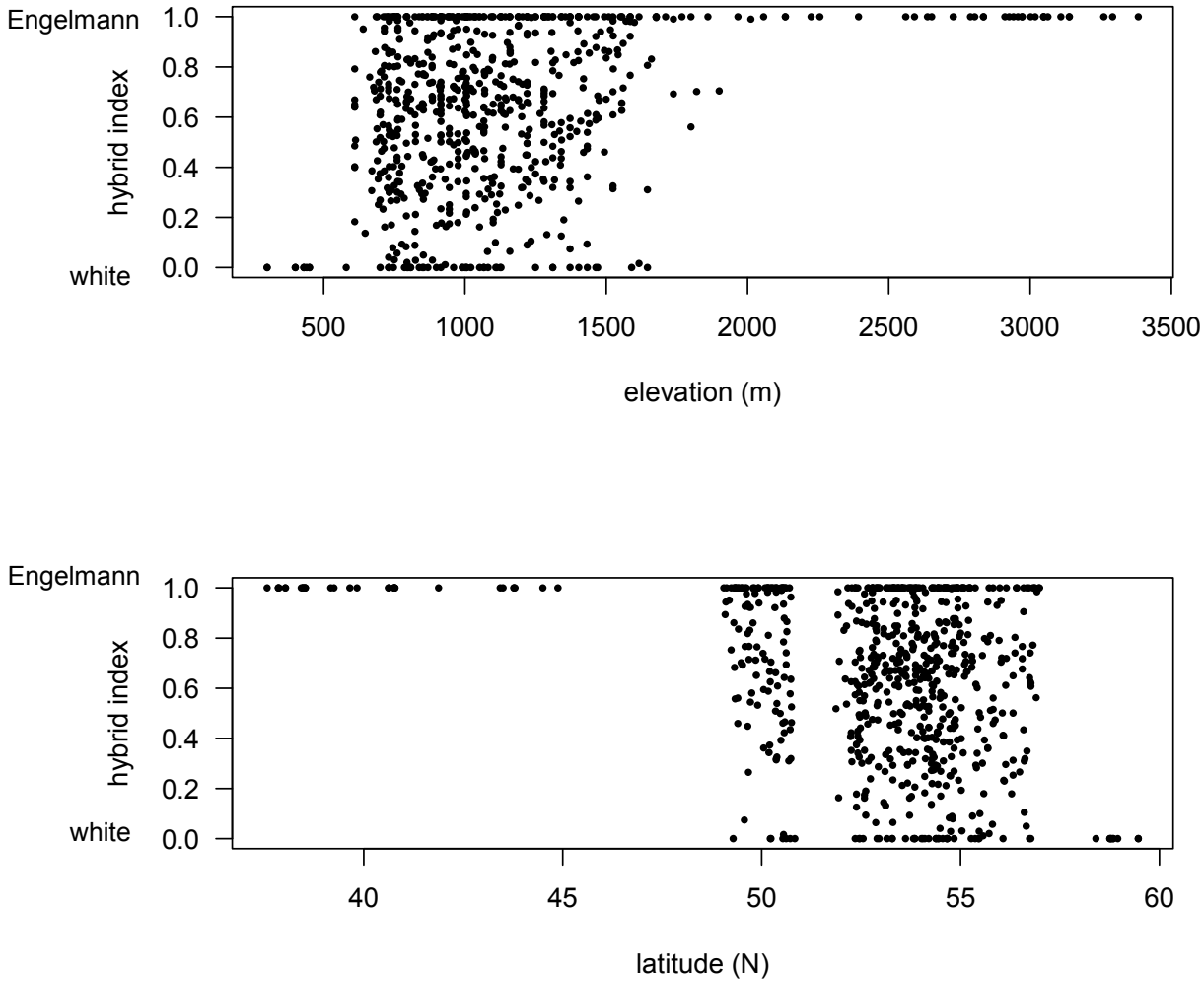


Figure 2.3 Analysis of population structure in the *Picea glauca* x *P. engelmanni* hybrid zone. A) Posterior estimates of cluster membership for K=2 in *STRUCTURE*. B) Posterior estimates of cluster membership for K=2 in *TESS*. Populations are ordered by decreasing latitude from left to right. Population names corresponding to two or three-letter codes can be found in Table 2.1.

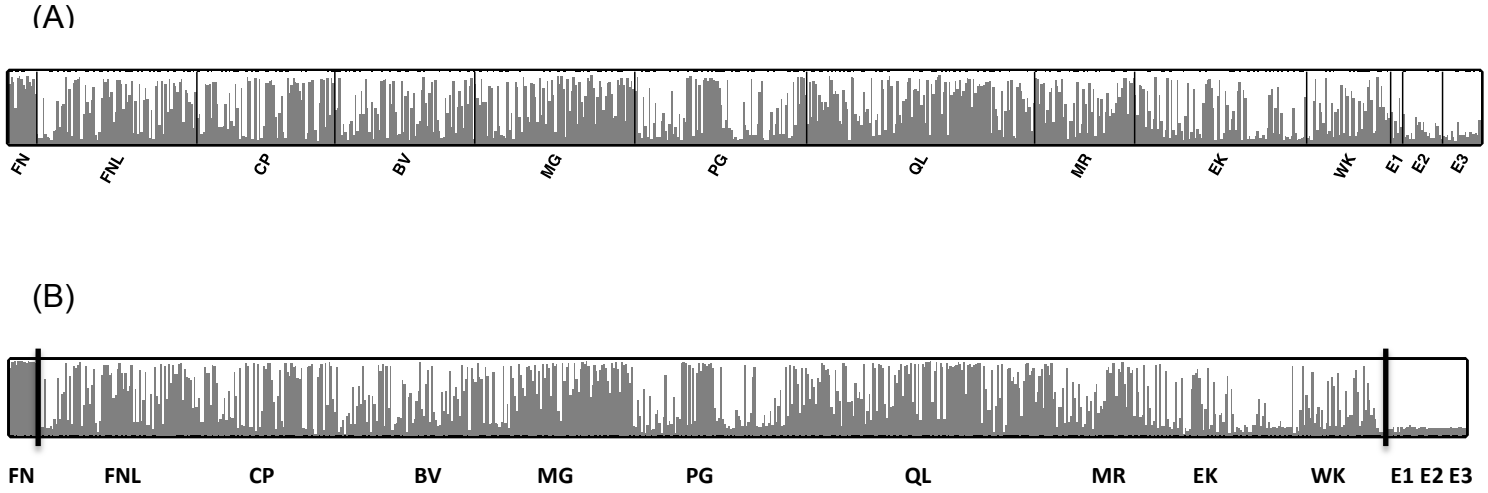
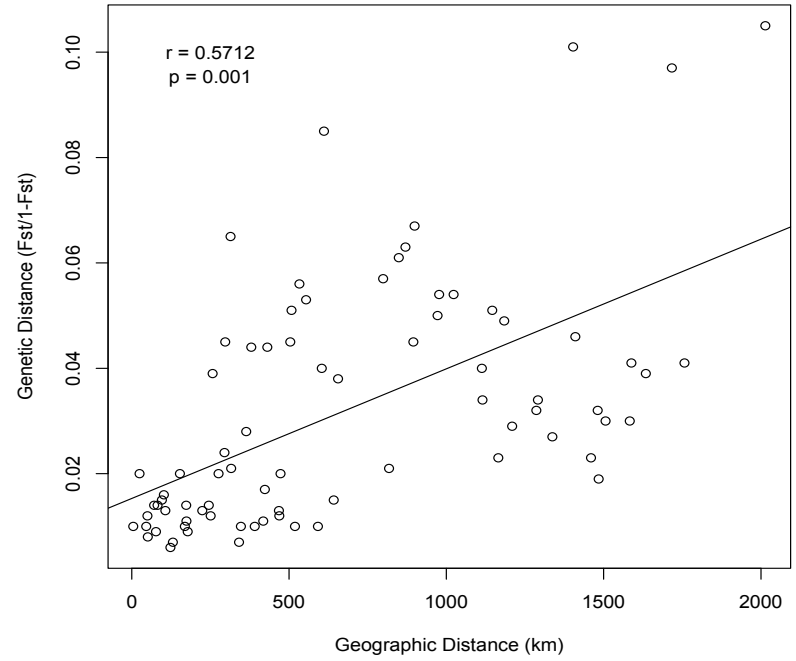


Figure 2.4 Scatterplots of isolation by distance for 13 populations within the *Picea glauca* x *P. engelmannii* hybrid zone based on F_{st} pairwise estimates using 10 SSR described in this study. Geographical distance (X-axis, top graph) is the distance along latitude from Fort Nelson (FN), British Columbia. Elevational distance (X-axis, graph at bottom) is the distance along elevation from Fort Nelson, BC.



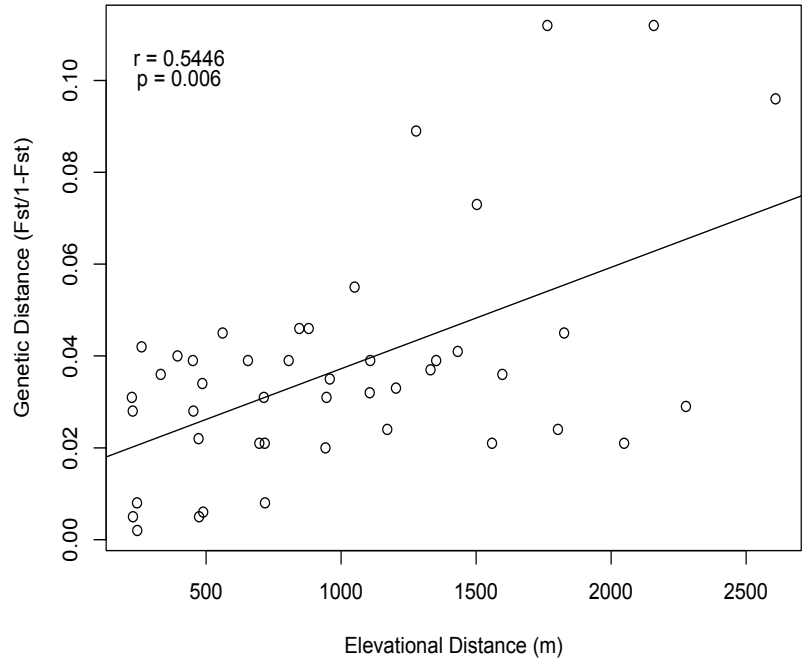


Figure 2.5 Plot of first two Principal Coordinates (PC1 and PC2) of a Principal Coordinates Analysis (PCA) based on Nei's genetic distances amongst populations. Circles represent *Picea engelmannii* populations; triangles, hybrid populations, and a cross, a pure *P. glauca* population.

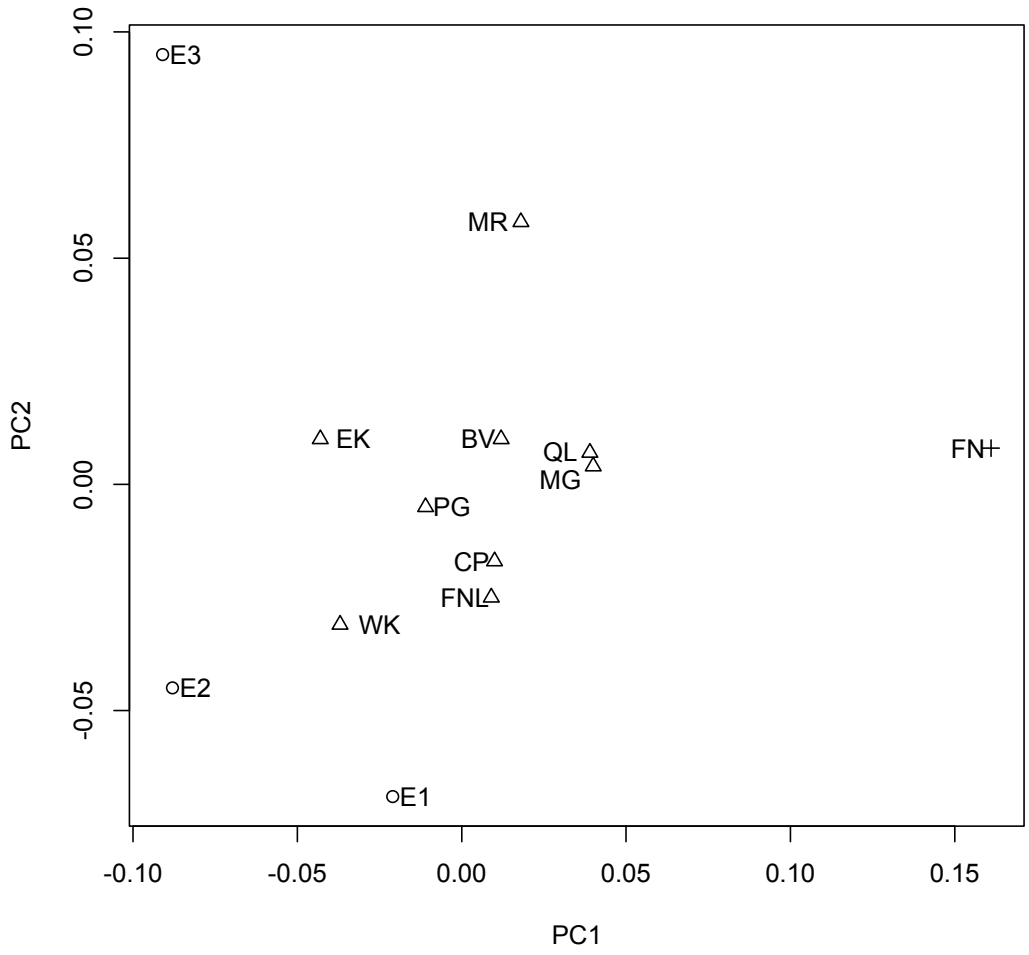
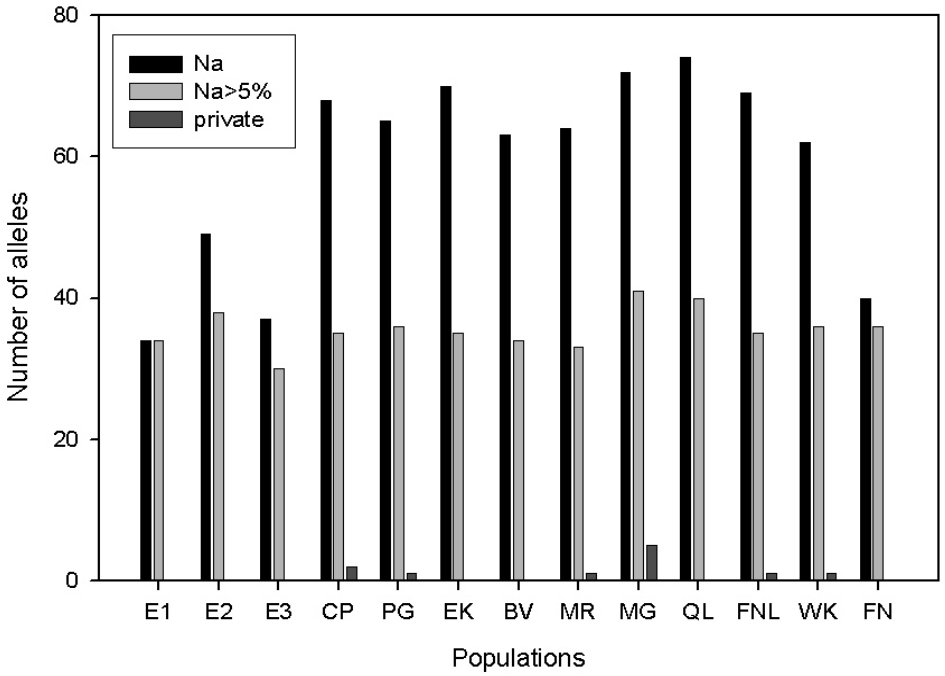
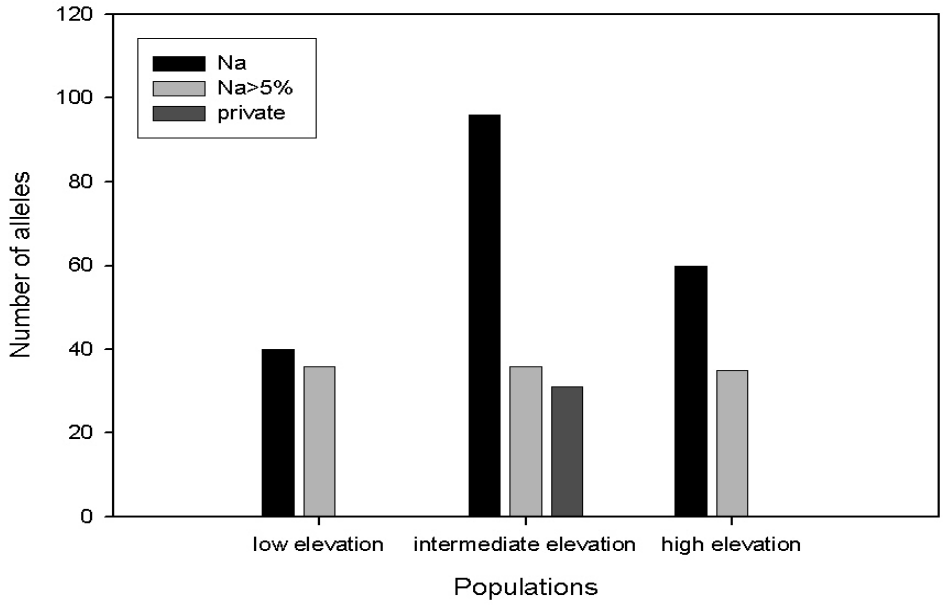


Figure 2.6 Allelic patterns across populations: number of different alleles (Na), number of different alleles with a frequency higher or equal than 5% (Na≥5%), and private alleles. Populations are stratified by A) latitude and B) by elevation.

(A)



(B)



3. Adaptation and exogenous selection in the *Picea glauca* x *Picea engelmannii* hybrid zone and its implications for natural and managed forests

3.1 Introduction

The role of hybridization in adaptive evolution has been a contentious issue in evolutionary biology. Some researchers have argued that hybridization is a potent evolutionary force that facilitates adaptive evolution and can lead to new species (Anderson 1949, Arnold 1997). According to this perspective, new gene combinations resulting from hybridization promote the development of novel adaptations (Rieseberg et al. 2003). In contrast, natural hybridization between divergent populations has been considered an evolutionary dead end or simply evolutionary noise (Mayr 1942).

Although definitive support of either point of view is lacking, recent studies have supported the first hypothesis for plant species. In sunflower (*Helianthus spp.*), new gene combinations generated by hybridization and introgression have contributed to ecological divergence (Rieseberg et al. 2003), and adaptation in several abiotic tolerance traits (Whitney et al. 2010). In iris species, adaptive introgression from *Iris fulva* to *I. brevicaulis* has increase flooding tolerance in some hybrid classes (Martin et al. 2005, 2006). Introgression and hybrid speciation are an important part of the evolutionary history of pines in North and Central America (Wang et al.1990; Wang and Szmidt 1994), and oaks in Europe (Whittemore and Schaal 1991; Kremer and Petit 1993).

The maintenance of hybrid zones has also been subject to considerable debate mainly because of different points of view about the power of natural selection and gene flow as a homogenizing force (Harrison 1993). Environment-independent models suggest that hybrid zones are maintained by a balance between dispersal and selection against hybrids, with selection being independent of the environment (Mayr 1942; Barton 1979). These models, also known as tension zones, assume that hybrids are less fit than their parents regardless of location (endogenous selection). Hybrid inferiority is thought to result from the break-up of co-

adapted gene complexes that affect fitness traits (Barton and Hewitt 1985, 1989; Hewitt 1988). Environment-dependent models involve genotype-by-environment interactions, where hybrid zones are maintained through selection gradients due to environmental heterogeneity (Endler 1973, 1977; Slatkin 1973; Moore 1977; Harrison 1986). In these models, hybrid fitness varies with the environment (exogenous selection). An example of an environment-dependent model is the Bounded Hybrid Superiority Model (Moore 1977), in which hybrid individuals are fitter than either parental species in environments that are intermediate to the parental habitats, but are less fit than parental species in their respective native habitats.

White spruce (*Picea glauca* (Moench) Voss) and Engelmann spruce (*P. engelmannii* Parry) are closely related, wind-dispersed, long-lived tree species that hybridize extensively in areas where the ranges overlap, in British Columbia and the western part of Alberta, Canada. White spruce and Engelmann spruce inhabit different ecological niches separated primarily by elevation. Engelmann spruce grows at high elevations and is restricted to cold, humid habitats because of its low tolerance to high temperature and drought (Alexander and Shepperd 1990). White spruce grows under highly variable conditions but is restricted to low elevations. Hybrids occupy ecological niches intermediate between those of the parental forms. Hybridization followed by backcrossing has produced hybrids with intermediate characteristics in morphology and phenotypic traits according to clinal variation along elevational gradients (Roche 1969; Daubenmire 1974; Ledig et al. 2006). Neutral molecular marker studies have found clines in admixture along elevation and latitude, with hybrids on average having a higher contribution from Engelmann spruce than from white spruce (Chapter 1). Although it is been suggested that hybrids are fitter than parentals in intermediate environments (Roche 1969; Daubenmire 1974; Xie et al. 1998, Ledig et al. 2006), this have never been tested.

White spruce and Engelmann spruce are economically important species in Canada. The complex of these two species and their hybrids represent the second most planted type of tree species in British Columbia, with millions of trees planted annually for reforestation that result from an intensive and large breeding program initiated in the early sixties. With British

Columbia getting warmer and in some areas drier as a result of climate change, there is a growing concern about how to manage these resources to cope with environmental change. In order to predict what is coming in the future, it is important to know what the effects of artificial selection are on the genetic architecture of the hybrid zone.

This study is the first to assess the evolutionary processes responsible for the maintenance of the ecologically and economically important white spruce x Engelmann spruce hybrid zone by combining molecular markers and phenotypic data. By doing that, I expect to contribute valuable information for current and future management of this species complex. The first objective was to assess whether the hybrid zone was maintained by exogenous selection. I compared the fitness of the hybrids and the parentals in common garden experiments in different locations within the hybrid zone by testing for correlations between hybrid index and quantitative traits, and by testing for differences between hybrid groups and pure species for each quantitative trait. The second objective was to elucidate the effect of climate on the genetic variation in the hybrid zone by identifying climate variables important in shaping spatial variation in hybrid index, and by creating genecological models that describe climate-related hybrid index variation across the zone. The third objective was to assess the effects of artificial selection on hybrid index; and the last objective was to predict future patterns of genetic variation in the zone using climate envelope models.

3.2 Materials and methods

3.2.1 Sample collection for genomic analysis

Newly flushed needle tissue of 745 samples of interior spruce were collected from field randomized block common garden experiments, previously established by the British Columbia Ministry of Forests, Lands and Natural Resources Operations' spruce breeding program for progeny testing. The experimental design of these common gardens is described in section 3.2.4 below. Up to four progeny were collected from 50 open-pollinated families (progeny of individual seed parents) in each of the West Kootenay (WK), East Kootenay (EK), Quesnel

Lakes (QL) and Mount Robson (MR) Seed Planning Zones (SPZ). A Seed Planning Zone (SPZ) is a geographical unit based on quantitative genetic similarities in adaptive traits. Seed parents of these progeny had a diffuse distribution across the SPZ in terms of latitude, longitude, elevation and climate, and geographic coordinates within SPZs are available for all parents. Out of the many families available in the progeny tests, families were selected for genotyping based on parent tree origins along elevational gradients (spanning a wide range of temperatures and growing season). Progeny test ages varied from 15 (West Kootenay) to 25 (East Kootenay, Mount Robson and Quesnel) years old. Also, 22 putatively pure white spruce from Fort Nelson (FN) and 40 putatively pure Engelmann spruce from southwest United States (E1, E2, E3) were obtained from grafts of mature trees sampled from natural populations for tree breeding, and archived in the British Columbia Ministry of Forests, Lands and Natural Resources Operations clone banks.

3.2.2 DNA extraction and genotyping

After sample collection, needles were stored at -80C° prior to DNA isolation. Each sample was isolated using a modified CTAB protocol (Doyle and Doyle 1987). After the extractions, DNA quality and concentration of each sample was assessed qualitatively using 0.8% agarose gels, and quantitatively using Nanodrop 2000C Spectrophotometer readings (Thermo Fisher Scientific Inc.). A total of 40 allopatric Engelmann, 22 allopatric white spruce and 745 samples from the Engelmann x white hybrid spruce DNA samples were sent to the Genome Quebec/ McGill Innovation Centre for SNP genotyping using an Illumina bead array chip (Illumina Inc.) in conjunction with the GoldenGate allele-specific assay in a 96-well, 768 SNP format (Fan et al. 2003; Shen et al. 2005). Samples from allopatric pure species populations and from hybrid populations were assayed in two different SNP arrays. In the first one, allopatric white spruce and Engelmann spruce samples were tested using 1536 SNPs from a large panel of genes putatively involved in cold hardiness (Holliday et al. 2009); insect

herbivory resistance (K. Ritland et al. Treenomix II project, unpublished data) and growth and bud set timing (Bousquet et al. Arborea project, Laval university, unpublished data). A total of 384 SNPs (138 from Holliday et al., 73 from Ritland et al., and 173 from Bousquet et al.) were selected based on their 1) genotyping quality (Gen Train score > 0.40); and 2) SNP frequency differences (interspecific Fst ≥ 0.20) between white spruce and Engelmann spruce for this study; and between white spruce and Sitka spruce (Hamilton and Aitken, unpublished). In the second SNP array (96-well, 386 SNP format), SNPs selected from the first array (384) were used to genotype 745 samples from the hybrid zone. Of 384 SNPs selected, 86 were successfully genotyped in both pure species and hybrids and met both genotyping quality and data normalization criteria.

3.2.3 Hybrid index and hybrid classes

In total, 86 SNP loci were used to calculate hybrid index with the *INTROGRESS* 1.1 (Gompert and Buerkle 2010) package in R 2.13.1 (R Development Core Team 2011). To estimate hybrid index, Fort Nelson (FN) population was used as parental white spruce population and E1, E2 and E3 were used as parental Engelmann spruce populations. Individuals were divided into four categories according to their hybrid index values as follows: 0-0.199 (pure white spruce); 0.2-0.499 (white spruce like-hybrids); 0.5-0.799 (Engelmann spruce like-hybrids); and 0.8-1 (pure Engelmann spruce).

Hybrid classes were estimated using the program *NewHybrids* 1.1 (Anderson and Thompson 2002). *NewHybrids* uses the Markov chain Monte Carlo (MCMC) simulation for computing the posterior distribution that individuals in a sample fall into different hybrid categories. Runs were tested using 20,000 sweeps. Individuals representing pure species were identified *a priori* and originated from populations outside of the hybrid zone. The default genotype frequency classes were used for the calculations. Following the *NewHybrids* assignment, individuals were divided in four groups: pure white spruce, pure Engelmann

spruce, F1 hybrids, and Fn hybrids (advanced generation hybrids including backcrosses and F2).

3.2.4 Common garden experiments

Randomized complete block common garden experiments previously established by the B.C. Ministry of Forests, Lands and Natural Resources Operations were used to test the fitness of pure species and their hybrids. Common garden experiments were established as half-sib progeny tests in which the same open-pollinated families were planted in three sites of contrasting elevation. Each site had 2 to 10 replications. Families within each replication were randomly assigned a planting order. Each family was represented by one 4-tree row plots (10-tree row plots in East Kootenay) within each replication. The spacing between trees was 2.5 x 2.5 m in all the Seed Planning Zones with the exception of West Kootenay, in which the spacing between trees was 1.25 x 1.25 m. A subset of fifty open-pollinated (OP) families that were also sampled for SNP genotyping were chosen in each of East Kootenay, West Kootenay, Mount Robson and Quesnel Seed Planning Zones (Table 3.1). A total of 12,082 individuals were phenotyped and analyzed in the four regions.

3.2.5 Phenotypic data

Growth data (height at ages 0-25) were provided by the B.C. Ministry of Forests, Lands and Natural Resources Operations. Survival was denoted as presence/absence of individuals at different ages.

Timing of bud burst was assessed in common garden experiments in the spring of 2009. Bud break was recorded from the apical bud of lateral branches using a system that classifies bud burst stages from 1 to 8 according to the amount of bud swelling, color and elongation (Alfaro et al. 2000).

Timing of bud set was measured as the Julian date of bud set at the end of the second growing season, in a previous seedling common garden experiment using several interior British Columbia white x Engelmann spruce populations (O'Neill and Aitken 2004). A subset of

the raw data (same East Kootenay families used for phenotyping and SNP genotyping) was reanalyzed in this study.

Needles from the current year's growth from the same families in East Kootenay, Mount Robson, Quesnel and West Kootenay were sampled in August 2010 to artificially test fall cold hardiness. A total of 931 individuals from 184 families (four to eight progeny per family) were evaluated. Cold hardiness was measured using electrolytic leakage as a proxy for cell death (Hannerz et al. 1999). Freeze-testing temperatures were chosen *a priori* based on the results of a cold-hardiness pre-test using five temperatures (-10°C, -20°C, -30°C, -40°C, -50°C) and 15 interior spruce individuals. The selected temperatures were -25°C, -35°C, and -45°C.

Five needles of upper lateral shoots were cut into 0.5 cm segments and place in vials containing 0.2 mL of dionized water and a small amount of an ice nucleator (AgL). Samples were placed in a programmable freezer (Tenney T20C-3 temperature chamber), in which temperature was reduced 4°C per hour until it reached the coldest temperature (-45°C). Starting temperature was 4°C. A control was kept at 4°C throughout the freezing program. After freezing, electrolytic conductivity was measured. All samples including the control ones were then heat-killed at 95°C. The conductivity was measured again. An index of injury (It) for each of the frozen samples was calculated as the percentage of injury that occur in the range from 0 (control) to 100 (heat-killed), as described by Flint et al. (1967):

$$I_t = 100(R_t - R_o)/(1 - R_o)$$

where $R_t = L_t/L_k$; $R_o = L_o/L_d$; Lt is the conductance of leachate from the sample frozen at temperature t; L_k is the conductance of the leachate from the sample frozen at temperature t and heat-killed; L_o is the control (unfrozen sample); and L_d is the conductance of the leachate from the heat-killed control (unfrozen sample) (Hannerz et al.1999).

3.2.6 Fitness analysis

Lifetime fitness in trees is very hard to measure because of their long generation lengths. In this study, growth (height), survival, cold hardiness, bud burst and bud set were

used as fitness proxies to compare the performance of pure species and hybrids at different ages and between different environments.

Differences between pure species and hybrids for height at different ages, bud burst, bud set, and cold hardiness traits were assessed using Analysis of Variance. The analysis of variance was conducted using the linear model:

$$y_{ijkl} = u + s_i + r(s)_{ij} + h_k + hs_{ik} + e_{ijkl}$$

where y_{ijkl} is the phenotype of individual l in hybrid class k in replication j of site i; s_i is the effect of site i, $r(s)_{ij}$ is the effect of replication j within site i, h_k is the effect of hybrid class k, hs_{ik} is the interaction between hybrid class k and site i, and e_{ijkl} is the error.

Population least square means with corresponding F tests and P values were estimated. Variance components estimated were calculated using the restricted maximum likelihood method with type III sum of squares. For bud burst, bud set and cold hardiness traits, population means instead of population least square means were used for the regressions. Tests of differences between means of pure species and hybrids were conducted using both Tukey and Bonferroni tests for multiple comparisons. Survival data was analyzed using binomial tests for contingency tables. All procedures were done using either *SAS Enterprise Guide* 4.2 or *SAS* 9.2 (SAS Institute Inc.) and graphed with R 2.13.1 (R Development Core Team 2011).

3.2.7 Climate analysis

ClimateWNA (Wang et al. 2012) was used to generate climate data for each sampling site and for a spatial grid (1 x 1 km) of the study areas. *ClimateWNA* downscales PRISM (Daly et al. 2002) monthly data (2.5 x 2.5 arcmin) for the reference period (1961-1990), and calculates a large number of additional climate variables for specific locations based on latitude, longitude and elevation for western North America. This program also downscales and integrates historical and future climate data (2020s, 2050s and 2080s) generated by various global circulation models. In this study, twenty annual climate variables were used, including

mean annual temperature (MAT), mean annual precipitation (MAP), and others (See Table A.8 in Appendix for a complete list of variables). Two climate datasets were generated for the reference period 1961-1990 (referred to as 1970s) and the future period 2041-2070 ("2050s"). The future climate data were generated using the Canadian third-generation coupled global climate model CGCM3 A2 run4 middle-of-the-road-change-scenario.

To relate hybrid index to climate, all climate variables, their combinations and transformations (quadratic and inverse forms) were screened using univariate and stepwise multivariate regression procedures in *SAS* (SAS Institute Inc). In the multivariate analysis, samples from different SPZ were pooled together. Hybrid indices were predicted based on the climate-hybrid index relationship developed in this study. Predicted hybrid indices were then mapped using *ArcGIS* (v. 10) for the two geographic regions where sampling sites were located (Figure 3.4). In order to demonstrate the impact of climate change on the climatic niche for the hybrids, hybrid index was projected for a middle-of-the-road future climate scenario for the 2050s using the CGCM3 A2 run4.

3.2.8 Effects of artificial selection on hybrid index

In order to assess the effect of artificial selection on hybrid index, family mean breeding values were plotted against mean hybrid index for each Seed Planning Zone (SPZ). Breeding value is a measure of an individuals' quantitative trait based on the mean quantitative trait of its progeny (Falconer 1996). Breeding values (BVs) for stem volume are estimated in order to select trees with higher growth potential (Xie and Yanchuk 2000). The Best Linear Prediction method (White and Hodges 1989) was used to calculate the breeding values of each family in each of the Seed Planning Zone (SPZ), according to:

$$BV=CV^{-1}(y-\alpha)$$

where **C** is a vector of genetic covariance between the observed half-sib family means and the breeding value being predicted, **V** is a s x s matrix of the phenotypic variances and covariances

of family means, **y** is the progeny mean of the parent (family mean) and **α**, is the progeny mean of all parents (population mean or overall family mean).

Breeding values for each Seed Planning Zone were provided by Barry Jaquish from the Ministry of Forests, Lands and Natural Resource Operations. To identify the families with breeding potential, the procedure generally used by tree breeders in British Columbia was followed. The best top 10 to 20 families with higher breeding values were selected for each Seed Planning Zone. The minimum cut-off breeding value was zero.

3.3 Results

3.3.1 Fitness analysis

3.3.1.1 Height

Significant differences in height at some or all ages were found between hybrid classes for all of the SPZ studied with the exception of West Kootenay (high elevation test site) (Table 3.2). Both methods of hybrid classification and hybrid index estimation, *NewHybrids* and *INTROGRESS*, showed very similar results. Only differences among classes for height at age six (West Kootenay at hybrid test site) was significant in the former classification and not significant in the latter.

White spruce and white-like hybrids generally tend to grow faster than Engelmann and Engelmann-like hybrids in intermediate environments (Figure 3.1, Tables A.6 and A.7 in Appendix). This trend in growth, however, is not always noticeable at young ages. When this happens, white spruce and white spruce-like hybrids won't show an advantage in growth in relation to Engelmann spruce until they reach ages 10 to 15. Engelmann and Engelmann-like hybrids may, in fact, show higher growth at early seedling and sapling stages.

However, in the high elevation test site, located at 1830m, this pattern of growth did not hold. There were no significant differences between the height of Engelmann individuals and hybrids. At this high elevation test site there were no pure white spruce individuals planted.

First generation hybrids (F1) were extremely variable (Table A.7 in Appendix). For example, F1 hybrids grew taller than advanced generations hybrids and Engelmann in Quesnel, but showed the opposite trend in West Kootenay. In East Kootenay, F1s grew similar to Engelmann, but then at age 20 surpassed Engelmann and reached similar sizes to the advanced generation hybrids.

3.3.1.2 Survival

The binomial tests indicated that there were significant differences in survival at different ages between white spruce, Engelmann spruce and their hybrids (Table 3.3). In most of the SPZ (East Kootenay and West Kootenay), hybrids had a higher survival rate than Engelmann at intermediate environments (hybrid habitat); and in West Kootenay, Engelmann had a higher survival rate than hybrids at high elevation environments (Engelmann habitat) (Table 3.4, Figure 3.2).

In Quesnel, the survival rate patterns differed from the other SPZ, with Engelmann having higher survival rates than hybrids, on average across all individual sites (Table 3.4). However, the differences between groups in each individual site were not significant for age 3. White spruce was present in lower numbers than Engelmann spruce and hybrids in the hybrid zone. Survival of white spruce was high (ages >3) in the SPZs where it was present, East Kootenay and Quesnel.

3.3.1.3 Bud burst and bud set timing

Bud burst timing was negatively correlated with hybrid index in Quesnel (R^2= 0.28, P= 0.0074) and East Kootenay (R^2=0.059, P= 0.0008), with Engelmann spruce and Engelmann-like hybrids having an earlier bud burst than white spruce and white-like hybrids in the same environment. It was also negatively correlated with elevation in the same SPZ: Quesnel (R^2=0.12, P=0.009) and East Kootenay (R^2= 0.2557, P=0.0002). Bud burst timing was

significantly different among white spruce, Engelmann spruce and their hybrids in East Kootenay and Quesnel (Table 3.2).

Similarly, bud set timing was also negatively correlated with hybrid index (R^2=0.126, P= 0.0003), elevation (R^2= 0.231, P=<. 0001) and latitude (R^2=0.025, P= 0.035) in East Kootenay. Engelmann spruce and Engelmann-like hybrids had an earlier bud set timing in comparison to white spruce and white-like hybrids while being in the same environment.

3.3.1.4 Cold hardiness

Although hybrids showed a pattern of being more cold hardy than pure species in intermediate environments; differences between hybrids and pure species were only significant in Quesnel (F=3.58, P=0.0297). Injury at -25°C was significantly correlated with elevation (R^2=0.1284, P=0.0072) in Quesnel; and with longitude (R^2=0.1079, P=0.0186) in East Kootenay.

3.3.2 Climatic analysis

Hybrid index showed strong clines corresponding to climatic gradients in temperature and precipitation (Table A.8 in Appendix). When considering all SPZs together, the main climatic variables associated with variation in hybrid index were precipitation as snow (PAS), summer heat-moisture index (SHM), and mean annual precipitation (MAP).

Results of a stepwise multivariate regression of hybrid index on climate variables showed that the inverse form of PAS together with SHM had the best model fit among all possible combinations of climate variables. The model explained 54% of the total variation in hybrid index (Table 3.5). Hybrid index was positively correlated with PAS (R^2=0.445, P<0.0001) (Figure 3.3) and SHM was significant but added little to the model (partial R^2=0.077, P<0.0001). Univariate regressions for SHM did not account for much of the total variation (R^2=0.108, P<0.0001). Maps of predicted current distribution of hybrid index showed good matches with observed ones (Figure 3.4). However, the matches were better in Mount Robson and Quesnel SPZ than in the East Kootenay and West Kootenay SPZ.

Optimal hybrid index values projected matched to future climate in 2050s were expected to decline to favor white spruce due to decrease in PAS. However, the decline in hybrid index was steeper at the lower end of PAS, while the change was hardly noticeable at the higher end of PAS, because of the nonlinear relationship between these (Figure 3.3).

3.3.3 Effects of artificial selection on hybrid index

Breeding values were negatively correlated with hybrid index in three of the four Seed Planning Zones (SPZs) studied. Pearson correlation coefficients (r) were 0.639 for East Kootenay, 0.654 for Mount Robson, and 0.53 for Quesnel (Figure 3.5). In these three SPZs, families with higher breeding potential were mainly composed of white spruce like hybrids, which is coincident with the fact than pure white spruce tends to grow faster than Engelmann spruce. It is important to note, though, that there is a fairly wide range of BVs for any given hybrid index value. Breeding values varied considerably among Seed Planning Zones, being the highest values in East Kootenay and the lowest in West Kootenay. In this last SPZ, correlations between hybrid index and breeding values were not significant due to a small range hybrid index (between 0.6 to 1), where all individuals were either pure Engelmann or advanced generation Engelmann-like hybrids, with a correspondingly small range in breeding values (-2 to 8);

3.4 Discussion

3.4.1 Fitness analysis

Temperate and boreal woody species have an outstanding capacity to survive winter freezing temperatures. Their relative fitness depends on the ability of the individuals to maximize their growth to be able to compete for light and yet avoid frost injury by synchronizing growth phenology with the local climate (Howe et al. 2003). Individuals with a higher fitness will

be those that grow taller and yet stop growing (i.e., set a terminal bud, enter dormancy, and initiate cold acclimation) before the first frost in that environment.

Hybrids have a combination of Engelmann and white spruce genomes; therefore, they have inherited a combination of genes that may provide them with an adaptive advantage in intermediate environments. Hybrids will generally grow taller than Engelmann spruce but will set bud earlier than white spruce. Both traits combined, faster growth and earlier bud set, may give the hybrids an environment-specific survival advantage during seedling and sapling stages. This likely explains the higher hybrid survival rates found in this study. These conditions hold in intermediate environments (hybrids habitats), however the story is different in pure species habitats.

In low-elevation environments (white spruce habitats), hybrids likely cannot compete with faster growing white spruce. In high-elevation environments (Engelmann habitats), hybrids likely cannot withstand the heavy snow loads or long, cold winters to which pure Engelmann is adapted. This may mean that the depth of the snowpack is keeping the hybrid zone from expanding to higher elevations in British Columbia. This explains why hybrids showed lower survival rates than Engelmann in the high-elevation West Kootenay test site. My inferences, however, are limited by the absence of common gardens in pure white spruce habitats in the study.

Another factor that indicates the advantage of white x Engelmann spruce hybrids over pure species is their abundance in the contact zone. Studies in natural populations using neutral markers (Chapter 2) have shown that hybrids are much more abundant than pure species within the zone. For example, from 805 individuals studied across the hybrid zone, around 700 were hybrids.

Although it was already well known that white spruce had faster growth than Engelmann spruce, what was not previously known is that this trend in growth rate is not evident at all life history stages. This study indicates that white spruce may not show a size advantage until later in life, around age 10. During the first years as sapling and juvenile, white spruce sometimes

grows less than hybrids or Engelmann spruce. This growth lag at early ages may be related to a lower capacity to withstand heavy snow loads and shorter growing seasons than Engelmann spruce and hybrids. Once a tree is well established, it will be less susceptible to frost injury and therefore more energy resources can be allocated to greater growth (Sakai and Larcher, 1987).

Bud set timing is generally under strong selection and is determined by a genetically determined response to photoperiod; whereas bud burst is triggered by the accumulation of a genetically determined heat sum after the exposure to chilling temperatures (Aitken and Hannerz 2001). With heat sum as the environmental cue, which is correlated with latitude and elevation, the timing of bud burst followed the same clinal pattern as bud set, with Engelmann and Engelmann-like hybrids burst and set buds earlier than white spruce and white-like hybrids.

3.4.2 Climate analysis

Results of the climate analysis suggest that the hybrid zone is maintained by adaptation to winter temperatures and precipitation, as well as summer aridity, with the most important variables being precipitation as snow (PAS) and summer heat-moisture index (SHM). These two climate variables are derived from directly calculated annual variables by the program *ClimateWNA*. PAS is based on monthly precipitation and monthly mean temperature (Wang et al. 2006). A larger value of PAS, or a deeper snowpack, can reflect either a larger amount of winter precipitation, cooler temperatures, or longer winter periods. PAS explained 46% of the total variation in hybrid index, and it is likely to be the main factor limiting white spruce survival in Engelmann's habitats (high elevation environments). SHM is a measure of aridity calculated using Mean Warmest Month Temperature (MWMT) divided by adjusted Mean Summer Precipitation (MSP/1000). The contribution of this variable to the hybrid index model is limited, varying between 8% (multivariate regression with PAS) and 10% (univariate regression). SHM is negatively correlated with hybrid index when it is the only variable in the model, while it turns positive when it is combined with PAS.

The relatively strong relationship between hybrid index and the two most explanatory climate variables enabled the prediction of hybrid index values for the entire sampling areas for the reference period 1961-1990. It also allowed the projection of future optimal hybrid index values by the incorporation of future climates. In this study, the trend of change in hybrid index with climate change is demonstrated using a single middle-of-the-road climate change scenario.

3.4.3 Exogenous selection as mechanism of maintenance of the hybrid zone

This chapter's results indicate that the white spruce x Engelmann spruce hybrid zone is maintained by selection gradients due to environmental heterogeneity (exogenous selection), where hybrids are fitter than parentals in intermediate environments. Adaptive introgression from Engelmann spruce to white spruce has produced hybrids with increased cold tolerance due to an earlier bud set. At the same time, adaptive introgression from white spruce to Engelmann spruce has resulted in hybrids with faster growth rates. Both traits, faster growth and increased cold tolerance, have given the hybrids an adaptive advantage over parental species (pure white and pure Engelmann spruce) in intermediate environments. This type of adaptive introgression has previously been seen in other plant species. Introgression from the flood tolerant *Iris fulva* into the dry-adapted *Iris brevicaulis* has increase flooding tolerance in *Iris fulva* backcrosses (Martin et al. 2005, 2006). Also, adaptive introgression from *Helianthus debilis* into *Helianthus annuus* has increased hervibory resistance (Whitney et al. 2006) and abiotic tolerance in several traits (Whitney et al. 2010).

According to my results, hybrids appear fitter than parentals in intermediate environments and less fit than both parentals in their respective native environments (although pure white spruce environments could not be tested). This suggests the white spruce x Engelmann spruce hybrid zone follows a bounded hybrid superiority model (Moore 1977). The most well known example of bounded hybrid superiority is the hybrid zone formed between subspecies of the long-lived, wind-dispersed big sagebrush, *Artemisia tridentata* (Wang et al.

1997). The *Artemisia* hybrid zone also occurs along elevational gradients; however, across a much smaller elevational range (from 1777 to 1870 m of elevation) than this study. In the *Artemesia* study, reciprocal transplant experiments have shown that hybrids are fitter than parentals in intermediate environments, suggesting that both parental and hybrid taxa are ecologically adapted to their own habitats.

Although I think that exogenous selection is the main factor responsible for the hybrid zone maintenance, I cannot rule out that some sort of endogenous selection is also acting in the zone. The main reason to believe that endogenous selection may be acting in the white spruce x Engelmann spruce hybrid zone is the low numbers of white spruce backcrosses. It may be that introgression from white spruce into Engelmann spruce, besides generating fit hybrids (hybrids with higher growth potential), also generates some unfavorable epistatic combinations that lead to unfit hybrids. Artificial crosses would be required to draw definitive conclusions about this viewpoint. Another explanation for the low numbers of white spruce backcrosses is simply the difference in density between white spruce and Engelmann spruce. If white spruce were more frequent in the zone, then there would be more opportunities for the hybrids to backcross with it. In fact, my previous molecular marker studies have found that pure Engelmann spruce appears to be in higher frequency than white spruce in the contact zone, assuming that the samples genotyped provide a proportional representation of the parental species and their hybrids (Chapter 2).

Given the complexity of this hybrid zone and the high levels of introgression, it was difficult to differentiate between F2 hybrids and backcrosses, therefore they were included in the same hybrid category. This differentiation between hybrid classes, however, was not so important, considering that most of the hybrids in the zone were advanced generation hybrids. As expected, only a small number of F1 hybrids were found. This is not surprising, considering that the formation of F1s is often a rare event in many species, due to prezygotic or postzygotic barriers between pure species (Arnold 2009); and that the hybrid zone is not likely to be of recent origin (Chapter 4). Even if F1s have low fitness, on average, they may mate with pure

species to form early backcrosses that will then mate again with pure species to form advanced generation hybrids. This process would allow the transfer of adaptations from one species to the other (Arnold 2009, 2011). In this study, apparent F1 hybrids showed high variability in the traits studied and did not show, on average, increased hybrid fitness. Advanced generation hybrids, on the other hand, were most abundant in the zone, showing, on average higher hybrid fitness than F1s. Therefore, my results indicate that advanced generation hybrids and not early generation hybrids are the ones with higher fitness than parentals at intermediate environments. These differences in fitness between hybrid classes are consistent with previous reports in natural hybrid zones (see Arnold 2011 for a complete set of references).

In contrast to artificial hybrid zones, in which only one or two generations are tested, individuals in this study comprised a vast array of recombinant hybrid genotypes, formed as a result of several to many generations of intercrossing (Rieseberg et al. 2000). Long-term phenotypic data (collected over a 25-year period) also provided a rare opportunity to study the adaptation of these long-lived woody species. On the other hand, because populations were highly admixed, this study failed to elucidate the processes that occurred early in hybrid zone formation, as is expected with studies using ancient natural hybrid zones; and some remaining questions about fitness of early-generation hybrids will require multiple generations of artificial crosses to answer (Carney et al. 2000, Kirk et al. 2005).

3.4.4 Effects of artificial selection on hybrid index

White spruce and Engelmann spruce are economically important species in Canada, with tens of millions of trees planted annually for reforestation. In British Columbia, the complex of white spruce, Engelmann spruce and their hybrids are managed as a single “interior spruce” species group in Seed Planning Zones (SPZ), based on geography and similarities in adaptive traits from provenance trials; and in Seed Planning Units (SPU), based on elevational bands within SPZs. SPZ classification is mainly used for managing natural-stand seed, whereas SPUs are used for managing seed orchards seedlots. Although this classification captures and

manages most of the genetic variation at the macro-scale, it does not reflect the fine scale picture. Knowledge of fine-scale population structure and differences in adaptive traits among white spruce, Engelmann spruce and their hybrids fills this gap, and also provides key information for the basis of genetic gains from artificial selection for volume growth within this hybrid zone.

By 2050, interior British Columbia is expected to get warmer and drier, with a projected temperature in 2 to 3°C according to projections of multiple General Circulation Models and greenhouse gas emissions scenarios (obtained through *ClimateWNA*). Due to a predicted decrease in precipitation as snow (PAS), new climates will favor white spruce (hybrid index closer to 0) over Engelmann spruce (hybrid index closer to 1). However, as a consequence of the non-linear relationship between hybrid index and PAS (Figure 3.3), and the moderate correlation between PAS and elevation (R^2= 0.416, P<0.0001), this trend favoring white spruce will only be evident in low and intermediate elevations. At high elevations (elevation> 1700m and PAS>700), hybrid index is not expected to change very much. Therefore, pure Engelmann populations growing at high elevations are expected to remain the same under new climatic conditions. However, due to the uncertainty of future climates, accurately projections for these changes will, at least, require the use of multiple climate change scenarios and even a more sophisticated model to better capture this relationship in future studies.

Future climatic conditions will affect natural and managed populations differently. A major concern is that managed forest tree populations will have problems adapting to changing climatic conditions. However, these data indicate that managed populations may in fact be somewhat pre-adapted to climate change compared to natural populations. My results indicate that artificial selection for breeding is currently selecting individuals that are more white-like hybrids because of their faster growth compared to Engelmann-like hybrids and pure Engelmann spruce. These individuals may have an advantage when adapting to the predicted warmer and drier climates in British Columbia, especially in low and intermediate elevations. This pattern of selection may counter the historical effects of natural selection on populations in

the hybrid zone. Studies using neutral and adaptive molecular markers in this hybrid zone had identified a strong asymmetry in introgression, with Engelmann-like hybrids much more abundant than the white-like hybrids (Chapters 2 and 3). Engelmann-like hybrids are adapted to shorter, cooler growing seasons and are slower growing than white-like hybrids; therefore they may have fewer opportunities to adapt to the predicted warmer climates in British Columbia. However, the high levels of genetic variation found at the contact zone (intermediate elevations and intermediate latitudes) may increase the ability of hybrid populations to adapt to new climates in comparison with single species populations (Aitken et al. 2008).

3.5 Conclusions

Results of this chapter indicate that the hybrid zone between white spruce and Engelmann spruce is maintained by selection gradients due to environmental heterogeneity (exogenous selection) along elevational and latitudinal gradients. White spruce, Engelmann spruce and their hybrids are fitter in their own habitats and show adaptations to different growing seasons lengths and snowpack persistence.

Hybrids are fitter in intermediate environments, but less fit than parentals in native parental environments, following the bounded hybrid superiority model of hybrid zone maintenance. Adaptive introgression from either white spruce or Engelmann spruce to the hybrids may likely be the cause of increased hybrid fitness in intermediate environments.

As a consequence of artificial selection, white spruce-like hybrids are more likely to be selected for breeding in British Columbia. This counteracts the effects of natural selection, as suggested by the higher frequency of Engelmann-like hybrids in natural populations of the hybrid zone (Chapter 2). This asymmetry towards white spruce in managed populations may confer an adaptive advantage over natural populations in future warmer temperatures, as it is expected to occur in British Columbia.

Table 3.1 Geographic coordinates and climatic variables for *Picea glauca* x *P. engelmannii* common garden experiments studied. The number of individuals (N) represents a subset of the total number of individuals planted at each site.

SPZ	Site	Reps	N	Elevation (m)	Latitude (degrees)	Longitude (degrees)	MAT (°C)	MAP (mm)	PAS (mm)	DD<0	AH:M	Hab.
EK	Bloom Ck	2	374	1676	49.02	115.45	2.1	1081	627	1111	11.2	hyb
	Perry Ck	3	542	1463	49.55	116.03	2.1	703	372	1138	17.2	hyb
	Red Rock	9	979	762	53.75	122.73	3.5	599	217	1008	22.5	hyb
WK	Hall Ck	10	1050	1200	49.267	116.367	3.8	820	393	895	16.8	hyb
	Duhamel	10	1099	1475	49.617	117.25	2.1	1103	608	1082	11	hyb
	Cortiana Ck	10	1207	1830	49.933	118.383	1.3	1100	562	1171	10.3	eng
QL	Little Benson	8	1744	960	52.53	122.3	3	590	212	996	22	hyb
	Camp Ck	8	1727	1080	51.42	120.3	3.3	638	260	936	20.8	hyb
	Ketcham Ck	8	1760	1380	53.15	121.41	1.4	864	382	1141	13.2	hyb
MR	Red Rock	8	1600	762	53.75	122.73	3.5	599	217	1008	22.5	hyb

Notes: EK= East Kootenay; WK=West Kootenay; QL= Quesnel Lakes; MR= Mount Robson (not included in fitness analysis); SPZ=Seed Planning Zone; Reps=Number of replications; N=Number of individuals studied in each replication; MAT=Mean Annual Temperature; MAP= Mean Annual Precipitation; PAS=Precipitation as snow; DD<0= Degree-days below 0°C; AH: M =Annual Heat: Moisture index (MAT+10)/(MAP/1000); Hab.= habitat; hyb=hybrid; eng=Engelmann.

Table 3.2 Differences among hybrid classes for phenotypic traits, based on ANOVA results. Hybrid classes are stratified as A) pure *Picea engelmannii*, pure *P. glauca*, advanced generation hybrids (Fn) and first generation hybrids (F1); based on the *NewHybrids*' assignment; B) pure *P. engelmannii*, pure *P. glauca*, *P. glauca*-like hybrids, and *P. engelmannii*-like hybrids; based on *INTROGRESS* hybrid index estimates. Traits showing significant differences are highlighted. Means and standard errors for individual traits are provided in Tables A.6 and A.7 (Appendix).

A) *NewHybrids* assignment

	Hybrid environment						*P. engelmannii* environment	
	East Kootenay		West Kootenay		Quesnel		West Kootenay	
Traits	F-value (df=3)	P-value	F-value (df=2)	P-value	F-value (df=2)	P-value	F-value (df=2)	P-value
Height at outplanting	--	--	--	--	32.02	<.0001	--	--
Height age 3	22.56	<.0001	3.96	0.0193	20.73	<.0001	1.89	0.1697
Height age 6	--	--	3.17	0.0421	23.68	<.0001	1.34	0.2464
Height age 10	13.71	<.0001	1.9	0.1502	46.56	<.0001	1.43	0.2326
Height age 15	--	--	--	--	42.65	<.0001	--	--
Height age 20	20.27	<.0001	--	--	32.05	<.0001	--	--
Height age 25	--	--	--	--	29.82	<.0001	--	--
Frost injury -25°C	1.23	0.2982	--	--	0.19	0.829	0.01	0.9332
Bud Burst	1.63	0.1983	--	--	3.28	0.0223	0.66	0.5182
Bud set	25.09	<.0001	--	--	--	--	--	--

B) *INTROGRESS* assignment

	Hybrid environment						*P. engelmannii* environment	
	East Kootenay		West Kootenay		Quesnel		West Kootenay	
Traits	F-value (df=3)	P-value	F-value (df=2)	P-value	F-value (df=3)	P-value	F-value (df=2)	P-value
Height at outplanting	--	--	--	--	15.33	<.0001	--	--
Height age 3	25.32	<.0001	4.05	0.0176	9.29	<.0001	0.88	0.4149
Height age 6	--	--	1.49	0.2259	15.18	<.0001	2.03	0.1313
Height age 10	14.45	<.0001	0.92	0.3968	24.7	<.0001	1.54	0.2152
Height age 15	--	--	--	--	22.06	<.0001	--	--
Height age 20	22.66	<.0001	--	--	21.74	<.0001	--	--
Height age 25	--	--	--	--	16.18	<.0001	--	--
Frost injury -25°C	1.38	0.2477	--	--	3.58	0.0297	0.01	0.9332
Bud Burst	8.2	<.0001	--	--	3.74	0.006	0.56	0.5735
Bud set	4.05	0.0204	--	--	--	--	--	--

Table 3.3 Binomial tests for differences in survival among hybrid classes. Hybrid classes compared were *Picea glauca, P. engelmannii* and their hybrids

	Hybrid environment						Engelmann environment	
	East Kootenay		West Kootenay		Quesnel		West Kootenay	
Traits	Z-value	P-value	Z-value	P-value	Z-value	P-value	Z-value	P-value
Survival age 3	3.538	0.0004	2.069	0.0385	2.210	0.0271	-2.166	0.0303
Survival age 6	--	--	2.575	0.005	-5.338	<0.0001	-2.680	0.0037
Survival age 10	3.618	0.0003	2.454	0.0141	2.450	0.0143	-2.836	0.0046
Survival age 20	2.884	0.0039	--	--	2.403	0.0162	--	--

Table 3.4 Differences in survival between *Picea engelmannii* and hybrids at different ages for all sites and regions studied in field common garden experiments. Significant differences are highlighted (P<0.05). N indicates the number of samples included in the study.

		% Survival of *P. engelmannii*				
Region	Site	N	Age 3	Age 6	Age 10	Age 20
East Kootenay	Bloom Ck	194	89.17	--	88.65	88.65
East Kootenay	Perry Ck	286	88.46	--	87.41	86.01
East Kootenay	Red Rock	538	93.68	--	91.26	90.33
East Kootenay	ALL sites	1018	91.35	--	89.68	88.8
West Kootenay	Hall Creek	895	80	70	49	--
West Kootenay	Duhamel Creek	919	93.47	88.46	76.27	--
West Kootenay	ALL sites	1814	86.93	79.38	63.07	--
Quesnel	Little Benson	347	95.1	94.81	93.08	91.93
Quesnel	Camp Creek	367	95.45	99.18	98.36	97.27
Quesnel	Ketcham Creek	367	89.64	88.01	86.37	85.55
Quesnel	ALL sites	1081	94.73	93.99	92.61	91.59
West Kootenay	Cortiana Creek	1031	91.85	89.55	86.16	--

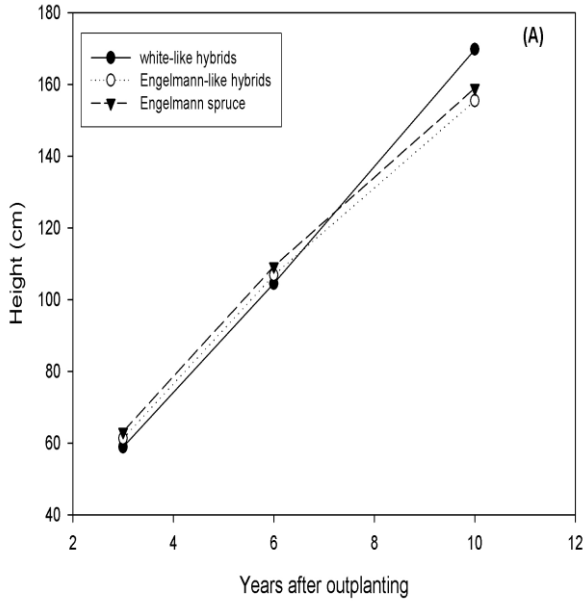
		% Survival of hybrids				
Region	Site	N	Age 3	Age 6	Age 10	Age 20
East Kootenay	Bloom Ck	174	95.97	--	93.67	92.52
East Kootenay	Perry Ck	247	93.11	--	91.49	87.44
East Kootenay	Red Rock	422	95.26	--	94.55	94.31
East Kootenay	ALL sites	843	94.78	--	93.48	91.93
West Kootenay	Hall Creek	155	85	78	58	--
West Kootenay	Duhamel Creek	180	95	90.55	78.88	--
West Kootenay	ALL sites	335	90.74	85.07	69.55	--
Quesnel	Little Benson	1364	92.88	92.08	90.46	89.73
Quesnel	Camp Creek	1342	98.8	98.58	98.13	97.39
Quesnel	Ketcham Creek	1373	87.47	85.5	82.81	81.06
Quesnel	ALL sites	4079	92.99	92.01	90.42	89.34
West Kootenay	Cortiana Creek	174	87.36	83.33	78.74	--

Table 3.5 Results of stepwise regression of hybrid index on precipitation as snow (PAS) and summer heat-moisture index (SHM)

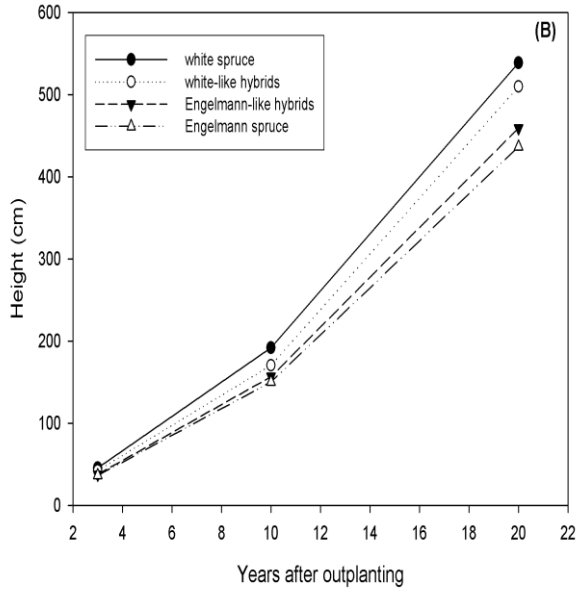
Independent variable	Parameter estimate	Partial R square	Model R square	F values (*P*)
Intercept	0.742			
1/PAS	-126.764	0.458	0.458	<0.0001
SHM	0.00524	0.078	0.536	<0.0001

Figure 3.1 Differences in height among *Picea engelmannii*, *P. glauca* and their hybrids in each geographic region (SPZ) represented in this study: (A) West Kootenay (WK), (B) East Kootenay (EK) and (D) Quesnel (QL), at the intermediate elevation hybrid habitat test sites; (C) West Kootenay at the high elevation test site. Hybrid classes were based on *INTROGRESS* assignment.

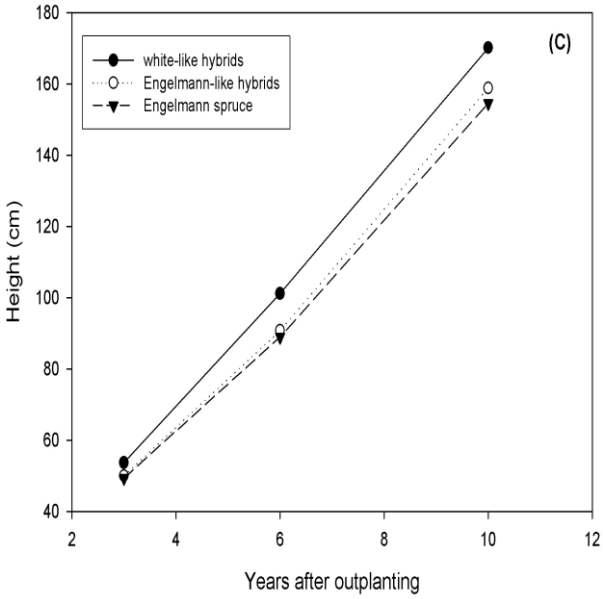
A) West Kootenay-Intermediate elevation



B) East Kootenay-intermediate elevation



C) West Kootenay- high elevation



D) Quesnel- intermediate elevation

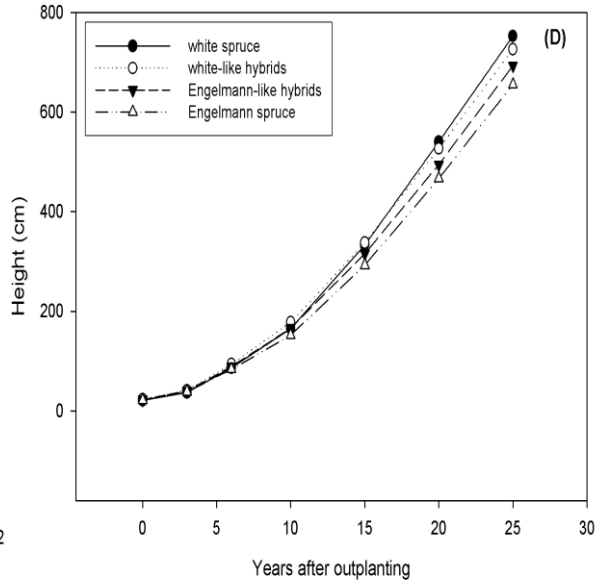


Figure 3.2 Results of the survival analysis for West Kootenay (WK). Percent survival was compared between hybrid and pure species *P. engelmannii* in two different habitats (*P. engelmannii* habitat: high elevation site and hybrid habitat: two intermediate elevation sites). Bottom graph summarizes the main results (average of all ages); both *P. engelmannii* and hybrids have higher survival rates in their own habitats. Differences between *P. engelmannii* and hybrids are significant at all ages.

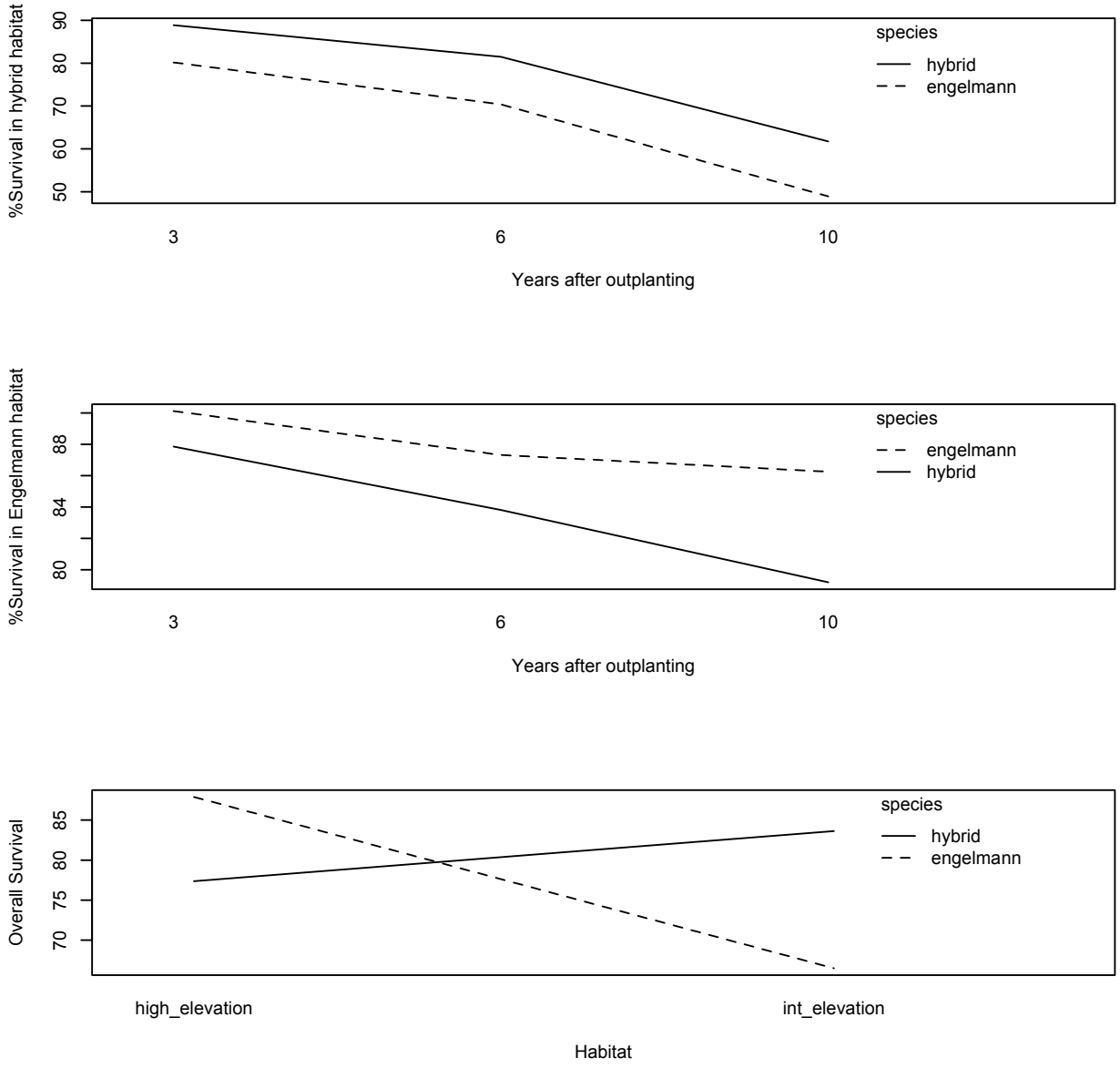


Figure 3.3 Observed (circles) and predicted (surface) spruce hybrid index against precipitation as snow (PAS) and summer heat-moisture index (SHM). Predicted hybrid index was generated based on the multiple linear relationships between hybrid index and climate variables shown in Table 3.2.

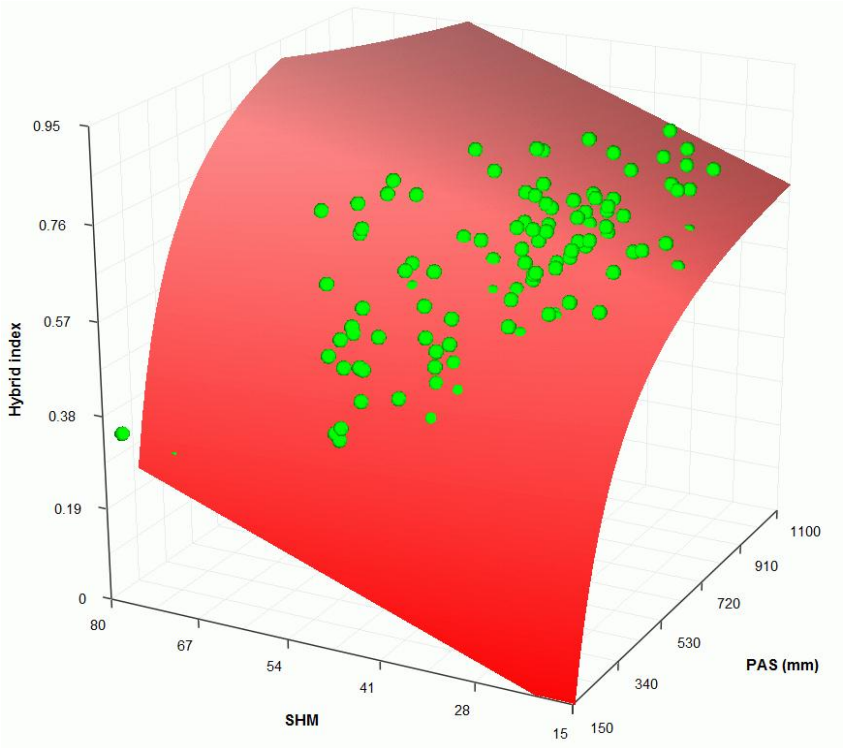


Figure 3.4 Observed and predicted hybrid index in the two sampling areas. Predicted hybrid index for 2050s was based on projected climate for this period by the Canadian third generation coupled global climate model CGCM3 A2 run4.

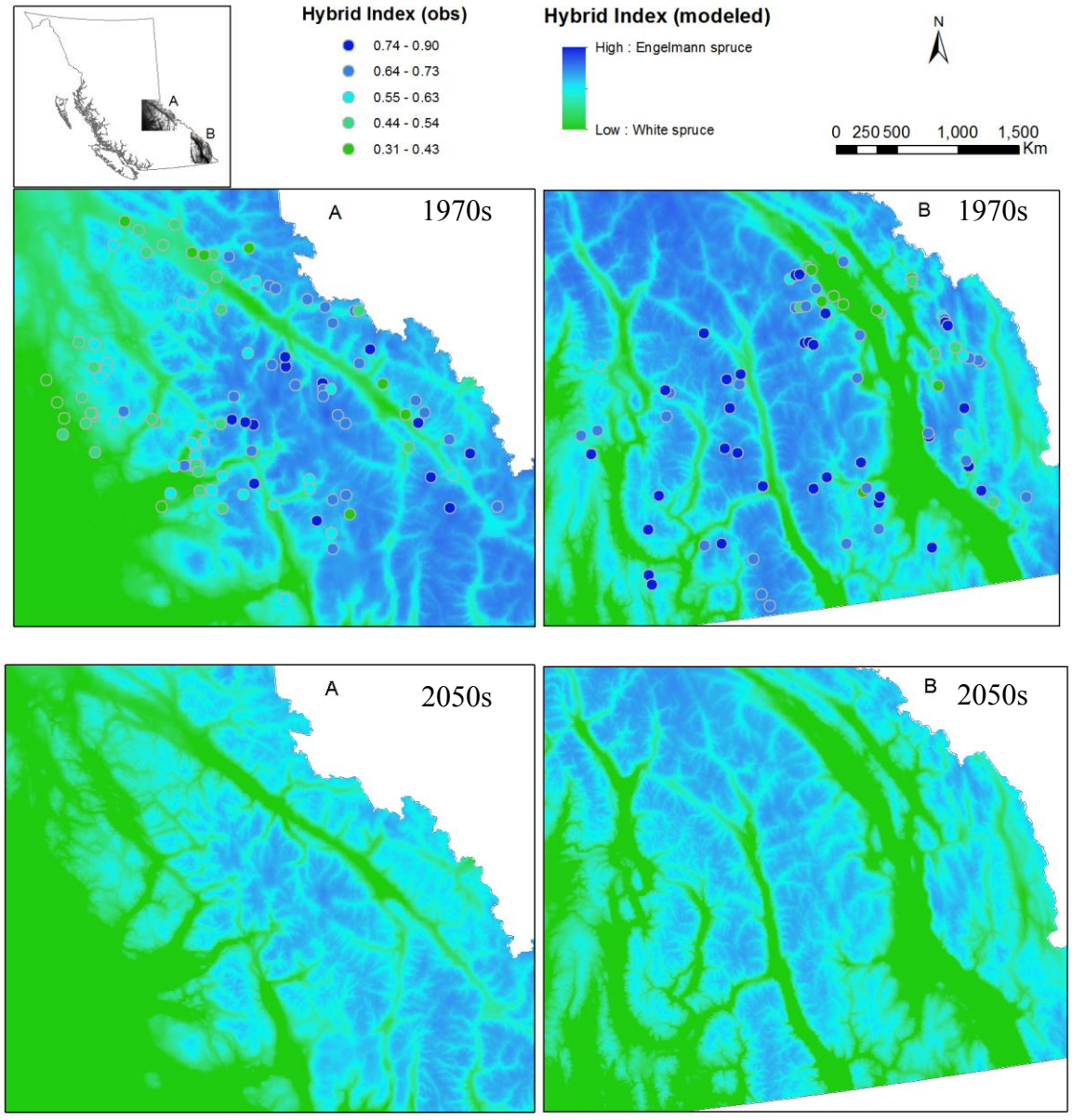
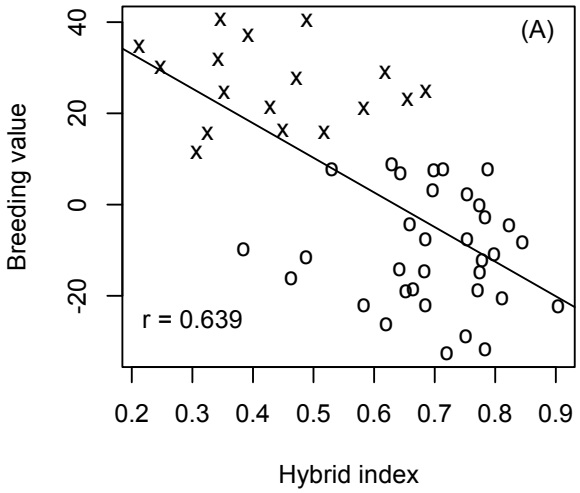
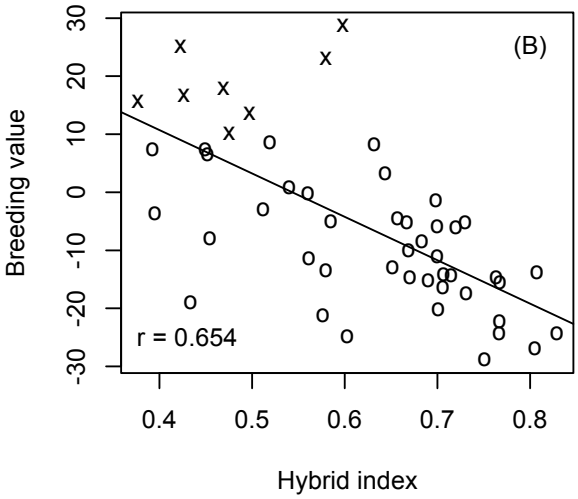


Figure 3.5 Breeding values vs. family mean hybrid index for spruce in four seed planning zones (SPZ) represented in this study: (A) East Kootenay (EK), (B) Mount Robson (MR), (C) Quesnel (QL) and (D) West Kootenay (WK). Crosses represent families with potential for breeding and empty circles represent families with no potential for breeding (negative or low breeding values). Individuals with a lower hybrid index are more *Picea glauca*-like, while those with higher hybrid indices are more *P. engelmannii*–like.

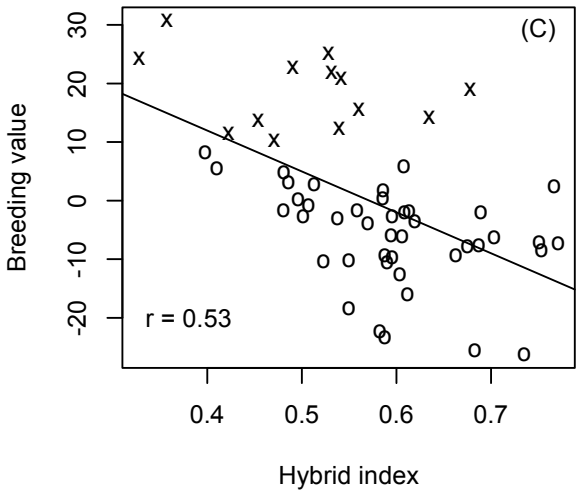
A) East Kootenay



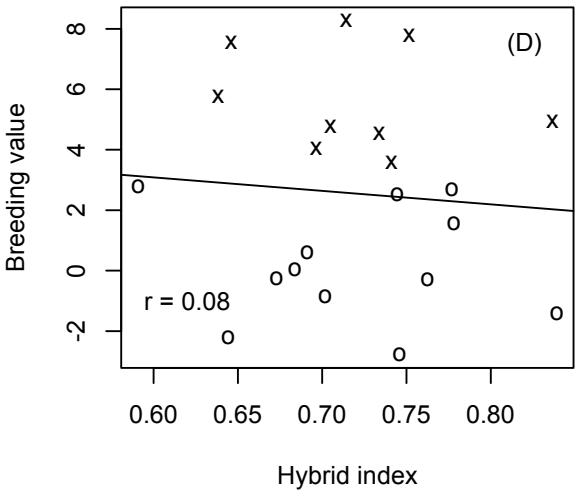
B) Mount Robson



C) Quesnel



D) West Kootenay



4. Genomic admixture analysis reveals the maintenance of species boundaries despite long history of interspecific gene flow

4.1 Introduction

The nature of genetic barriers that isolate species from interspecific gene flow is of great biological interest (Minder and Widmer 2008, Milne et al. 2003). Understanding the maintenance of species boundaries would not be possible without asking a fundamental question: are the genomes or genes the units of specific differentiation? Under the most widely recognized species concept, the Biological Species Concept (Mayr 1942), the genomes of species are co-adapted units that are separated from other units by reproductive barriers. This concept implies that species divergence only occurs through whole-genome isolation and therefore hybridizing species are not “true” species. By contrast, the genic view of speciation proposes the gene is the unit of species differentiation (Wu 2001), and reproductive isolation is a consequence of natural selection acting on individual genes. Species boundaries are “semi-permeable”; some genomic regions share introgressed genes between species, whereas other regions accumulate divergence between species in response to natural selection (Harrison 1986, Wu 2001).

Despite recent advances in population genomics and genetics, little is known about how species boundaries are maintained between closely related species that hybridize (Minder and Widmer 2008). One common hypothesis is that in hybridizing species showing differential adaptations, divergent selection acts on a subset of genes, counteracting the homogenizing effect of gene flow and preventing introgression in surrounding genomic regions. As a result, species boundaries are maintained despite hybridization and introgression (Minder and Widmer 2008). Species that hybridize can also coexist without merging or being swamped in environments where hybrids are favored over pure species by natural selection (fitness higher

than pure species), i.e., in intermediate environments (Moore 1977, Arnold 1997, Milne et al. 2003).

White spruce (*Picea glauca* (Moench) Voss) and Engelmann spruce (*P. engelmannii* Parry) are closely related species that came into secondary contact during the last glacial maximum or at the end of the Pleistocene (Wisconsin time) (Daubenmire 1974; Ledig et al. 2006). These wind-dispersed, long-lived, closely related tree species hybridize extensively in British Columbia and the western part of Alberta. This extensive hybrid zone is mainly composed of hybrids with a clinal intergradation of morphological, physiological and genetic characteristics along elevational gradients between parental species' habitats (Roche 1969; Ledig et al. 2006). White spruce and Engelmann spruce inhabit different ecological niches; Engelmann spruce is restricted to high elevations due to low tolerance to warm temperatures and drought, and high tolerance of deep snowpacks and short growing seasons (Chapter 3); whereas white spruce is restricted to low elevations. Elevation is the most important geographical factor defining the levels of admixture within the contact zone, whereas latitude is most important across the hybrid zone (Chapter 2).

Recent studies using neutral microsatellite markers have found that introgression is extensive in the hybrid zone and asymmetric towards Engelmann spruce (Chapter 2). Despite extensive interspecific gene flow, natural selection acting along environmental gradients (exogenous selection) is responsible for the hybrid zone maintenance. White spruce, Engelmann spruce and their hybrids are each adapted to different environments (Chapter 3). This hybrid zone follows a bounded hybrid superiority model in which hybrids are fitter than pure species in intermediate environments (Chapter 3).

Over the last century, the study of the evolutionary and genetic relationships between white spruce and Engelmann spruce has focused on the contentious issue of whether these two closely related species represent extreme phenotypes along a genetic continuum (Taylor 1959; Rajora and Dancik 2000) or if they deserve specific recognition (Wright 1955; Roche 1969). Although previous studies in the white spruce x Engelmann spruce contact zone had

given a broad idea of the evolutionary relationships between these species, they have failed to identify the factors contributing to isolating barriers between species. Some limitations of previous studies included the use of small numbers of selectively neutral markers that lacked species-specific diagnostic markers. Also, many of these studies sampled only a small geographic area within the hybrid zone.

This study uses both genomic and palaeoclimatic analyses to assess the past and current evolutionary relationships between white spruce and Engelmann spruce. My main question resided in understanding how white spruce and Engelmann spruce maintain their species integrity despite interspecific gene flow. To answer this question, I addressed the several objectives. The first objective was to better understand the recent evolutionary history of white spruce and Engelmann spruce by using palaeoclimatic analysis to study the potential for glacial and post-glacial demographic processes with range expansions and contractions, and to infer past opportunities for secondary contact between species. The second objective was to study the current patterns of interspecific gene flow, by determining the extent and direction of introgression and by comparing these results with previous studies using neutral markers (Chapter 2). The third objective was to use outlier analysis to identify some of the genes that may be responsible for isolating barriers and adaptive differences between the species. The final objective was to use genomic cline analysis to identify the main types of selection that have resulted in deviations from neutrality in outlier loci.

4.2 Materials and methods

4.2.1 Evolutionary history of white spruce and Engelmann spruce

To study past demographic processes during glacial and postglacial range contractions and expansions, and to determine recent potential opportunities for secondary contact between species, ecological niche modelling was used. The ecological niche model was built using mapped ecoregion delineations for North America from various public sources. Frequencies for

both species were based on forest inventory plot records falling geographically within the ecoregion boundaries (see Roberts and Hamann 2011 for detailed data sources). Environmental predictors consisted of ten climate variables, interpolated at 1km resolution. Climate for the modern period was based on the 1961-1990 climate record. Palaeoclimate data was generated by overlaying the modern climate data with climatic anomaly data generated by two general circulation models (GCMs): the Community Climate Model (CCM1) (Kutzbach et al. 1998) and the Geophysical Fluid Dynamics Laboratory model (GFDL) (Bush and Philander 1999).

Models were trained with data from the present day species distributions, and projected with the palaeoclimate data for past periods. Ecoregion model projections were made by a majority-vote of three modelling strategies, capable of generating class-based model projections (Roberts and Hamann, unpublished). Models were evaluated with palaeoecological data using the area under the receiver-operating characteristic (AUC) (Table A.9 in Appendix). While AUC values were highly variable, sensitivity was generally low and specificity generally very high, indicating that models were better predictors of species absences than species presences. This is to be expected, given the limited number of validation points available for each species, particularly in the earlier periods immediately following the last glacial maximum.

4.2.2 Sample collection for genomic analyses

Newly flushed needle tissue of 745 samples of interior spruce were collected from field randomized block common garden experiments, previously established by the British Columbia Ministry of Forests, Lands and Natural Resources Operations' spruce breeding program (Table 4.1, Figure 4.1). Three to four surviving progeny with representative growth rates were collected from 50 open-pollinated families (progeny of individual seed parents) in the West Kootenay (WK), East Kootenay (EK), Mount Robson (MR) and Quesnel Lakes (QL) Seed Planning Zones (SPZ) selected from those available to sample representatively across elevational gradients. Progeny test age varied from 15 (West Kootenay) to 25 (East Kootenay,

Mount Robson and Quesnel) years old. A Seed Planning Zone (SPZ) is a geographical unit based on similarities in adaptive traits used to manage seed transfer from natural stands for reforestation. Seed parents of these progeny had a diffuse distribution across the SPZ in terms of latitude, longitude, elevation and climate, and geographic coordinates within SPZs are available for all parents. In this study, a population was defined as all individuals that occur in the same SPZ. Also, 40 putatively pure white spruce (22 Fort Nelson (FN) and 11 Prince George (PG) individuals from British Columbia; and 7 individuals from the Ottawa valley (45°38'N, 75°71'W) in Eastern Canada), and 40 putatively pure Engelmann spruce from southwest United States (E1, E2, E3) were obtained from grafts of mature trees sampled from natural populations for tree breeding and archived in the British Columbia Ministry of Forests, Lands and Natural Resources Operations clone banks. After the population structure and admixture analyses, the Prince George population was reassigned as a hybrid population (Table 4.1). All samples, with the exception of the Prince George and Eastern Canada samples, were the same as those analyzed in Chapter 3.

4.2.3 DNA extraction and genotyping

After sample collection, needles were stored at -80°C prior to DNA isolation. Each sample was isolated using a modified CTAB protocol (Doyle and Doyle 1987). After the extractions, DNA quality and concentration of each sample was assessed using 0.8% agarose gels and quantified based on Nanodrop 2000C spectrophotometer readings (Thermo Fisher Inc.). A total of 40 allopatric Engelmann spruce, 40 allopatric white spruce and 745 Engelmann x white hybrid spruce DNA samples were sent to the Genome Quebec/ McGill Innovation Centre for single nucleotide polymorphism (SNP) genotyping using an Illumina bead array chip (Illumina Inc.) in conjunction with the GoldenGate allele-specific assay in a 96-well, 768 SNP format (Fan et al. 2003).

Samples from allopatric pure species populations and from hybrid populations were assayed in two different SNP arrays. In the first one, white spruce and Engelmann spruce

samples were tested using 1536 SNPs from a large panel of genes putatively involved in cold hardiness (Holliday et al. 2009); insect herbivory resistance (K. Ritland et al. Treenomix II project, unpublished data) and growth and bud set timing (J. Bousquet et al., Arborea project, Laval university, unpublished data). A total of 384 SNPs (138 from Holliday et al., 73 from Ritland et al., and 173 from Bousquet et al.) were selected based on: 1) genotyping quality (GenTrain score > 0.40); and 2) SNP frequency differences (interspecific Fst ≥ 0.20) between white spruce and Engelmann spruce for this study; and between white spruce and Sitka spruce for J. Hamilton and S.N. Aitken, unpublished). In the second SNP array (96-well, 386 SNP format), SNPs selected from the first array (384) were used to genotype 745 samples from the hybrid zone.

4.2.4 Data normalization

Data files with florescence intensity for each SNP were loaded directly into Illumina's genotype analysis software, *GenomeStudio* Genotyping Module v1.0 (Illumina 2010). The intensity data for each SNP was normalized to minimize BeadChip to BeadChip variability. Once the normalization was complete, the clustering algorithm was run to evaluate cluster positions for assigning individual genotypes and generating quality scores for each locus. The minimum threshold for the GenTrain score was set at 0.4 for the current assay. SNPs were only accepted that had call rates of greater than 90%; however, most of them (91%) had call rates greater than 98%. All SNPs had minor allele frequencies above 5%, reflecting the SNP selection process. SNPs showing heterozygote excess (Ht>0.25) were discarded to avoid confounding results due to gene duplications. SNPs showing low clustering differentiation between homozygotes and heterozygotes were not considered for later analyses. Non-polymorphic SNPs were also discarded. Of 384 SNPs included in the GoldenGate array, 311 were successfully genotyped and met both genotyping quality and data normalization criteria.

4.2.5 Admixture and population structure

Genotypes were available for samples of putatively pure species from allopatric populations for a subset of 86 of the SNPs used to genotype individuals from the hybrid zone. These SNPs were used to assess population structure and admixture levels. Population structure was evaluated using the program *STRUCTURE* 2.3.3 (Pritchard et al. 2000). Models with a putative number of clusters (K) from one to 16 were tested using 10,000 iterations for the pre- and post-burn periods using the admixture model. The degree of admixture alpha was inferred from the data. Each run was replicated twenty times in order to estimate K using the method developed by Evanno et al. (2005) with the program *Structure Harvester* version 0.6.7 (Earl 2010).

Geographical coordinates (longitude and latitude) and genetic data for each sample were used to estimate admixture coefficients and population structure with *TESS* version 2.3 (Chen et al. 2007). *TESS* is a Bayesian program that calculates membership probabilities and geographical cluster assignments of individuals from individual multilocus genotypes sampled at distinct geographic locations (Durand et al. 2009). The admixture model was performed for values of Kmax ranging from 2 to 14. Markov chain Monte Carlo (MCMC) algorithms were run for a length of 50,000 sweeps with burn-in periods of 30,000 sweeps. Each run was replicated twenty times. Twenty percent lowest DIC (Deviance Information Criterion, Spiegelhalter et al. 2002) runs were averaged and plotted against the number of clusters (Kmax) to decide which Kmax may provide the best fit to the genetic data.

Principal Component Analysis (PCA) was conducted using a correlation matrix of allele frequencies with *SAS Enterprise Guide* 4.2. The first twenty Principal Components were then tested for correlation with elevation using *PROC CORR*. Population sum of squares and corresponding F-test and P-values were calculated using *PROC GLM*, in a model in which elevation was explained by the first three Principal components. F_{st} values were calculated using *GenAlEx* version 6.4 (Peakall and Smouse 2006).

In order to look for differences in allele frequencies between white spruce, Engelmann spruce and their hybrids, allele frequencies for each population were estimated using the program *GenAlEx* version 6.4 (Peakall and Smouse 2006).

Based on the results of the Evanno test and the Principal Component Analysis, alignments of clusters for K=2 and K=3 using 50,000 pre- and post-burn iterations were optimized using the program *CLUMPP* 1.1.2b (Jakobsson and Rosenberg 2007) and graphed with the program *distruct* (Rosenberg 2004).

4.2.6 Hybrid index and Interspecific heterozygosity

Based on *STRUCTURE* results for K=2, individuals with Bayesian admixture proportions (Q) greater than 0.8 from Fort Nelson (white spruce) and E1, E2, E3 (Engelmann spruce) were used as reference genotypes for "pure species" to calculate hybrid index within the zone with 86 SNPs using the *INTROGRESS* 1.1 (Gompert and Buerkle 2010) package in R 2.13.1 (R Development Core Team 2011). Although the cut-off was set to Q>0.8, most of the individuals had Q>0.9. Hybrid index ranged from 0 (all alleles inherited from white spruce) to 1 (all alleles inherited from Engelmann spruce). Individual hybrid index estimates were regressed on geographical variables in each of the seed planning zones (East Kootenay, West Kootenay, Quesnel and Mount Robson) using *SAS PROC REG*.

Interspecific heterozygosity, defined as the proportion of the individual's genome with alleles inherited from both parental populations, was calculated and plotted as a function of hybrid index. The diagonal lines of the triangle plot indicated the maximum heterozygosity that the progeny of each of the pure species could theoretically reach. Hybrid index was plotted against elevation and latitude to visualize the structure of the hybrid zone. Patterns of introgression for all markers and individuals in the admixed population were also plotted, in which each rectangle denotes an individual's genotype at a given locus.

4.2.7 Detection of outlier loci

In order to test for signatures of selection, two sets of analyses were conducted. First, populations from within the hybrid zone (sympatric and parapatric populations) were analyzed using all candidate 311 SNPs. In the second analysis, populations across the hybrid zone (allopatric, parapatric and sympatric) were analyzed using 86 SNPs from candidate genes in cold hardiness that were assayed in all of those populations. Populations used in previous analysis were subdivided according to their elevation into five groups: 350-600m, 600-1600m, 1600-1800m, 1800-2250m, >2250m. *BayeScan* v2.0 (Foll and Gaggiotti 2008) was used to identify loci that deviated significantly from neutrality. This Bayesian program decomposes locus-population F_{st} coefficients into a population-specific component (β) and a locus-specific component (α) using logistic regression. When the pattern of diversity cannot be explained by β alone (α significantly different from 0), the locus is considered to be under selection. Positive values of α suggest diversifying selection and negative values, balancing selection. Several runs were performed to ensure consistency, with 5,000 iterations and burn-in period of 50,000 iterations. False discovery rate (q), defined as the expected proportion of false positives among outlier markers, was 0.05. The gene annotations for outlier loci were compiled.

4.2.8 Genomic clines

Genomic Clines were calculated using the parametric procedure with 1,000 permutations with the *INTROGRESS* 1.1 (Gompert and Buerkle 2009) package in R 2.13.1 (R Development Core Team 2011). This method uses multinomial regression to predict the probability of a given genotype for a marker as a function of hybrid index between a pair of species (Gompert and Buerkle 2010). Expected genomic clines based on a null model are used as a reference to identify loci that deviated from neutral expectations. Genomic clines from loci showing deviations from neutrality in the outlier analysis were selected in order to assess patterns of selection between the two species. The genomic cline analysis was used as an alternative to the traditional geographic cline method (Barton & Hewitt 1985), which is not

applicable to the white spruce x Engelmann spruce hybrid zone due to the violation of assumptions (additive underdominance as main model of selection).

4.3 Results

4.3.1 Evolutionary history of white spruce and Engelmann spruce

Results of the climate niche modelling suggest that white spruce and Engelmann spruce potentially came into secondary contact as early as 21,000 YBP in the southern part of the Rocky Mountains (Colorado and Wyoming), east of the current hybrid zone location (Figure 4.2). As a result of white spruce and Engelmann spruce range expansions and contractions, the climatic niche of the hybrid zone likely moved west and then north until reach its current location in British Columbia and Alberta by 14,000 YBP, after the recession of the Cordilleran Ice Sheet. These results are consistent in both general circulation models used, CCM1 and GFDL. After 14,000 YBP the hybrid zone expanded its range in latitude and longitude until reach its current northern expanse (Figure 4.3). By 11,000 YBP, white spruce populations from the southern refugia extended their range until Alaska; and the range of Engelmann spruce started to become fragmented at the southern part of the Rocky Mountains.

4.3.2 Admixture, hybrid index and population structure

High levels of admixture and introgression were found within the contact zone between white spruce and Engelmann spruce, where most of the individuals had a hybrid ancestry (Figures 4.4, 4.5, 4.6). Northern hybrid populations (PG, QL and MR) showed a higher genetic contribution from white spruce, and southern hybrid populations (EK, WK), which also originated from higher elevations on average, showed a higher genetic contribution from Engelmann spruce (Figure 4.4). Admixture followed clines along elevation and latitude, where Engelmann spruce occurs at elevations of more than 1800 m, white spruce occurs at elevations of less than 600 m and the intermediate elevations are covered by hybrids (Figure 4.5).

Both the admixture and the hybrid index analysis showed asymmetry in introgression towards Engelmann spruce, meaning that in general in the hybrid zone, there were more hybrid individuals with a higher genetic contribution from Engelmann spruce than those with a higher genetic contribution from white spruce (Figures 4.5, 4.6). This asymmetrical introgression occurred along both latitudinal and elevational gradients, but it was more noticeable with elevation. The histogram of hybrid classes showed that the majority of the hybrids (75%) had a hybrid index (measure of admixture) between 0.4 and 0.7 towards Engelmann spruce, where the classes between 0.1 and 0.2 (putatively white spruce backcrosses) were relatively rare (4%) in the zone (Figure 4.7).

Hybrid index was correlated with elevation of origin in Mount Robson (R^2=0.47, P<0.0001), East Kootenay (R^2=0.42, P<0.0001), West Kootenay (R^2=0.19, P=0.013) and Quesnel (R^2=0.14, P<0.0001). It was also correlated with latitude in Mount Robson (R^2=0.34, P<0.0001) and East Kootenay (R^2=0.11, P=0.0175) and longitude in Mount Robson (R^2=0.32, P<0.0001) and Quesnel (R^2=0.25, P<0.0001). Hybrid index was also correlated with several climatic variables, including Mean Annual Temperature (MAT) in Mount Robson (R^2=0.50, P<0.0001), East Kootenay (R^2=0.24, P=0.0002) and Quesnel (R^2=0.19, P=0.0009); Annual Heat: Moisture Index (AHM) in Mount Robson (R^2=0.53, P<0.0001), Quesnel (R^2=0.24, P=0.0001) and East Kootenay (R^2=0.24, P=0.0003).

Despite high levels of introgression, both Engelmann spruce and white spruce are genetically well differentiated, as evidenced by the results from *STRUCTURE*, *TESS*, PCA and F_{st} analyses. *TESS* and *STRUCTURE* results showed separate clusters for Engelmann spruce and white spruce, with the hybrids showing a combination of both genomes (Figure 4.4). The Evanno (Evanno et al. 2005) test for *STRUCTURE* indicated there were two clusters clearly differentiated (Delta K showed a peak in K=2), each cluster representing one of the pure species (Figure A.14 in Appendix). This estimation of clusters was not evident in the *TESS* results. The DIC curve decreased and did not show a plateau until Kmax=10. Sometimes, the variability in DIC may lead *TESS* to select models in which Kmax is greater than K (Durand et

al., 2009). The PCA analysis, showed similar results but the hybrids formed a separate cluster from pure species (Figure A.15 in Appendix). The model formed by the first three principal components was significantly correlated with elevation (R^2=0.52, F=282.38, P<0.0001). The F_{st} analysis showed a higher genetic distance between pure species than among pure species and hybrids (0.188±0.02 vs 0.095±0.04 between white spruce and hybrids; and 0.08±0.02 between Engelmann spruce and hybrids).

Allele frequencies from the 86 SNPs assayed suggested that the majority of alleles (149 from 172) were shared between Engelmann spruce, white spruce and their hybrids (Table A.10 in Appendix). Of the alleles that were not shared by all of them, Engelmann spruce and hybrids shared 29 alleles; white spruce and hybrids shared 3 alleles; and 11 were found only in the hybrids. As was expected, hybrid populations had on average more alleles than pure species populations (169.4 vs. 142.0). All loci had higher than 5% minor allele frequencies.

4.3.3 Outlier loci and genomic clines

Seven of 311 SNP were identified as outliers in the first analysis including sympatric and parapatric populations within the hybrid zone. In the second analysis, which included all sympatric, allopatric and parapatric populations across the hybrid zone, seven outliers were identified from a total of 86 SNPs (Figure 4.8). From these, 2 loci (SNPs 17 and 62) showed deviations from neutrality in both set of analyses (within and across the hybrid zone). Therefore, a total of 12 different outlier loci were found.

The majority of outlier loci indicated diversifying selection; only 3 outlier loci indicated the presence of balancing selection as suggested by their low F_{st} and negative α values. As expected, outliers indicating diversifying selection showed lower F_{st} values within the contact zone than outliers across the hybrid zone. For example, outlier F_{st} estimates within the contact zone ranged from 0.08 to 0.18, whereas outliers F_{st} across the contact zone ranged from 0.21 to 0.46.

Loci suggesting diversifying selection were linked to various gene functions including signal transduction, transcription regulation and carbohydrate metabolism. Balancing selection loci were linked to phytohormone signaling, post-transcriptional regulation and protein turnover, and amino acid transportation (e.g., nitrogen intake from soil) (Table 4.2).

The genomic clines analysis indicated that overdominance and directional selection were responsible for observed deviations in genomic clines away from expectations under neutrality (Figure 4.9). Directional selection towards the Engelmann spruce allele was observed in loci 68 and 70, whereas selection towards the white spruce allele was present in locus 17. Overdominance was observed in loci 45 and 62. Loci 7 and 76 did not show a pattern consistent with overdominance or any other mode of selection; in the former the clines were flat, and in the latter the clines were absent (figures not shown). In locus 76, one allele is fixed in all white spruce and Engelmann spruce populations, and the other allele is close to fixation in all the hybrid populations (frequencies from 0.726 to 0.837), with the exception of Prince George (Table A.10 in Appendix).

4.4 Discussion

4.4.1 Evolutionary history of white spruce and Engelmann spruce

The evolutionary histories of white spruce and Engelmann spruce are intimately related. According to this study, these species most recently had the potential to come into secondary contact by 21,000 YBP, earlier than previously thought (Ledig et al. 2006), in the southern Rocky Mountains in Colorado and Wyoming, considerably south and east of the current hybrid zone. During the last Glacial Maximum, white spruce and Engelmann spruce populations were pushed south of the present distribution, with the exception of scattered relicts (Nienstaedt and Zasada 1990). White spruce appears to have survived the Last Glacial Maximum in two refugia, the unglaciated Yukon Valley, Alaska, north from the ice front, and the Appalachian Mountains Wisconsin refugium (Anderson et al. 2006). After the last glaciation, by 11,000 YBP, white spruce was distributed as far north as Alaska and had re-established its transcontinental

range. While the white spruce range expanded, the Engelmann spruce range likely contracted. This chapter's results indicate that Engelmann spruce range may have become fragmented at the southern part of the Rocky Mountains by 11,000 YBP. This is coincident with previous reports that suggest Engelmann spruce populations, which were more abundant and occupied lower elevations in the Rocky Mountains and the Great Basin during the Pleistocene, became fragmented when the temperature rose during the Xerothermic Period (10,000-7,000 years B.P) (Hamrick et al. 1994, Ledig et al. 2006),

As a result of white spruce and Engelmann spruce range expansions and contractions, it appears likely that the hybrid zone moved west and then north until reaching British Columbia by 14,000 YBP after the recession of the Cordilleran Ice Sheet. Our estimates of timing of re-colonization of British Columbia by white spruce and Engelmann spruce based on climate modelling is coincident with previous reports (Ledig et al. 2006; Pellatt et al. 2001). After 14,000 YBP the hybrid zone expanded its range in latitude and longitude until reach its current northern and eastern expanse.

Although the climate niche modelling and palaeoclimatic analysis used in this study contributes to a deeper understanding of the evolutionary history of white spruce and Engelmann spruce and the early dynamics in hybrid zone formation, it also had some limitations. For example, this study did not identify a white spruce refugium in Alaska. Both the CCM1 and the GFDL model indicated the presence of Engelmann spruce rather than white spruce in Alaska between 21,000 and 14,000 YBP. This inconsistency with previous genetic reports of a white spruce refugium (Anderson et al. 2006) may be due to fewer data points and lower resolution in the Alaska ecoregion in comparison with other ecoregions; or due to white spruce ecotypes in Alaska that had a different climatic niche than those elsewhere in the species range (Aitken et al. 2008). It is important to mention that, because the models were based on ecoregions, they were not that flexible to community reorganization. Therefore any no-analogue climates in the past may have served to confound the model (Roberts and Hamann 2011). Furthermore, the models used only predicted "climate habitat" for species,

which means that even though there appeared to be a suitable climate available in a given location, there is a chance that the species may not have reached those locations at that time.

4.4.2 Extension and direction of introgression

Extensive admixture and introgression was observed in the contact zone, with most of the alleles being shared by white spruce, Engelmann spruce and their hybrids. In general, individuals from below 600 m elevation are largely white spruce, and those above 1800 m elevation are Engelmann spruce. The vast majority of genetic resources from this zone showed an elevational cline in admixture corresponding to climatic gradients in temperature and precipitation as well. Despite extensive admixture and introgression, both pure species remained well differentiated from each other, as suggested by the *STRUCTURE, TESS*, F_{st} and PCA results. These results are coincident with previous recent studies using neutral markers (Chapter 2).

Results of the genomic analysis indicated a strong asymmetry in introgression suggesting that hybrid individuals backcross primarily to Engelmann spruce and only rarely to white spruce. This asymmetry is more noticeable along elevational gradients (within the contact zone) than along latitudinal gradients (across the hybrid zone) (Figure 4.5). The steep cline with latitude is probably indicating strong selection acting across the hybrid zone, whereas a wider gradient in admixture along elevation may indicate higher dispersal within the contact area.

The asymmetry in introgression in the white spruce x Engelmann spruce hybrid zone may be caused by one or more of several factors: reproductive barriers, cline movement, demographic processes during secondary contact, or density differentials between pure species. Within possible pre-zygotic barriers, temporal isolation seems to be the most logical explanation for asymmetry. Temporal isolation has been identified as the cause of asymmetrical introgression in several genera including *Populus* (Lexer et al. 2005), *Iris* (Martin et al. 2007) and *Quercus* (Bacilieri et al. 1996). White spruce and Engelmann spruce are monoecious, having female and male strobili in the same individuals. Previous studies have

indicated differences in vegetative phenology between white spruce and Engelmann spruce, with Engelmann spruce having earlier bud burst and bud set timing than white spruce in the same environment (Chapter 3). Although there is no data for reproductive phenology, it is likely that it follows the same pattern (both vegetative and reproductive phenology are correlated in several temperate tree species). This difference in reproductive phenology may mean that when Engelmann spruce pollen is shed, white spruce female strobili in the same environment are not receptive. However, female strobili of hybrids are likely receptive before white spruce, giving the hybrids a higher chance to be fertilized by Engelmann rather than by white spruce. This hypothesis, however, does not explain how the gene flow moves from high to low elevations.

There may also be some postzygotic barriers such as intrinsic postzygotic isolation. The reduced number of apparent backcrosses to white spruce, in comparison with the other hybrid classes, may indicate the presence of epistatic interactions beyond the F1 generation of hybridization. Artificial crosses would be required to test this possibility.

Another possible explanation for the asymmetry in introgression is hybrid zone movement. According to hybrid zone theory, hybrid zones will move to minimize their length towards regions of low density, in the direction of dispersal (Barton and Hewitt 1985). In the bounded hybrid superiority model (Moore 1977), the hybrid zone is stable because the fitness of the hybrids is “bounded” in environments intermediate to that occupy by the parental taxa, assuming environments are stable (Buggs 2007). However, if the fitness of the hybrids is not bounded, and one of the parental species has lower fitness than the other one, hybrids will extend their range towards one of the parental species. Repeated and more frequent backcrossing between the hybrids and that parental form will produce advanced generation hybrids that resemble one of the parental species. This would produce asymmetry in introgression as a consequence of hybrid zone movement. This hypothesis would explain why there is a small number of white spruce backcrosses in the zone, and would suggest that the hybrid zone is moving towards white spruce. However, given that white spruce, Engelmann

spruce and their hybrids show strong adaptations to their own habitats (Chapter 3), it is likely that the fitness of the hybrids is "bounded" to intermediate environments and therefore the hybrid zone is stable. This "stability" however may have only been achieved in the last 10,000 years, as our results indicate that the hybrid zone had in fact moved in its early history, as a result of range expansion and contraction of white spruce and Engelmann spruce in a changing climate.

Demographic processes during range expansion and contraction can also cause asymmetric introgression (Excoffier and Petit 2009). If a species expands its range into another species range, and the two species hybridize, this would produce a moving hybrid zone during the expansion (Buggs 2007). Simulation studies have shown that if the hybrids have moderate or high fitness, the local species will have little sign of introgression, whereas the invader species will be massively introgressed by genes of the local species (Currat and Excoffier 2004, Currat et al. 2008). This holds true even if there are differences in population density between the pure species and the invading species, with the latter having a higher density. In this scenario, asymmetric introgression seems to be the rule rather than the exception. Only when introgression is rare are the patterns expected to be symmetrical (Currat et al. 2008). In this study, although it is clear that the hybrid zone has moved in its early formation as a result of past and post-glacial climate changes and resulting white spruce and Engelmann spruce range shifts, it is unclear whether demographic processes have had an effect on the current levels of admixture. Our models suggest Engelmann spruce range has not changed its distribution much in the past 21,000 years. White spruce, on the other hand, greatly expanded its range after the recession of the Cordilleran Ice Sheet, recovering its transcontinental range across Canada. In this scenario, we would expect white spruce (the invading species) to be massively introgressed by Engelmann spruce genes (the local species). This, however, has not been seen for the white spruce x Engelmann spruce hybrid zone, in which both species have maintained their species integrity within their climatic niches. It may have happened, however, that because the hybrids are fit in intermediate environments and the pure species inhabit

different environments that the direction of introgression was from Engelmann to hybrids instead of Engelmann to white spruce. This would explain the massive introgression of Engelmann spruce alleles found in the hybrids. These however are just hypothesis that would need further verification.

Finally, differences in population density may also be the cause of asymmetric introgression, as was observed in species of *Morus* (Burgess et al. 2005). Previous studies using neutral markers have found that Engelmann spruce likely occurs at a higher frequency than white spruce in the hybrid zone (Chapter 2). This would mean that hybrids would have more opportunities to mate with Engelmann spruce than with pure white spruce just because Engelmann spruce is more abundant in the area. This is the simplest explanation for the observed patterns.

4.4.3 Overdominance and directional selection in the hybrid zone

Results of the F_{st} outlier loci and genomic clines analyses revealed a small percentage of candidate loci (8.1% when analyzed across the hybrid zone including allopatric populations, and 2.2% within the hybrid zone) that deviate from selective neutrality. Overdominance and directional selection were found to be the main factors responsible for shifting genomic clines away from expectations under neutrality.

Overdominance has been suggested as an explanation for increased fitness in hybrids (Rieseberg and Carney 1998; Rieseberg et al. 1999). Caused by the non-additivity of allelic effects within a locus, overdominance seems to be more important in first-generation hybrids (heterosis) than in advanced-generation crosses (transgressive segregation) (Rieseberg and Carney 1998; Rieseberg et al. 1999). In this study, evidence of overdominance was found for loci 45 and 62, suggesting that these loci may contribute to the increased hybrid fitness previously reported in the hybrid zone (Chapter 3). Although locus 76 did not show a pattern consistent with overdominance or any other mode of selection, it is possible that this locus may

also contribute to increased hybrid fitness. In locus 76, one allele has reached fixation in both parental species, whereas the alternative allele is close to fixation in the hybrids.

Directional selection towards Engelmann spruce was observed for loci 68 and 70 (although the genomic cline was not significant for the latter), whereas selection towards white spruce was detected for locus 17. Directional selection may be an indication of adaptive introgression from one parental species to the hybrids. Adaptive introgression has been suggested to be an important process in promoting adaptive differences between white spruce, Engelmann spruce and their hybrids (Chapter 3). It is important to note that any interpretation of deviations of neutrality given in terms of candidate genes in this work is premature and needs to be validated with genome-wide marker scans.

4.4.4 Functional roles of genes exhibiting outlier behavior

Loci suggesting diversifying selection were linked to signal transduction, transcription regulation and carbohydrate metabolism, important gene functions in adaptation to climate. Immunophilins (SNP 17) are intracellular receptors for the immunosupressants FK506 and rapamycin, which inhibit different signaling pathways required for T-cell activation (Luan et al. 1996). Studies in *Arabidopsis* and rice have suggested that FK506 homologs are encoded by a small gene family in higher plants (Luan et al. 1996). Leaf-specific immunophilins are regulated by light in fava beans (Luan et al. 1994), suggesting immunophilins may play an important role in plants light signal transduction.

No-apical meristem (Nam) genes (SNP 62) are part of the NAC domain proteins, which are plant-specific transcriptional factors known to play important role in plant developmental processes (Hu et al. 2010). NAC transcription factors comprise a large gene family in Arabidopsis, rice and soybeans (Hu et al. 2010). It is been suggested that NAC genes may also play an important role in wood formation and secondary cell wall biosynthesis in *Populus trichocarpa* (Hu et al. 2010).

Carbohydrates play an important role in protecting cellular membranes from freezing injury by reducing osmotic potential across the membranes and by stabilizing them (Bryant et al. 2001, Holliday 2009). Triggered by the cessation of growth and the onset of dormancy, carbohydrate metabolism is modified towards the accumulation of storage compounds, cryoprotective or dehydration-protective solutes and reductive co-factors, increasing cold hardiness in spruce (Dauwe et al. 2012). In this study, I have found two loci with functions related to carbohydrate metabolism, SNP 68 (alpha-amylase) and SNP 76 (glycosyl hydrolase). Sugar content and cold hardiness are positively correlated in several tree species as Scots pine (*Pinus silvestris*), lodgepole pine (*Pinus contorta*), Norway spruce (*Picea abies*) and red spruce (*Picea rubens*) (Dehayes 1992; Ogren et al. 1997).

4.4.5 Genomic islands and the maintenance of species boundaries in spruce

This study contributes to the long-standing debate on the maintenance of white spruce and Engelmann spruce species identities despite hybridization, by viewing the evolutionary relationships between these two species based on the genic view of speciation (Wu 2001). I believe white spruce and Engelmann spruce have highly porous genomes, in which a small number of genes under selection counteract the homogenizing effects of gene flow and prevent introgression in surrounding regions, maintaining species differences despite extensive interspecific gene flow.

This pattern of maintenance of species integrity between hybridizing species has been reported for several plant species, challenging the view that species differentiation is a genome-wide phenomenon. Yatabe et al. (2007) found that species differences between hybridizing species *Helianthus annuus* and *H. petiolaris* were maintained despite extensive interspecific gene flow. Minder and Widmer (2008) found a small percentage of outlier loci between closely related species *Silene latifolia* and *S. dioica*, and concluded that differentiation between species had been shaped by a combination of introgression and selection. Scotti-Saintagne et al. (2004) using a genome scan found 12% of outlier loci from a total of 389

markers between closely related species of oaks (*Quercus robur* and *Q. petraea*), suggesting the existence of porous genomes.

White spruce and Engelmann spruce have a long history of interspecific gene flow, which may date to more than 21,000 years ago. The hybrid zone appears to have moved as a result of climatic changes and resulting white spruce and Engelmann spruce range expansions and contractions to finally reach its present location in British Columbia and Alberta by 10,000 YBP. After this time, the hybrid zone likely expanded its area in latitude and longitude to reach its present area. Although the amount of gene flow between species during past and post-glacial times is unknown, it is known that the current levels of admixture and introgression, as suggested by the genomic analysis, are high.

When analyzing allele frequency differentials between white spruce, Engelmann spruce and hybrids, I found that most of the alleles freely cross species barriers, whereas a small number of them are only shared by one parental species and their hybrids, accumulating divergence between species. A subset of these loci apparently impermeable to introgression between species were identified in the outlier analysis, suggesting they are under selection and in relation to adaptive differences between white spruce, Engelmann spruce and their hybrids. There are likely many more such loci across the genome as this study included a relatively small number of candidate loci.

These outlier loci point to some important gene functions in adaptation to winter regimes. According to results in Chapter 3, both parental species and their hybrids are adapted to different environments along elevational gradients, in which the key factor for survival is the adaptation to the length of the growing seasons and the depth of the snowpack. Selection acting in some of the genes has favored the hybrids in intermediate environments, preventing the swamping of parental species (Chapter 3).

The use of SNPs from candidate genes facilitates finding some of the loci involved in adaptation across this hybrid zone and had allowed me to identify gene functions and relate them with my previous results from the study of adaptive differences between white spruce,

Engelmann spruce and their hybrids. However, because these SNP were not randomly chosen and because their location in the chromosomes is unknown (although linkage maps are close to completion), the amount of the genome that was covered by this study remains obscure. Future genome-wide studies including linkage and genetic maps will contribute with a wider perspective of this topic.

4.5 Conclusions

Results of this chapter suggest that the closely related and hybridizing species white spruce and Engelmann spruce have highly porous genomes, in which a small number of genes under selection may counteract the homogenizing effects of gene flow and prevent introgression in surrounding regions, maintaining species differences despite extensive interspecific gene flow potentially over tens of thousands of years. Several outlier loci with patterns suggesting diversifying or balancing selection were identified. Outlier loci suggesting diversifying selection were linked to signal transduction, transcription regulation and carbohydrate metabolism; which are important gene functions in adaptation to climate. These loci likely represent a fraction of the genes responsible for the adaptive differences between white spruce, Engelmann spruce and their hybrids found in this study. Outlier loci suggesting balancing selection were linked to phytohormone signaling, post-transcriptional regulation and protein turnover, and amino acid transportation.

Table 4.1 Geographical coordinates and climatic variables of parent trees for *Picea glauca, P. engelmanni* and their hybrids analyzed with 311 SNP loci. Two-letter codes are used to identify populations in subsequent tables and graphs.

Population	Province	Elevation range (m)	Latitude (degrees)	Longitude (degrees)	MAT (°C)	MAP (mm)	Sample size
Picea glauca							
Fort Nelson (FN)*	B.C	350-600	58.4-59.4	120.5-126.3	0.3	509	22
P.glauca x P.engelmannii							
Prince George (PG)	B.C	610-793	53.5-54	121.6-122	2.8	769	11
Quesnel Lakes (QL)	B.C	680-1555	51.8-53.2	119.4-122.1	2.3	914	220
Mount Robson (MR)	B.C	701-1525	52.2-53.8	118.4-121.5	1.7	1167	197
East Kootenay (EK)	B.C	1006-1677	49.4-50.8	115.1-116.6	1.9	944	204
West Kootenay(WK)	B.C	690-1966	49-50.5	114.9-118.4	2.5	1168	124
Picea engelmannii							
Salmon River (E1)*	Idaho	1859-2530	43.8-46.2	113.7-115.9	2.8	1028	9
Teton-Wasatch (E2)*	Wyo.	2347-3048	40.4-43.8	109.5-111.6	2.6	885	13
Fishlake-Lasal (E3)*	Col.	2606-3383	37.5-39.8	109.2-112.8	3.4	768	18

Notes: MAT= Mean annual Temperature; MAP=Mean Annual Precipitation; B.C= British Columbia; Wyo.=Wyoming; Col=Colorado; *parental populations used to estimate hybrid index.

Table 4.2 Significant SNP outliers detected using A) graph on top: 311 SNP loci within the contact zone (only sympatric populations); and B) graph at bottom: 86 SNP loci across the hybrid zone (sympatric and allopatric populations) with *BayeScan*. A positive value of α suggests diversifying selection, and a negative value, balancing selection. Cut-off for Bayesian posterior probability (prob(α≠0)) was 0.7. This probability cannot be compared with traditional p-values. SNP ID identifies SNPs in Figure 4.7.

SNP ID	SNP name	prob(α≠0)	log (PO)	α	Fst	BLASTX vs Arabidopsis	position	type	gene function
17	13_496_NS	0.96779	1.4778	1.29	0.10417	immunophilin	496	NS	signal transduction
56	208pg12875c	1	1	1.9987	0.1767				
62	295_78_S	0.89898	0.94934	1.045	0.084892	no-apical meristem	78	S	transcription regulation
129	C2270-contig1.NC1-384	0.79056	0.57687	0.94254	0.080339				
146	C6522-contig1.NC1-269	0.96999	1.5096	1.317	0.10647				
200	WS-2.0-GQ0024.B3.r-D12.1-239	0.989	1.9537	1.2692	0.10083				
240	WS-2.0-GQ0064.B3.r-I13.1-1236	0.9806	1.7036	1.2661	0.10062				

SNP ID	SNP name	prob(α≠0)	log (PO)	α	Fst	BLASTX vs Arabidopsis	position	type	gene function
7	124_495_S	0.70594	0.38033	-1.076	0.053519	auxin efflux carrier	495	S	phytohormone signaling
17	13_496_NS	1	1	1.6777	0.29291	immunophilin	496	NS	signal transduction
45	234_171_S	0.70314	0.37449	-0.98515	0.055809	AN1-like zinc finger	171	S	post-transciptional regulation and protein turnover
62	295_78_S	0.78496	0.56232	0.87226	0.19316	no-apical meristem	78	S	transcription regulation
68	45_1067_NS	0.976	1.6091	1.0779	0.21397	alpha-amylase	1067	NS	carbohydrate metabolism
70	50_135_S	0.86517	0.80733	-1.2689	0.043362	aminoacid permease	135	S	aminoacid transportation (nitrogen intake)
76	68_286_S	1	1	2.7706	0.4658	glycosyl hydrolase	286	S	carbohydrate metabolism

Figure 4.1 Geographic locations for 9 populations of *Picea glauca* (FN), *P. engelmannii* (E1, E2 and E3), and their hybrids (all other populations). Population names corresponding to two-letter codes can be found in Table 4.1.

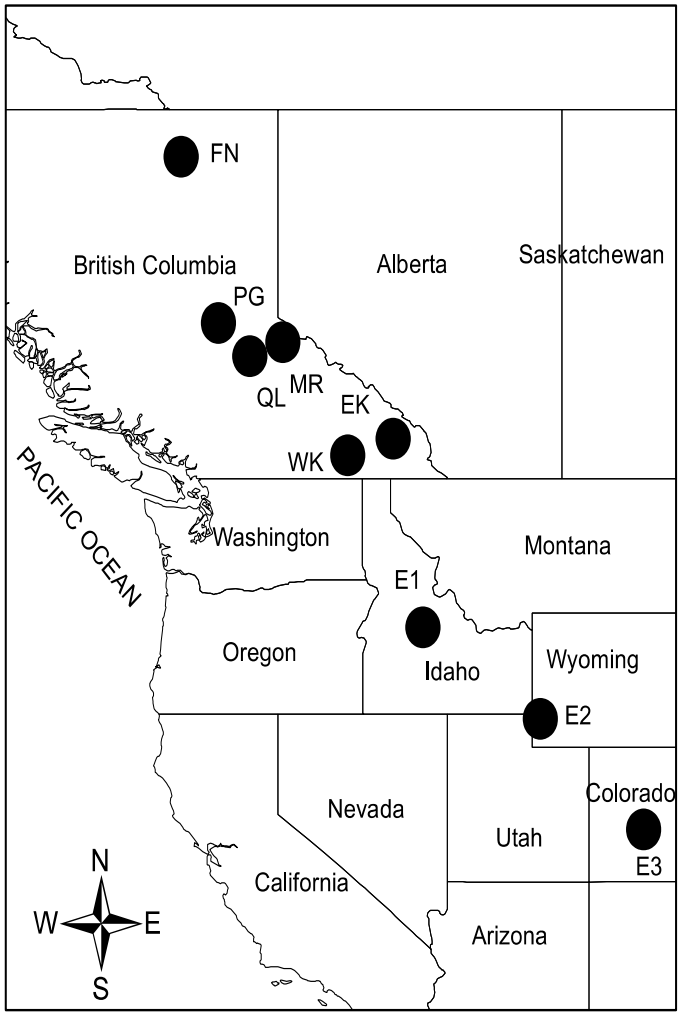


Figure 4.2 Inferred history of *Picea glauca*, *P. engelmannii* and their hybrids from 21,000 to 14,000 years before present based on climate niche modelling (CCM1 model) and palaeoclimate data. Years before present are in bold at the top of the graph. *P. engelmannii* is in green, *P. glauca* is in blue and the hybrid zone is in brown.

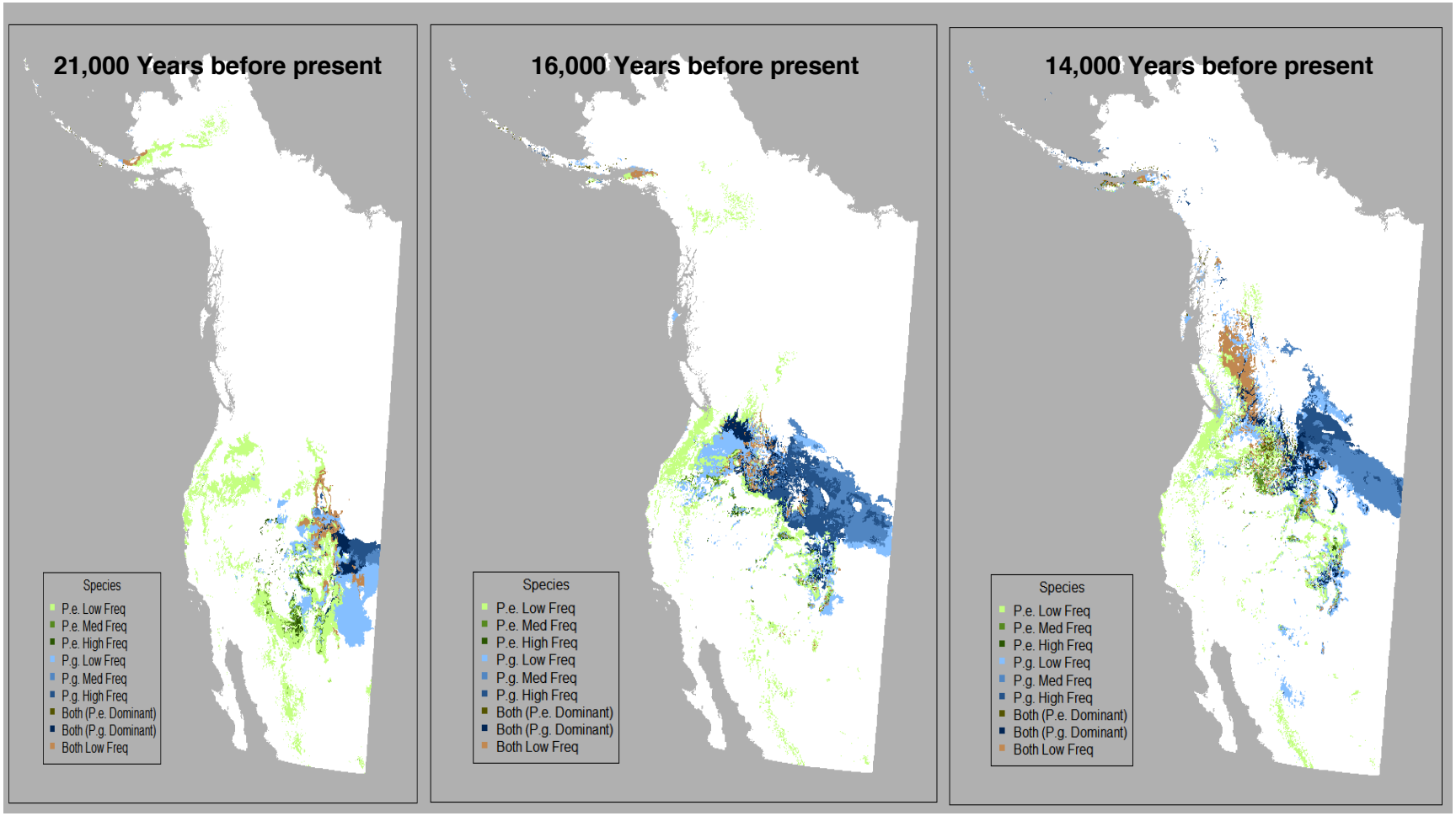


Figure 4.3 Post-glacial evolutionary history of *Picea glauca*, *P. engelmannii* and their hybrids based on climate niche modelling (CCM1 model) and palaeoclimate data. Years before present are in bold at the top of the graph. *P. engelmannii* is in green, *P. glauca* is in blue and the hybrid zone is in brown.

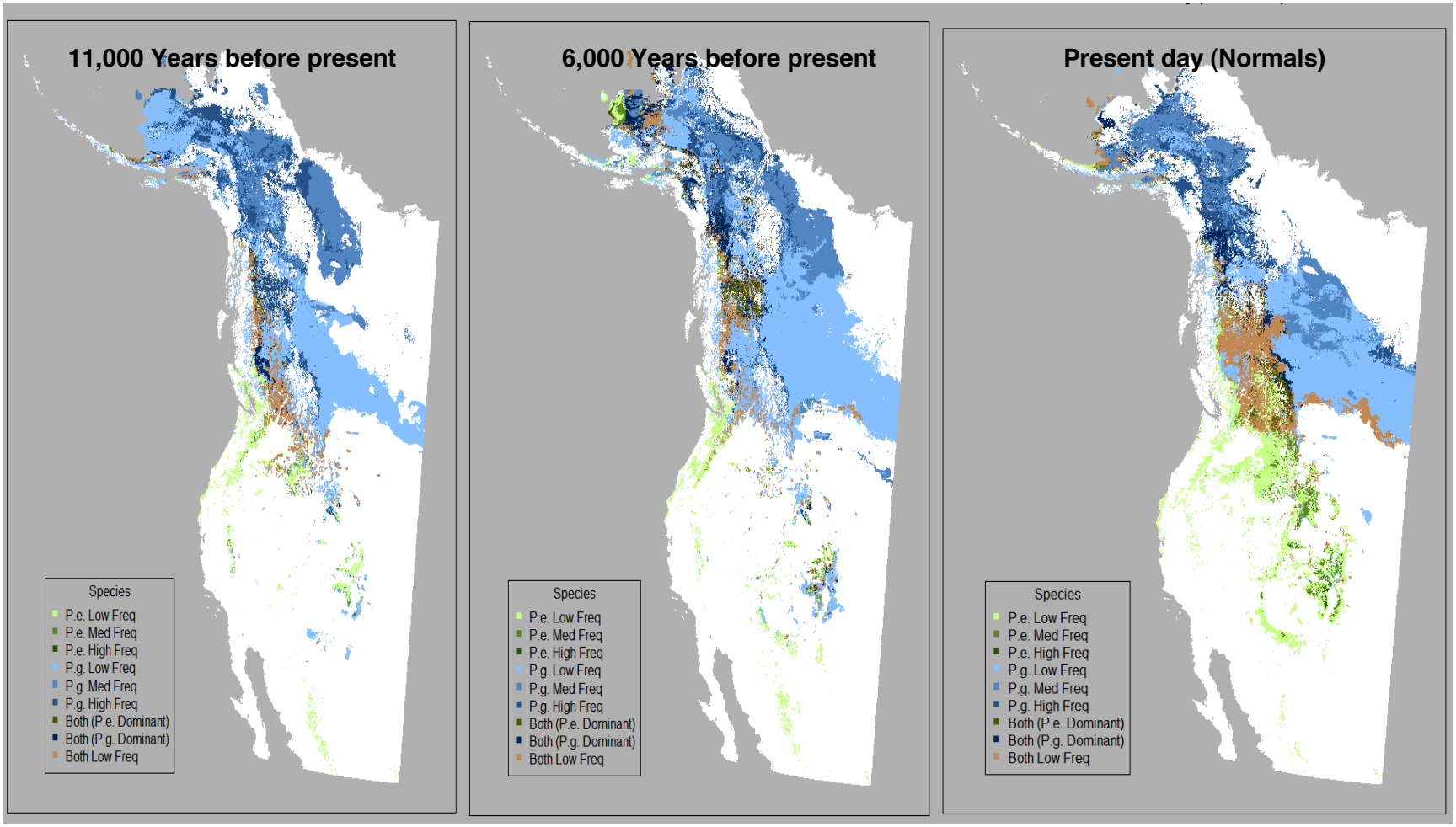
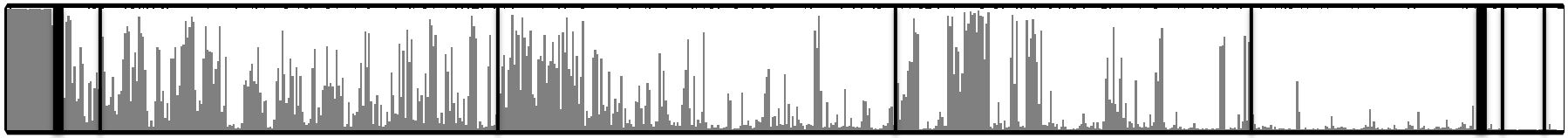


Figure 4.4 Posterior estimates of cluster membership for the *Picea glauca* x *P. engelmannii* hybrid zone with *TESS* for K=2. *P. engelmannii* is represented by white and *P. glauca* by grey. Populations are ordered by decreasing latitude from left to right. Population names corresponding to two-letter codes can be found in Table 4.1. Wide vertical black lines separate the *P. glauca* population (FN), hybrids (PG, QL, MR, EK and WK) and *P. engelmannii* populations (E1, E2, E3).



FN PG QL MR EK WK E1 E2 E3

Figure 4.5 Hybrid index plotted against A) interspecific heterozygosity; B) elevation; and C) latitude for *Picea glauca x P. engelmannii* individuals and 86 SNP loci. Circles represent individuals. Hybrid index ranges from 0 (pure *P. glauca*) to 1 (pure *P. engelmannii*).

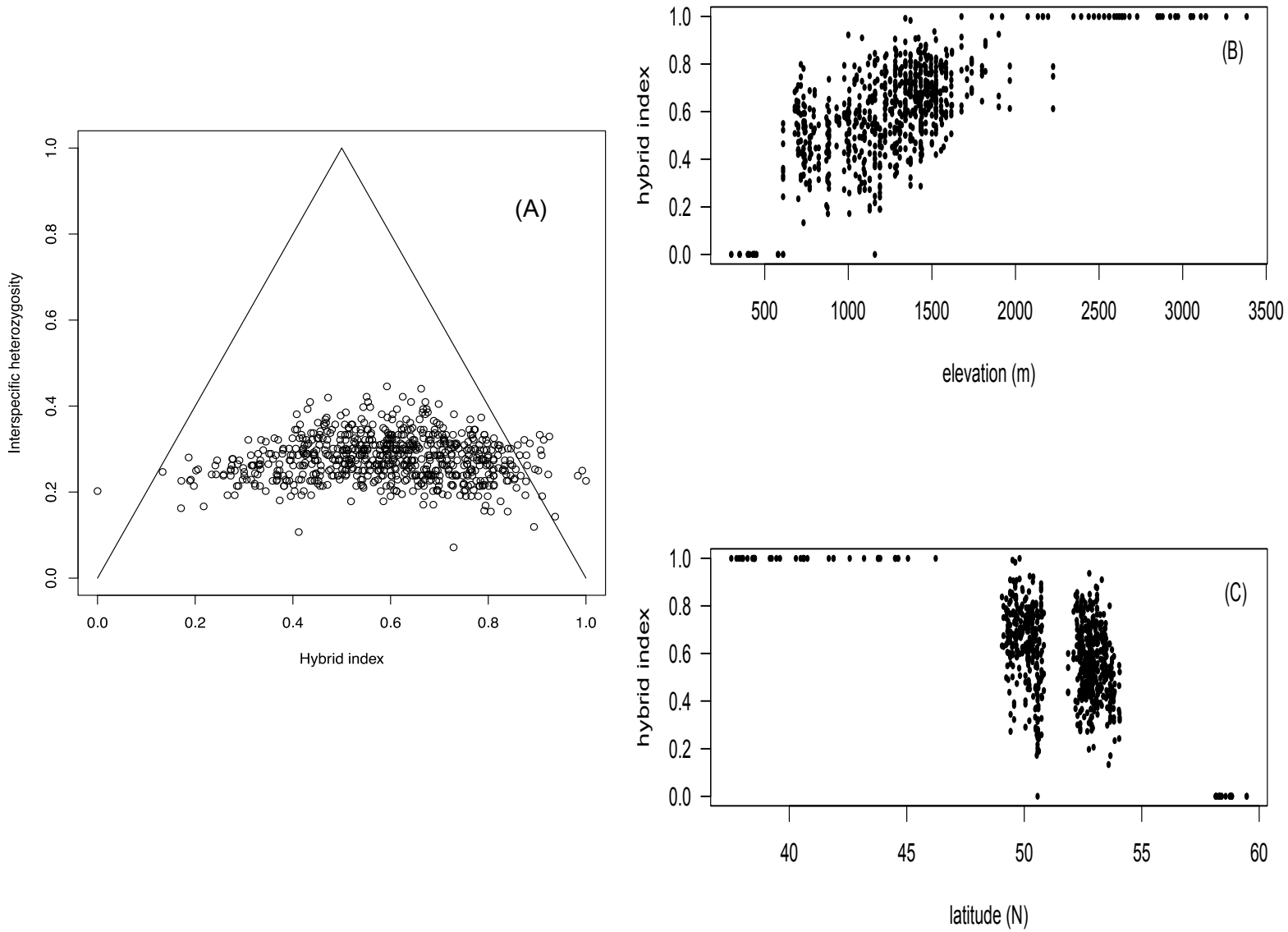


Figure 4.6 Patterns of introgression in the *Picea glauca* x *P. engelmannii* hybrid zone for 86 SNP loci. Each rectangle denotes an individual's genotype at a given locus: light green (A_1A_1= *P. glauca*), green (A_1A_2= hybrids) and dark green (A_2A_2= *P. engelmannii*). On the right is a plot of hybrid index (proportion of *P. engelmannii* ancestry, where "0" is pure *P. glauca* and "1" is pure *P. engelmannii*).

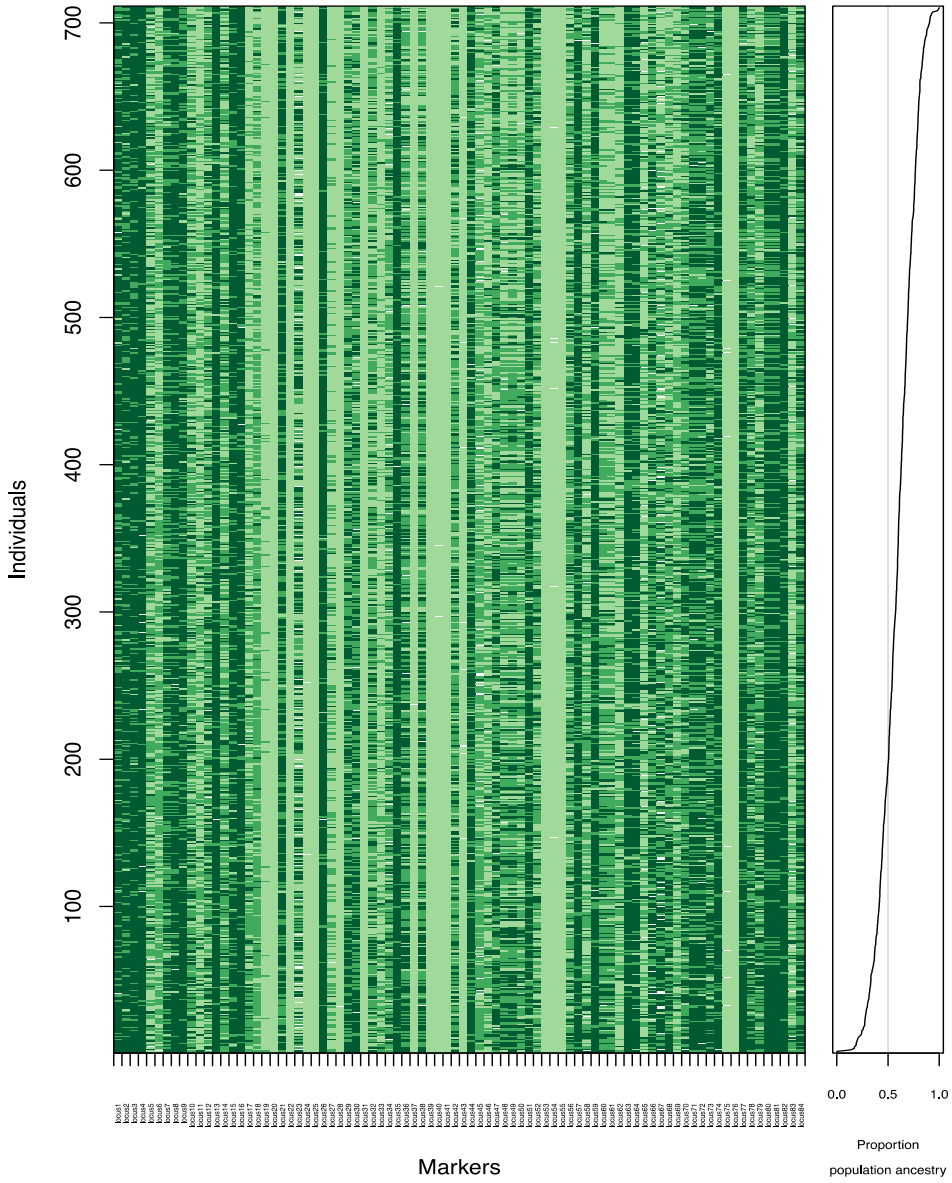


Figure 4.7. Hybrid classes in the *Picea glauca* x *P. engelmannii* hybrid zone based on 86 single nucleotide polymorphism markers. Individual hybrid index was divided into 11 classes with an interval of 0.1, where 0 is pure *P. glauca* and 1 is pure *P. engelmannii*.

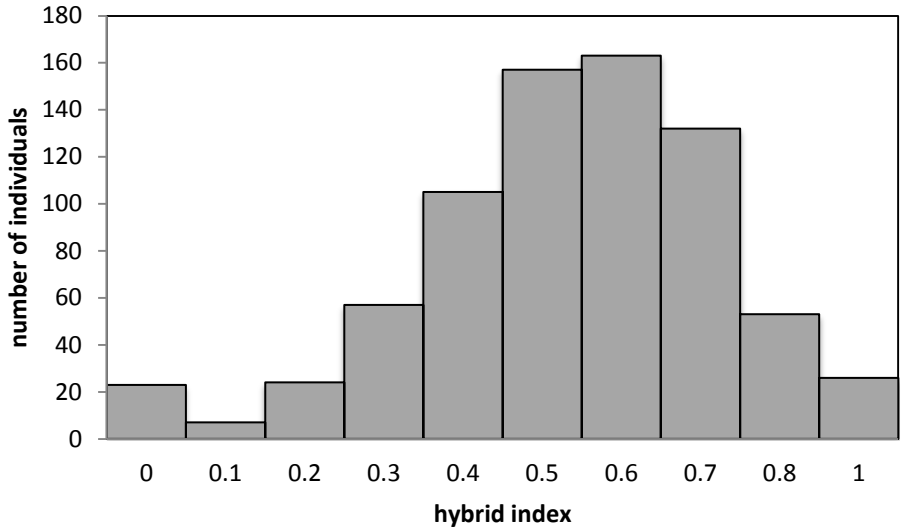


Figure 4.8 Results of Bayesian outlier detection analysis A) within contact zone using 311 SNP loci; and B) across hybrid zone, using 86 SNP loci. Estimate of F_{st} plotted against transformed p-values, where PO=p/(1-p). Loci (full circles) at the right of the vertical line showed significant deviations from neutrality.

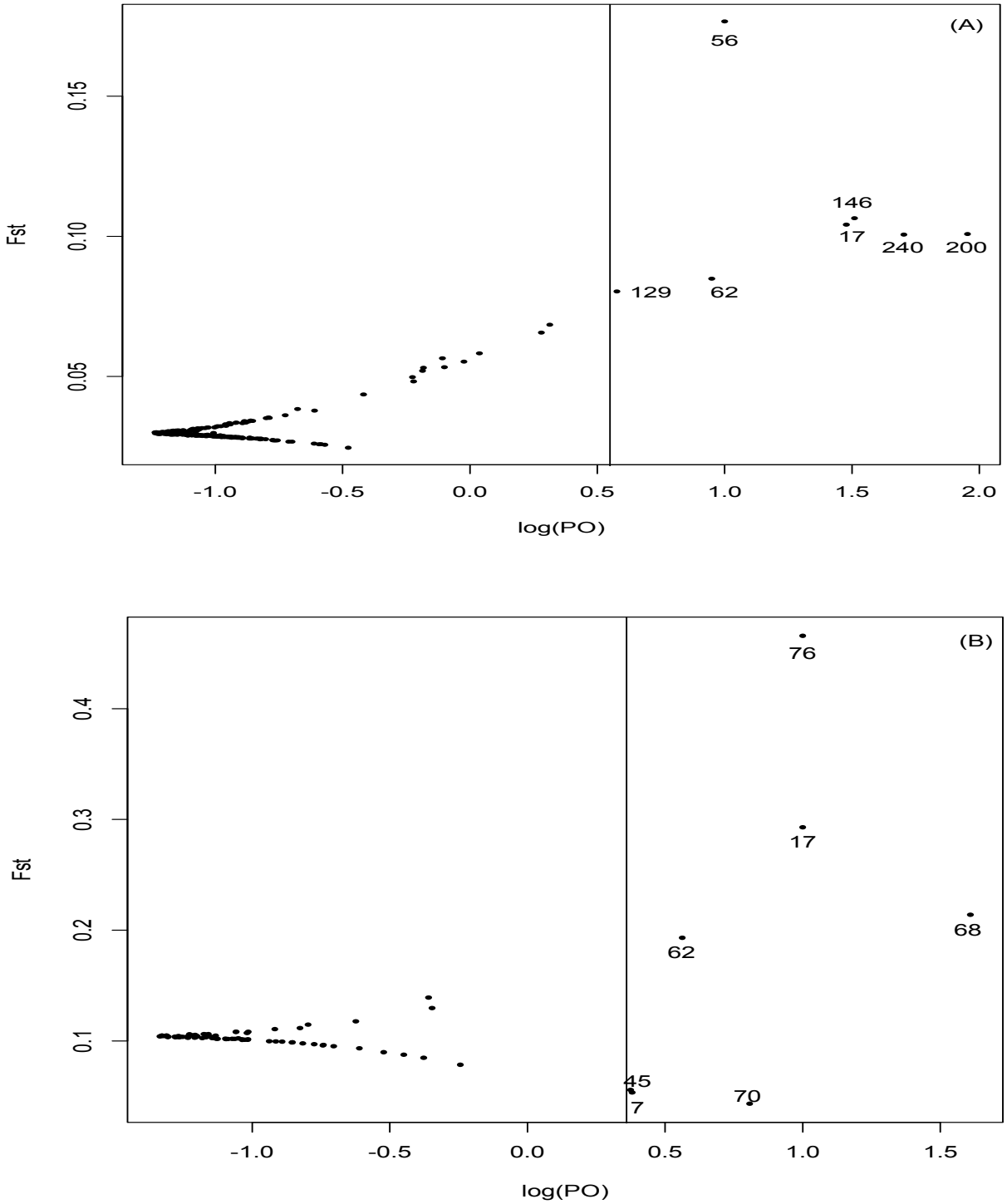
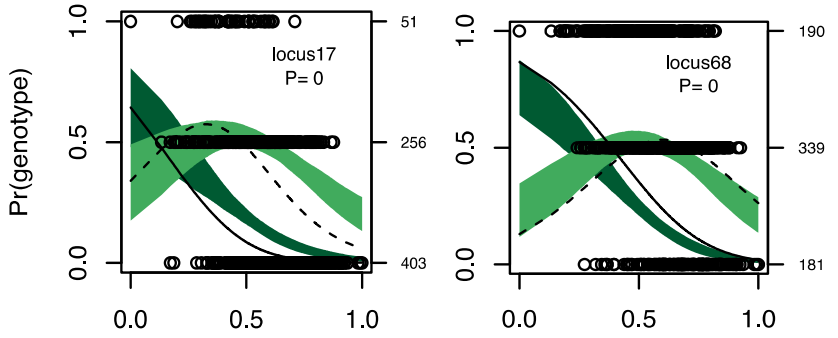
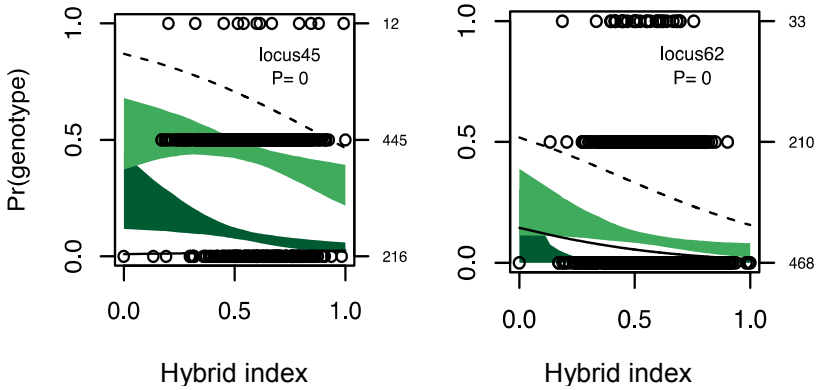


Figure 4.9 Genomic clines of the loci that deviated from neutrality in the *Picea glauca* X *P. engelmannii* hybrid zone and suggested modes of selection, based on 86 SNP. Solid coloured regions represent the 95% confidence intervals for A_1A_1 (dark green) and A_1A_2 (light green) genomic clines given neutral introgression. The solid and dashed lines denote fitted genomic clines from observed genotype frequencies for A_1A_1 and A_1A_2 genotypes respectively. Empty circles indicate the raw genotype data, with counts of individuals on the right vertical axis. Hybrid index indicates the fraction of alleles derived from *P. engelmannii* population, where "0" is pure *P. glauca*, and "1" is pure *P. engelmannii*.

Directional selection



Overdominance



5. Thesis conclusions

5.1 Introduction

Given the economic importance of these species, there is growing concern about how the genetic resources are managed in the interior spruce hybrid zone. By treating the pure species and their hybrids as the same species, the effects of artificial selection on the genetic resources of the zone cannot be predicted. This adds more uncertainty to the future of interior spruce in a time when forest breeders are keen to take actions that may mitigate the effects of climate warming on managed forests. Conservation efforts of natural forests are also limited because of the lack of information about past and current evolutionary relationships between white spruce and Engelmann spruce.

This study represents a significant step forward in our understanding of the evolutionary relationships between closely related white spruce and Engelmann spruce and on the genomic basis of local adaptation to climate in conifers. By doing this, I have contributed valuable information for current and future management of this species complex in British Columbia.

5.2 Population structure and levels of admixture and introgression

Both the SSR (chapter 2) and the SNP (chapters 3 and 4) results indicate that the hybrid zone between white spruce and Engelmann spruce follows elevational and latitudinal clines in admixture corresponding to climatic gradients in temperature and precipitation; in which Engelmann spruce occurs at high elevations (>1800 m), white spruce occurs at low elevations (>600 m) and hybrids occupy the intermediate environments. Admixture and introgression in the hybrid zone are extensive, with southern populations having, on average, a higher Engelmann genetic contribution; and populations in the north having, on average, similar Engelmann and white spruce genetic contributions.

Despite extensive introgression between the species, Engelmann spruce and white spruce have maintained their species integrity, according to both the SSR and the SNP results.

Hybrids show, on average, a higher genetic contribution from Engelmann spruce indicating that introgression is asymmetric towards this species. Although the reasons behind this asymmetry are unknown, some possible explanations include temporal isolation (differences in phenology), hybrid zone movement, demographic processes during secondary contact, or population density differentials between pure species.

Results of the paleoclimatic analysis suggest that the hybrid zone is not of recent origin. White spruce and Engelmann spruce came into secondary contact as early as 21,000 years ago. This is consistent with the results of the genetic and genomic analyses, which indicate that the hybrid zone is mainly formed by advanced generation hybrids. As a result of range expansions and contractions (mainly from white spruce), my results suggest that the hybrid zone has moved from its unglaciated refugium in the southern region of the Rocky Mountains to its current distribution in British Columbia and Alberta. After 14,000 YBP the hybrid zone had the potential to expand its range in latitude and longitude until reach its current broad area in British Columbia and the western part of Alberta. This hybrid zone represents one of the biggest and more economically important hybrid zones studied to date.

5.3 Exogenous selection and adaptation as mechanisms of maintenance of the hybrid zone

Results of this study indicate that the hybrid zone between white spruce and Engelmann spruce is maintained by selection gradients due to environmental heterogeneity (environment dependent model of hybrid zone maintenance) (Endler 1973, 1977; Slatkin 1973; Moore 1977; Harrison 1986). This model assumes exogenous selection, which means that the hybrid fitness varies with the environment. Hybrid individuals are fitter than either parental species in environments that are intermediate to the parental habitats (intermediate elevation) but are less fit than parental species in their respective native habitats (low and high elevation), which suggests the hybrid zone follows a bounded hybrid superiority model (Moore 1977).

White spruce, Engelmann spruce and their hybrids are all fitter in their native environments than the others, as a result of different adaptations to the length of the growing season and the persistence of the snowpack. White spruce is predominant at low elevations due to its adaptation to a longer growing season and its faster growth than Engelmann and the hybrids. Engelmann spruce is adapted to a shorter growing season and deeper, more persistent snowpacks, which allows it to survive in high-elevation environments. Hybrids grow faster than Engelmann spruce and are cold hardier than white spruce as a result of adaptive introgression from pure species. This combination of phenotypic traits allows the hybrids to survive in intermediate environments.

Results of the climatic analysis also indicate the importance of environmental gradients (climate) in the adaptation of pure species and hybrids, and in the maintenance of the hybrid zone. Precipitation as snow (PAS) is the most important variable shaping the levels of admixture in the white spruce x Engelmann spruce zone (PAS explained 46% of the total variation in hybrid index), and is likely to be the main factor limiting white spruce survival in Engelmann's habitats. A larger value of PAS mainly reflects a deeper winter snowpack and shorter growing season. Summer Heat: Moisture Index (SHM) is the second most important variable defining hybrid index. SHM is a measure of aridity in an area and is negatively correlated with hybrid index, which is consistent with the well-established view in forestry that Engelmann spruce is adapted to cooler, more humid environments than white spruce.

5.4 Maintenance of species boundaries

Results of this study suggest that closely related and hybridizing species white spruce and Engelmann spruce have highly porous genomes. As the results of the SSR and SNP analysis suggest, white spruce and Engelmann spruce share the majority of alleles, which means that most of their genomes are permeable to introgression between species. Only a small number of genes are shared by one pure species and hybrids, and this group of genes is

responsible for differences between species. Some of these genes are under selection, as suggested by the outlier and genomic cline analyses, and are responsible for adaptive differences between white spruce, Engelmann spruce and their hybrids. Although interspecific gene flow occurs frequently and extensively between these two closely related species, genes under selection counteract the homogenizing effects of gene flow and prevent introgression in surrounding regions, maintaining species differences despite interspecific gene flow.

White spruce and Engelmann spruce have a long history of interspecific gene flow, which may date to more than 21,000 years ago. These two species may also have had previous contact in previous interglacial periods during the Pleistocene. Despite this extensive admixture, the hybrid zone neither lead to the fusion of the parental types, nor did it lead to the extinction of one or the other parental form (Mayr 1942, 1963; Harrison 1993). This indicates that selection must be strong in parental environments, making it possible for the hybrid zone to persist without genetic swamping and genomic extinction of one or both of the parental species.

Gene functions of adaptive loci (diversifying selection) include signal transduction, transcription regulation and carbohydrate metabolism; which are important functions in adaptation to climate. Outlier loci suggesting balancing selection were linked to phytohormone signaling, post-transcriptional regulation and protein turnover, and amino acid transportation (including nitrogen intake from soil). These outlier loci deviated from neutrality as a consequence of directional selection and overdominance. Overdominance may be the mechanism of increased hybrid fitness found in intermediate environments; and directional selection may be in relation with adaptive introgression from parental species to the hybrids.

5.5 Limitations of the current study

The number of molecular markers used in this study is substantial and represents a significant step forward in comparison with the low number of markers generally used in hybrid

zone studies. However, when it comes to identifying adaptive loci, this study must be considered as preliminary and caution should be taken when trying to extrapolate these results to a genome-wide scale. Boreal and temperate conifers have very large genomes (10-30 Gb) (Holliday et al. 2011) therefore this study likely covered a small fraction of white spruce and Engelmann spruce genomes. The use of linkage and genetic maps of the SNPs assayed (which are close to completion by the Arborea project, Quebec) will clarify their relative locations in the genome, and provide an idea of the genomic coverage provided by this set of loci.

The use of SNPs from candidate genes previously identified in other studies of spruce species improved the chances of finding adaptive SNPs, and facilitated identification of gene functions and their potential relationships with adaptive differences between white spruce, Engelmann spruce and their hybrids. However, there are some limitations in the use of candidate genes from non-model systems. Orthology of candidate genes is uncertain when comparing non-model systems with those in model systems (Holliday 2009). Although this problem seems to be negligible when genes are single copy, caution should be taken when making functional inferences based on orthology.

Climate models are considered one of the best tools when predicting species habitat shifts under climate change, extinction rates and geographic areas for conservation of tree species (Aitken et al. 2008). Aside from their valuable contributions to forest conservation and management, climate modelling also has some drawbacks. Climate models do not take into consideration biological or evolutionary processes as migration, seed and pollen dispersal or local adaptation. Furthermore, the uncertainty of future climates makes accurate projections more difficult to make, requiring the use of multiple climate change scenarios and more sophisticated models. Climate niche models, as the ones used to study the historic evolutionary relationships between white spruce and Engelmann spruce also present some limitations. Because the models are based on ecoregions, they are not flexible to community reorganization. Therefore, any no-analogue climates in the past may serve to confound the

model (Roberts and Hamann 2011). Moreover, these models only predict “climate habitat” for species; even when there is suitable climate available, the species may not be able to reach it and establish populations. Climate models are very useful for providing information on climatic habitat and potential distributions when they are interpreted with caution and considering their limitations.

Foresters have long used provenance trials to study local adaptation. These studies have provided extensive information on the genetic differentiation of tree populations and on the effects of seed transfer, as a result of long-term data measurement (Savolainen et al. 2007). Traditional reciprocal transplant experiments differ from provenance trials in the availability of *a priori* pedigree and genetic information, and the use of several generations of interspecific crosses (Lexer et al. 2004). The first difference is reduced with the use of accurate assignment methods based on molecular markers (Gompert and Buerkle 2009; Anderson and Thompson 2002). The study of several generations of interspecific crosses is extremely difficult with trees because of their long generation times. This becomes an almost impossible task when the goal is to measure the fitness of mature trees. In the absence of lifetime fitness measurements, the use of fitness proxies, such as those used in this study, becomes the best alternative.

5.6 Future research

This study contributes with a better understanding of the evolutionary relationships and genetic interactions between white spruce and Engelmann spruce, and constitutes an initial step towards identifying genes responsible for adaptation to climate in spruce species. Future genome-wide studies including linkage and genetic maps will significantly contribute to a deeper understanding of the genomic basis of local adaptation in spruce and also with identifying the genes responsible of maintaining species differences between white spruce and Engelmann spruce. An ongoing team project (AdapTree, led by Sally Aitken at UBC) will use

high-throughput genomic techniques to assess thousands of SNPs to identify adaptive genes in interior spruce. The main goal of this project is to match current natural populations of interior spruce (as well as lodgepole pine) with appropriate climate areas under climate change. A similar approach in *Arabidopsis* has led to the identification of important climate-adaptive loci and pathways, and a better understanding of the genomic architecture of local adaptation (Hancock et al. 2011).

In this study, the causes of asymmetric introgression remained unknown. A thorough study of the reproductive phenology of white spruce and Engelmann spruce, which takes into account differences in pollen shed and pollen receptivity timing between pure species *in situ*, would be valuable. Also multi-generation cross-fertilization and pollen competition experiments would determine if endogenous selection and some sort of hybrid incompatibilities are present in this hybrid zone. An alternative study to assess these topics is a paternity analysis, in which at least two generations with known hybrid class are assayed. There might be opportunities for some seed orchard work with the same families included in this study in 2012. By comparing hybrid index (already estimated in chapter 4) with reproductive success, this work may shed light on the causes of asymmetric introgression and endogenous selection in the zone.

Cytoplasmic genomes (mitochondrial and chloroplast) are often maternally inherited in higher plants (Neale and Sederoff 1989). Spruce species, however, possess the advantage of having cpDNA and mtDNA inherited differently; cpDNA is inherited paternally and mtDNA is inherited maternally (Szmidt et al. 1988). This makes spruce species ideal for the study of paternal and maternal genetic lineages; and past and current patterns of gene flow. Organelle markers are also useful for the study of hybrid zones, when alleles are fixed in alternate species. Studies including cpDNA and mtDNA markers will likely contribute with the study of the white spruce and Engelmann spruce hybrid zone. Potential useful markers have already been identified for the study of introgression between these two species (Jaramillo-Correa et al. 2003). Also, organelle markers could be used to assess the relationships between hybridizing spruce species in North America. In western North America, white spruce is known to hybridize

with black spruce (Northern British Columbia) and Sitka spruce (coastal British Columbia). Engelmann spruce is known to hybridize with blue spruce (*Picea pungens*) in western United States. With the exception of the Sitka x white spruce hybrid zone (Szmidt et al. 1988; Sutton et al. 1994), and the Engelmann x white spruce hybrid zone (this study), there is scarce information about North American spruce hybrid zones.

My next and last step in contributing with a deeper knowledge of the local adaptation of white spruce and Engelmann spruce would be to conduct an association study of cold hardiness traits. Association studies have gained considerable attention because of their suitability for dissecting complex traits in conifers. The candidate gene based-association approach has several important advantages that include adequate levels of nucleotide diversity, rapid decay of linkage disequilibrium, and accurate phenotype identification when working with progeny tests (Neale and Savolainen, 2004). A recent association study in Sitka spruce, which used some of the same SNPs assessed in this study, found significant association in 28 candidate genes, which cumulatively explained 28% and 34% of the phenotypic variance for cold hardiness and bud set, respectively (Holliday et al. 2010).

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Appendix

Table A.1 Characteristics of 10 nuclear microsatellites markers used in this study (adapted from Rungis et al. 2004). Microsatellite ID is used to identify individual loci in subsequent tables.

ID	Code	Primer sequence (5' 3')	SSR Motif	Allele size range	Annealing T(°C)
SSR01	WS0011.P12	F:cgataagatggctcctcaaa R:ggaggctgaaaagtggttaca	$(AGGA)_{32}$	291-295	62
SSR02	WS0033.A18	F:ggctgctctcttatccgtttt R:tggctctcatccagaaaagaa	$(TA)_{26}$	145-149	55
SSR03	WS0046.M11	F:cactagggcattgggaagaa R:atgagaggctggggtatgaa	$(AAG)_6$	287	60
SSR04	WS0061.C21	F:tttttagcctcatggacgtt R:ggttaaacggacgctgaaag	$(CTTT)_5$	259-279	60
SSR05	WS0082.O23	F:agtgacagttgtcttagcacatca R:aaggtttccgatcgcatcta	$(TA)_{15}$	214-224	60
SSR06	WS0092.A19	F:tgtggttttctgcttggaaa R:cccattttgactttgaaataagc	$(AC)_9$	215-223	60
SSR07	WS0092.M15	F:gatgttgcaggcattcagag R:gcaccagcatcgattgacta	$(TCC)_6$	212-218	60
SSR08	WS0092.H13	F:ccacgatgtcgttgaaagaa R:tttcagtcttcctgcattcg	$(GCT)_8$	220-226	55
SSR09	WS00111.K13	F:gactgaagatgccgatatgc R:ggccatatcatctcaaaataaagaa	$(AT)_9$	215-225	62
SSR10	PAAC17	F:gaaacaaaaattattacgcg R:atgccctcctaatgaatg	$(AC)_{36}$	132-148	53

Notes: F= Forward primer; R= Reverse primer

Table A.2 Sample size, number of alleles, number of expected alleles, observed heterozygosity, expected heterozygosity, unbiased expected heterozygosity and inbreeding coefficient for 10 microsatellite loci in populations of *Picea glauca* x *P. engelmannii*

Pop	Locus	N	N_a	A_e	H_o	H_e	UH_e	F_{is}
E1	SSR01	2	2	2	1	0.5	0.667	-1
	SSR02	6	7	5.143	0.667	0.806	0.879	0.259
	SSR03	5	4	1.923	0.4	0.48	0.533	0.2727
	SSR04	5	2	1.923	0	0.48	0.533	1
	SSR05	4	4	2.909	0.25	0.656	0.75	0.7
	SSR06	6	2	1.385	0.333	0.278	0.303	-0.111
	SSR07	6	3	1.946	0.667	0.486	0.53	-0.29
	SSR08	4	2	1.28	0.25	0.219	0.25	-0.143
	SSR09	4	3	2.462	0.25	0.594	0.679	0.66
	SSR10	6	5	3.789	0.5	0.736	0.803	0.4
E2	SSR01	16	2	1.992	0.688	0.498	0.514	-0.3525
	SSR02	17	8	6.721	0.882	0.851	0.877	-0.0063
	SSR03	20	7	4.255	0.8	0.765	0.785	-0.0201
	SSR04	19	3	1.74	0.263	0.425	0.437	0.404
	SSR05	17	8	3.828	0.941	0.739	0.761	-0.245
	SSR06	20	4	1.61	0.25	0.379	0.388	0.362
	SSR07	18	2	1.057	0.056	0.054	0.056	-0.029
	SSR08	16	5	1.391	0.313	0.281	0.29	-0.079
	SSR09	17	2	1.125	0*	0.111	0.114	1
	SSR10	18	8	5.735	0.444*	0.826	0.849	0.483§
E3	SSR01	17	2	1.94	0.471	0.484	0.499	0.058
	SSR02	18	7	4.025	0.5*	0.752	0.773	0.359
	SSR03	18	3	2.88	0.667	0.653	0.671	0.0073
	SSR04	17	2	1.335	0.176	0.251	0.258	0.3239
	SSR05	17	5	3.124	0.882	0.68	0.701	-0.269
	SSR06	19	3	2.155	0.368	0.536	0.55	0.337
	SSR07	16	3	1.21	0.188	0.174	0.179	-0.046
	SSR08	17	3	1.533	0.294	0.348	0.358	0.183
	SSR09	15	2	1.8	0*	0.444	0.46	1.000§
	SSR10	19	7	4.402	0.737	0.773	0.794	0.073
CP	SSR01	65	2	1.996	0.307	0.499	0.503	0.389§
	SSR02	69	9	5.198	0.667*	0.808	0.813	0.1816
	SSR03	67	7	3.285	0.522	0.696	0.701	0.256§
	SSR04	66	4	1.96	0.258*	0.49	0.493	0.479§
	SSR05	65	8	3.762	0.769*	0.734	0.74	-0.04
	SSR06	69	10	2.476	0.435*	0.596	0.6	0.277§
	SSR07	68	4	1.077	0.044*	0.072	0.072	0.39
	SSR08	62	5	1.243	0.161*	0.195	0.197	0.182
	SSR09	55	9	4.21	0.436*	0.762	0.769	0.435§
	SSR10	69	10	4.392	0.493	0.772	0.778	0.368§

Table A.2 (Continued) Sample size, number of alleles, number of expected alleles, observed heterozygosity, expected heterozygosity, unbiased expected heterozygosity and inbreeding coefficient for 10 microsatellite loci in populations of *Picea glauca* x *P. engelmannii*

Pop	Locus	N	N_a	A_e	H_o	H_e	UH_e	F_{is}
PG	SSR01	83	4	2.073	0.446	0.518	0.521	0.144
	SSR02	86	8	4.964	0.534*	0.799	0.803	0.335§
	SSR03	85	6	3.539	0.682	0.717	0.722	0.0548
	SSR04	81	6	1.778	0.395*	0.437	0.44	0.103§
	SSR05	82	10	4.492	0.732*	0.777	0.782	0.0648
	SSR06	86	7	2.691	0.477*	0.628	0.632	0.247§
	SSR07	82	4	1.315	0.146*	0.24	0.241	0.395§
	SSR08	79	4	1.263	0.114*	0.209	0.21	0.458§
	SSR09	63	8	2.763	0.253*	0.638	0.643	0.607§
	SSR10	85	8	4.778	0.518*	0.791	0.795	0.35§
EK	SSR01	74	3	1.97	0.446	0.492	0.496	0.101
	SSR02	85	9	4.377	0.776*	0.772	0.776	-0.0005
	SSR03	84	8	3.964	0.785	0.748	0.752	-0.0448
	SSR04	70	7	2.015	0.286*	0.504	0.507	0.438§
	SSR05	73	9	4.448	0.726*	0.775	0.781	0.0703
	SSR06	86	8	2.574	0.418*	0.611	0.615	0.3207
	SSR07	75	4	1.192	0.093*	0.161	0.162	0.426§
	SSR08	71	5	1.225	0.155	0.184	0.185	0.163
	SSR09	49	9	1.953	0.204*	0.488	0.493	0.588§
	SSR10	83	8	5.811	0.675*	0.828	0.833	0.19§
BV	SSR01	56	3	2.107	0.304*	0.525	0.53	0.429
	SSR02	68	8	5.492	0.853	0.818	0.824	-0.0354
	SSR03	68	6	2.797	0.721	0.643	0.647	-0.1142
	SSR04	57	3	1.637	0.263*	0.389	0.393	0.331§
	SSR05	65	9	3.703	0.831*	0.73	0.736	-0.1305
	SSR06	67	8	3.267	0.567*	0.694	0.699	0.189§
	SSR07	63	4	1.138	0.095*	0.121	0.122	0.223
	SSR08	55	5	1.322	0.109*	0.244	0.246	0.558§
	SSR09	42	10	3.749	0.405*	0.733	0.742	0.457§
	SSR10	69	7	4.087	0.681*	0.755	0.761	0.105§
MR	SSR01	41	4	1.963	0.39*	0.49	0.497	0.216§
	SSR02	50	8	4.912	0.8*	0.796	0.804	0.005
	SSR03	48	8	3.491	0.83*	0.714	0.721	-0.157
	SSR04	41	4	1.527	0.17	0.345	0.349	0.514
	SSR05	46	7	3.04	0.696*	0.671	0.678	-0.0256
	SSR06	49	8	2.689	0.428	0.628	0.635	0.3269
	SSR07	44	4	1.149	0.068*	0.129	0.131	0.482§
	SSR08	43	4	1.181	0.163	0.153	0.155	-0.05
	SSR09	35	9	4.861	0.629	0.794	0.806	0.222

Table A.2 (Continued) Sample size, number of alleles, number of expected alleles, observed heterozygosity, expected heterozygosity, unbiased expected heterozygosity and inbreeding coefficient for 10 microsatellite loci in populations of *Picea glauca* x *P. engelmannii*

Pop	Locus	N	N_a	A_e	H_o	H_e	UH_e	F_{is}
MR	SSR10	45	8	5.728	0.644	0.825	0.835	0.229
MG	SSR01	51	2	1.841	0.392	0.457	0.461	0.151
	SSR02	75	9	5.496	0.827*	0.818	0.824	-0.0038
	SSR03	74	8	3.511	0.811*	0.715	0.72	-0.127
	SSR04	71	8	2.069	0.324*	0.517	0.52	0.379§
	SSR05	52	9	3.128	0.673*	0.68	0.687	0.0203§
	SSR06	61	9	3.712	0.475	0.731	0.737	0.356§
	SSR07	65	2	1.063	0.062	0.06	0.06	-0.024
	SSR08	69	6	1.402	0.246*	0.287	0.289	0.148
	SSR09	44	11	6.977	0.659	0.857	0.867	0.241§
	SSR10	71	8	4.996	0.577*	0.8	0.806	0.284§
QL	SSR01	76	2	1.699	0.316	0.411	0.414	0.238
	SSR02	113	9	6.062	0.832	0.835	0.839	0.0082
	SSR03	109	8	3.974	0.716	0.748	0.752	0.0484
	SSR04	89	8	2.839	0.539*	0.648	0.651	0.172
	SSR05	83	8	2.81	0.807*	0.644	0.648	-0.2475
	SSR06	93	11	3.521	0.569*	0.716	0.72	0.209§
	SSR07	95	4	1.161	0.105	0.138	0.139	0.244
	SSR08	90	4	1.058	0.056	0.054	0.055	-0.014
	SSR09	59	10	5.343	0.54*	0.813	0.82	0.340§
	SSR10	101	10	4.734	0.673	0.789	0.793	0.151§
FNL	SSR01	63	3	1.986	0.556	0.496	0.5	-0.111
	SSR02	75	9	5.887	0.827	0.83	0.836	0.0109
	SSR03	67	7	3.378	0.701	0.704	0.709	0.011
	SSR04	69	8	2.476	0.536	0.596	0.601	0.107
	SSR05	64	7	3.057	0.75*	0.673	0.678	-0.107§
	SSR06	74	7	2.332	0.595	0.571	0.575	-0.0343
	SSR07	70	4	1.044	0.043	0.042	0.043	-0.007
	SSR08	66	5	1.225	0.121*	0.184	0.185	0.348
	SSR09	43	10	3.64	0.372*	0.725	0.734	0.495§
	SSR10	78	9	5.892	0.744*	0.83	0.836	0.11§
WK	SSR01	27	2	1.957	0.185	0.489	0.498	0.632§
	SSR02	41	9	5.18	0.854	0.807	0.817	-0.0456
	SSR03	41	6	3.58	0.61	0.721	0.73	0.166
	SSR04	29	6	1.56	0.345	0.359	0.365	0.057
	SSR05	36	8	3.323	0.917*	0.699	0.709	-0.298§
	SSR06	41	9	1.81	0.439*	0.448	0.453	0.0316
	SSR07	32	4	1.253	0.219	0.202	0.205	-0.069

Table A.2 (Continued) Sample size, number of alleles, number of expected alleles, observed heterozygosity, expected heterozygosity, unbiased expected heterozygosity and inbreeding coefficient for 10 microsatellite loci in populations of *Picea glauca* x *P. engelmannii*

Pop	Locus	N	N_a	A_e	H_o	H_e	UH_e	F_{is}
WK	SSR08	30	3	1.226	0.133*	0.184	0.188	0.2927
	SSR09	25	6	2.753	0.28*	0.637	0.65	0.574§
	SSR10	39	9	5.654	0.744	0.823	0.834	0.109
FN	SSR01	13	2	1.988	0.462	0.497	0.517	0.111
	SSR02	14	7	4.962	0.786	0.798	0.828	0.053
	SSR03	14	4	2.8	0.429	0.643	0.667	0.365§
	SSR04	14	2	1.849	0.429	0.459	0.476	0.1034
	SSR05	14	4	2.074	0.571	0.518	0.537	-0.0667
	SSR06	14	6	4.041	0.429	0.753	0.78	0.460§
	SSR07	14	1	1	0	0	0	--
	SSR08	6	2	1.18	0.167	0.153	0.167	-0.091
	SSR09	7	7	5.444	0.571	0.816	0.879	0.368
	SSR10	14	5	3.379	0.5	0.704	0.73	0.323

Notes: N= sample size, Na= Number of different alleles, Ae = Number of effective alleles= 1/(Sum pi^2), Ho= Observed heterozygosity= Number of Hets/N, He= Expected heterozygosity= 1-Sum pi^2, UHe= Unbiased Expected Heterozygosity= (2N/(2N-1)*He; Fis=Inbreeding coefficient; where pi is the frequency of the ith allele for the population

* Significant deviation from Hardy-Weinberg equilibrium (P<0.01)

§ Significant deviation of inbreeding coefficient from zero (P<0.01). A positive value indicates heterozygote deficit and a negative value, heterozygote excess.

Table A.3. Allele frequencies and sample sizes by population, when populations are divided by SPZ

Locus	Allele/n	E1	E2	E3	CP	PG	EK	BV	MR	MG	QL	FNL	WK	FN
SSR01	**N**	2	16	17	65	83	74	56	41	51	76	63	27	13
	304	0	0	0	0	0.018	0.007	0	0.012	0	0	0.008	0	0
	308	0.5	0.469	0.412	0.477	0.434	0.412	0.473	0.268	0.353	0.289	0.421	0.574	0.538
	312	0.5	0.531	0.588	0.523	0.542	0.581	0.5	0.659	0.647	0.711	0.571	0.426	0.462
	316	0	0	0	0	0.006	0	0.027	0.061	0	0	0	0	0
SSR02	**N**	6	17	18	69	86	85	68	50	75	113	75	41	14
	171	0	0.059	0.028	0.007	0.041	0.024	0.007	0	0.033	0.062	0.007	0.037	0
	173	0.083	0.088	0.139	0.13	0.186	0.094	0.132	0.09	0.16	0.119	0.053	0.049	0.321
	175	0.167	0.235	0.361	0.268	0.291	0.335	0.199	0.33	0.22	0.235	0.26	0.256	0.071
	177	0.333	0.176	0.139	0.217	0.157	0.182	0.221	0.2	0.213	0.181	0.153	0.171	0.143
	179	0.083	0.147	0.278	0.203	0.227	0.265	0.235	0.19	0.233	0.217	0.193	0.28	0.143
	181	0.083	0.118	0	0.116	0.058	0.035	0.125	0.04	0.06	0.089	0.14	0.11	0.214
	183	0.167	0.088	0	0.036	0.035	0.041	0.074	0.03	0.053	0.058	0.12	0.037	0.071
	185	0.083	0.088	0.028	0.014	0.006	0.018	0.007	0.08	0.02	0.018	0.067	0.049	0.036
	187	0	0	0.028	0.007	0	0.006	0	0.04	0.007	0.022	0.007	0.012	0
SSR03	**N**	5	20	18	67	85	84	68	48	74	109	67	41	14
	232	0	0.025	0	0	0	0.012	0	0.01	0.014	0.032	0.007	0	0
	235	0	0.075	0	0.007	0.018	0.042	0.007	0.021	0.027	0.064	0.007	0.049	0
	238	0.1	0.1	0	0.172	0.135	0.101	0.044	0.031	0.108	0.083	0.254	0.073	0.143
	241	0.7	0.325	0.333	0.336	0.406	0.381	0.478	0.333	0.432	0.376	0.396	0.415	0.5
	244	0.1	0.3	0.25	0.396	0.288	0.22	0.338	0.333	0.277	0.284	0.269	0.256	0.286
	247	0	0.15	0.417	0.075	0.124	0.214	0.11	0.25	0.061	0.128	0.052	0.183	0.071
	250	0.1	0.025	0	0.007	0.029	0.024	0.022	0.01	0.068	0.028	0.015	0	0
	253	0	0	0	0.007	0	0.006	0	0.01	0.014	0.005	0	0.024	0
SSR04	**N**	5	19	17	66	81	70	57	41	71	89	69	29	14
	234	0	0	0	0	0	0	0	0.037	0.007	0.022	0	0	0
	238	0	0	0	0	0.006	0	0	0	0	0	0	0	0
	242	0	0	0	0	0	0.007	0	0	0.014	0.006	0	0	0

Table A.3 (Continued) Allele frequencies and sample sizes by population, when populations are divided by SPZ

Locus	Allele/n	E1	E2	E3	CP	PG	EK	BV	MR	MG	QL	FNL	WK	FN
SSR04	**246**	0	0	0	0	0	0.021	0	0	0.007	0.034	0.022	0.017	0
	250	0.4	0.263	0.147	0.098	0.031	0.086	0.105	0.159	0.183	0.174	0.174	0.086	0
	254	0.6	0.711	0.853	0.689	0.722	0.679	0.763	0.793	0.662	0.534	0.587	0.793	0.357
	258	0	0.026	0	0.144	0.198	0.164	0.132	0.012	0.106	0.185	0.167	0.052	0.643
	262	0	0	0	0.068	0.019	0.014	0	0	0.007	0.017	0.022	0.034	0
	266	0	0	0	0	0.025	0.029	0	0	0	0.028	0.007	0	0
	270	0	0	0	0	0	0	0	0	0	0	0.014	0.017	0
	274	0	0	0	0	0	0	0	0	0	0	0.007	0	0
	286	0	0	0	0	0	0	0	0	0.014	0	0	0	0
SSR05	**N**	4	17	17	65	82	73	65	46	52	83	64	36	14
	224	0	0.029	0	0.046	0.061	0.034	0.015	0	0	0.03	0.039	0	0
	226	0.125	0.147	0.059	0.285	0.232	0.199	0.3	0.25	0.317	0.367	0.258	0.292	0.25
	228	0	0.176	0.353	0.031	0.012	0.068	0.062	0.065	0.096	0.03	0.078	0.083	0
	230	0	0.029	0.029	0	0.012	0.034	0.015	0	0.01	0	0	0.028	0.036
	232	0	0.029	0	0	0.012	0.007	0.008	0.022	0	0	0.016	0.014	0
	234	0	0.059	0	0	0.006	0	0	0	0.01	0.006	0	0.028	0
	236	0.125	0	0	0.031	0.055	0.027	0.015	0.011	0	0.006	0.016	0	0
	238	0.25	0.088	0	0.223	0.213	0.123	0.162	0.076	0.067	0.096	0.102	0.097	0.071
	240	0.5	0.441	0.147	0.362	0.335	0.384	0.385	0.5	0.452	0.458	0.492	0.444	0.643
	242	0	0	0.412	0.015	0.061	0.123	0.038	0.076	0.01	0.006	0	0.014	0
	250	0	0	0	0	0	0	0	0	0.019	0	0	0	0
	256	0	0	0	0.008	0	0	0	0	0	0	0	0	0
	264	0	0	0	0	0	0	0	0	0.019	0	0	0	0
SSR06	**N**	6	20	19	69	86	86	67	49	61	93	74	41	14
	223	0	0	0	0.014	0	0	0	0.01	0	0	0	0	0
	225	0	0	0	0	0	0	0	0	0.008	0	0	0.012	0
	227	0	0	0	0	0	0	0	0	0.008	0	0	0	0

Table A.3 (Continued) Allele frequencies and sample sizes by population, when populations are divided by SPZ

Locus	Allele/n	E1	E2	E3	CP	PG	EK	BV	MR	MG	QL	FNL	WK	FN
SSR06	**229**	0	0	0	0.014	0	0	0.022	0	0.033	0.011	0	0.012	0.143
	231	0	0	0	0.014	0.064	0.012	0.007	0.01	0.016	0.059	0.047	0.024	0.036
	233	0	0.125	0.053	0.116	0.099	0.058	0.06	0.041	0.189	0.108	0.027	0.11	0.071
	235	0.833	0.775	0.553	0.609	0.57	0.564	0.448	0.429	0.426	0.457	0.608	0.732	0.286
	237	0	0.05	0.395	0.123	0.169	0.244	0.284	0.429	0.189	0.231	0.23	0.049	0.357
	239	0.167	0.05	0	0.051	0.058	0.081	0.142	0.041	0.123	0.075	0.041	0.037	0.107
	241	0	0	0	0.036	0.035	0.023	0.03	0.031	0.008	0.016	0.041	0.012	0
	243	0	0	0	0.014	0.006	0.012	0.007	0.01	0	0.011	0	0	0
	245	0	0	0	0	0	0	0	0	0	0.005	0	0.012	0
	247	0	0	0	0.007	0	0	0	0	0	0.022	0.007	0	0
	251	0	0	0	0	0	0.006	0	0	0	0.005	0	0	0
SSR07	**N**	6	18	16	68	82	75	63	44	65	95	70	32	14
	210	0	0	0	0	0	0	0	0.011	0	0	0.007	0	0
	222	0.083	0	0	0.007	0	0.007	0.016	0	0	0	0	0	0
	225	0	0	0.031	0	0.006	0	0	0.011	0	0.005	0	0	0
	228	0.667	0.972	0.906	0.963	0.866	0.913	0.937	0.932	0.969	0.926	0.979	0.891	1
	231	0.25	0.028	0.063	0.022	0.098	0.067	0.016	0.045	0.031	0.058	0.007	0.063	0
	234	0	0	0	0.007	0.03	0.013	0.032	0	0	0.011	0.007	0.016	0
	237	0	0	0	0	0	0	0	0	0	0	0	0.031	0
SSR08	**N**	4	16	17	62	79	71	55	43	69	90	66	30	6
	234	0	0.031	0	0.008	0	0.021	0.009	0	0.022	0.006	0.015	0	0
	237	0.125	0.063	0.118	0.032	0.07	0.056	0.1	0.023	0.058	0	0.03	0.067	0
	240	0.875	0.844	0.794	0.895	0.886	0.901	0.864	0.919	0.841	0.972	0.902	0.9	0.917
	243	0	0.031	0.088	0.04	0.038	0.007	0.018	0	0.036	0.006	0.045	0.033	0.083
	246	0	0.031	0	0.024	0.006	0.014	0.009	0.047	0.036	0.017	0.008	0	0
	249	0	0	0	0	0	0	0	0	0.007	0	0	0	0
	252	0	0	0	0	0	0	0	0.012	0	0	0	0	0

Table A.3 (Continued) Allele frequencies and sample sizes by population, when populations are divided by SPZ

Locus	Allele/n	E1	E2	E3	CP	PG	EK	BV	MR	MG	QL	FNL	WK	FN
SSR09	**N**	4	17	15	55	63	49	42	35	44	59	43	25	7
	232	0.375	0.941	0.667	0.418	0.54	0.704	0.452	0.229	0.273	0.364	0.488	0.56	0
	234	0.5	0.059	0.333	0.118	0.238	0.092	0.202	0.329	0.114	0.136	0.093	0.18	0.286
	236	0	0	0	0.145	0.095	0.061	0.048	0.171	0.114	0.11	0.081	0.06	0.071
	238	0.125	0	0	0.127	0.048	0.031	0.036	0.086	0.125	0.059	0.081	0.06	0.214
	240	0	0	0	0.091	0.008	0.02	0.107	0.029	0.091	0.042	0.035	0.06	0.143
	242	0	0	0	0.036	0	0.041	0.06	0.029	0.08	0.051	0.047	0.08	0.143
	244	0	0	0	0	0.016	0.031	0.036	0.014	0.057	0.085	0.035	0	0.071
	246	0	0	0	0.036	0.008	0.01	0.024	0.043	0.011	0.034	0.093	0	0
	248	0	0	0	0.018	0.048	0	0.024	0.071	0.091	0.042	0.023	0	0.071
	250	0	0	0	0.009	0	0	0.012	0	0.034	0.076	0.023	0	0
	252	0	0	0	0	0	0.01	0	0	0.011	0	0	0	0
SSR10	**N**	6	18	19	69	85	83	69	45	71	101	78	39	14
	147	0	0.028	0	0.007	0	0.024	0	0	0	0.025	0	0.013	0
	149	0.167	0.056	0	0.007	0.029	0.084	0.043	0.033	0.049	0.015	0.026	0.013	0
	151	0.417	0.167	0.026	0.058	0.071	0.102	0.101	0.156	0.085	0.119	0.083	0.051	0
	153	0	0.083	0.053	0.145	0.159	0.145	0.087	0.067	0.099	0.064	0.109	0.154	0.036
	155	0.167	0.167	0.342	0.37	0.276	0.259	0.42	0.233	0.246	0.366	0.276	0.269	0.393
	157	0.083	0.278	0.158	0.225	0.294	0.133	0.159	0.244	0.324	0.188	0.186	0.231	0.321
	159	0.167	0.167	0.237	0.109	0.118	0.217	0.145	0.156	0.092	0.139	0.173	0.103	0.179
	161	0	0.056	0.158	0.065	0.024	0.036	0.043	0.067	0.077	0.05	0.09	0.077	0.071
	163	0	0	0.026	0.007	0.029	0	0	0.044	0.028	0.03	0.026	0.09	0
	165	0	0	0	0	0	0	0	0	0	0.005	0.032	0	0
	171	0	0	0	0.007	0	0	0	0	0	0	0	0	0

Table A.4. Allele frequencies and sample sizes by population, when populations are divided by elevational band

Locus	Allele/n	pop1 350-600m	pop2 600-850m	pop3 850-1100m	pop4 1100-1350m	pop5 1350-1600m	pop6 1600-1800m	pop7 1800-2050m	pop8 2050-2300m	pop9 2300-2800m	pop10 2800-3292m
SSR01	**N**	13	139	205	117	58	13	7	5	8	21
	304	0	0	0.01	0.004	0.009	0	0	0	0	0
	308	0.538	0.424	0.402	0.427	0.267	0.577	0.643	0.4	0.5	0.429
	312	0.462	0.561	0.583	0.556	0.724	0.423	0.357	0.6	0.5	0.571
	316	0	0.014	0.005	0.013	0	0	0	0	0	0
SSR02	**N**	14	168	238	147	87	16	9	10	8	22
	171	0	0.021	0.025	0.027	0.046	0.031	0	0.05	0.063	0.023
	173	0.321	0.113	0.134	0.119	0.092	0.094	0	0.1	0.063	0.136
	175	0.071	0.268	0.267	0.245	0.276	0.25	0.278	0.1	0.375	0.341
	177	0.143	0.164	0.204	0.204	0.161	0.125	0.222	0.35	0.063	0.159
	179	0.143	0.253	0.197	0.221	0.241	0.281	0.333	0.1	0.063	0.273
	181	0.214	0.107	0.082	0.071	0.075	0.094	0.056	0.1	0.125	0.023
	183	0.071	0.042	0.05	0.061	0.075	0.125	0.056	0.15	0.125	0
	185	0.036	0.021	0.025	0.044	0.029	0	0.056	0.05	0.125	0.023
	187	0	0.012	0.015	0.007	0.006	0	0	0	0	0.023
SSR03	**N**	14	163	228	148	84	14	8	10	9	24
	232	0	0.009	0.002	0.007	0.042	0	0	0	0.056	0
	235	0	0.025	0.013	0.024	0.083	0.036	0.125	0.05	0.056	0
	238	0.143	0.104	0.121	0.115	0.107	0.179	0	0.1	0.167	0
	241	0.5	0.411	0.41	0.402	0.333	0.393	0.25	0.55	0.333	0.313
	244	0.286	0.313	0.329	0.264	0.208	0.286	0.313	0.15	0.222	0.313
	247	0.071	0.11	0.105	0.149	0.173	0.107	0.25	0.1	0.167	0.354
	250	0	0.025	0.018	0.027	0.042	0	0.063	0.05	0	0.021
	253	0	0.003	0.002	0.014	0.012	0	0	0	0	0

Table A.4 (Continued) Allele frequencies and sample sizes by population, when populations are divided by elevational band

Locus	Allele/n	pop1 350-600m	pop2 600-850m	pop3 850-1100m	pop4 1100-1350m	pop5 1350-1600m	pop6 1600-1800m	pop7 1800-2050m	pop8 2050-2300m	pop9 2300-2800m	pop10 2800-3292m
SSR04	**N**	14	150	214	132	62	11	7	8	9	23
	234	0	0.003	0.005	0.019	0	0	0	0	0	0
	238	0	0.003	0	0	0	0	0	0	0	0
	242	0	0	0.005	0.008	0	0	0	0	0	0
	250	0	0.14	0.126	0.095	0.121	0.091	0.214	0.313	0.278	0.196
	254	0.357	0.65	0.689	0.644	0.702	0.864	0.714	0.688	0.667	0.804
	258	0.643	0.167	0.117	0.182	0.129	0.045	0	0	0.056	0
	262	0	0.017	0.028	0.019	0.008	0	0	0	0	0
	266	0	0.013	0.012	0.011	0.016	0	0	0	0	0
	270	0	0	0.002	0.004	0.008	0	0	0	0	0
	274	0	0	0.002	0	0	0	0	0	0	0
	286	0	0	0.005	0	0	0	0	0	0	0
SSR05	**N**	14	151	204	127	66	13	9	8	7	21
	224	0	0.033	0.034	0.024	0.015	0.038	0	0	0.071	0
	226	0.25	0.301	0.289	0.287	0.212	0.154	0.111	0.125	0	0.119
	228	0	0.023	0.071	0.047	0.061	0.077	0.167	0.063	0.286	0.31
	230	0.036	0.01	0.005	0.008	0.03	0.038	0	0	0.071	0.024
	232	0	0.007	0.01	0.008	0	0	0.056	0.063	0	0
	234	0	0	0.005	0.008	0	0.038	0	0.063	0.071	0
	236	0	0.03	0.022	0.012	0.015	0	0	0.063	0	0
	238	0.071	0.202	0.103	0.106	0.144	0.231	0.056	0.25	0.071	0
	240	0.643	0.381	0.429	0.421	0.439	0.346	0.611	0.375	0.429	0.214
	242	0	0.013	0.029	0.063	0.083	0.077	0	0	0	0.333

Table A.4 (Continued) Allele frequencies and sample sizes by population, when populations are divided by elevational band

Locus	Allele/n	pop1 350-600m	pop2 600-850m	pop3 850-1100m	pop4 1100-1350m	pop5 1350-1600m	pop6 1600-1800m	pop7 1800-2050m	pop8 2050-2300m	pop9 2300-2800m	pop10 2800-3292m
SSR05	**250**	0	0	0	0.008	0	0	0	0	0	0
	256	0	0	0.002	0	0	0	0	0	0	0
	264	0	0	0	0.008	0	0	0	0	0	0
SSR06	**N**	14	169	220	132	84	15	9	10	9	25
	223	0	0	0.005	0.004	0	0	0	0	0	0
	225	0	0	0.002	0.004	0	0	0	0	0	0
	227	0	0	0	0.004	0	0	0	0	0	0
	229	0.143	0.006	0.016	0.011	0	0	0	0	0	0
	231	0.036	0.053	0.027	0.019	0.03	0	0.056	0	0	0
	233	0.071	0.068	0.12	0.064	0.083	0.1	0.056	0.05	0.167	0.06
	235	0.286	0.479	0.55	0.545	0.524	0.733	0.611	0.85	0.833	0.58
	237	0.357	0.225	0.218	0.235	0.214	0.067	0.056	0.05	0	0.34
	239	0.107	0.107	0.041	0.072	0.101	0.033	0.167	0.05	0	0.02
	241	0	0.044	0.009	0.027	0.024	0.067	0.056	0	0	0
	243	0	0.009	0.005	0.004	0.018	0	0	0	0	0
	245	0	0	0	0.004	0.006	0	0	0	0	0
	247	0	0.009	0.005	0.004	0	0	0	0	0	0
	251	0	0	0.002	0.004	0	0	0	0	0	0
SSR07	**N**	14	156	230	124	66	12	8	9	9	22
	210	0	0	0.002	0	0.008	0	0	0	0	0
	222	0	0	0.007	0	0.008	0	0.063	0	0	0
	225	0	0.003	0.002	0	0.008	0	0	0	0	0.023
	228	1	0.952	0.926	0.956	0.879	0.792	0.938	0.833	0.944	0.909

Table A.4 (Continued) Allele frequencies and sample sizes by population, when populations are divided by elevational band

Locus	Allele/n	pop1 350-600m	pop2 600-850m	pop3 850-1100m	pop4 1100-1350m	pop5 1350-1600m	pop6 1600-1800m	pop7 1800-2050m	pop8 2050-2300m	pop9 2300-2800m	pop10 2800-3292m
SSR07	**231**	0	0.022	0.052	0.04	0.068	0.167	0	0.167	0.056	0.068
	234	0	0.022	0.011	0.004	0.015	0.042	0	0	0	0
	237	0	0	0	0	0.015	0	0	0	0	0
SSR08	**N**	6	144	213	132	65	6	8	6	7	23
	234	0	0.007	0.007	0.008	0.031	0	0	0.083	0	0
	237	0	0.09	0.023	0.027	0.069	0	0.063	0.083	0.071	0.087
	240	0.917	0.837	0.927	0.943	0.846	1	0.938	0.833	0.786	0.848
	243	0.083	0.031	0.033	0.004	0.031	0	0	0	0.071	0.065
	246	0	0.035	0.009	0.015	0.015	0	0	0	0.071	0
	249	0	0	0	0.004	0	0	0	0	0	0
	252	0	0	0	0	0.008	0	0	0	0	0
SSR09	**N**	7	115	154	87	46	10	5	7	9	20
	232	0	0.348	0.503	0.46	0.489	0.5	0.6	0.714	0.944	0.75
	234	0.286	0.13	0.169	0.178	0.174	0.3	0.2	0.286	0	0.25
	236	0.071	0.13	0.11	0.098	0.022	0	0	0	0	0
	238	0.214	0.104	0.045	0.075	0.087	0	0.1	0	0.056	0
	240	0.143	0.07	0.058	0.023	0.043	0	0.1	0	0	0
	242	0.143	0.057	0.013	0.052	0.087	0.1	0	0	0	0
	244	0.071	0.039	0.023	0.029	0.043	0.1	0	0	0	0
	246	0	0.048	0.029	0.023	0	0	0	0	0	0
	248	0.071	0.061	0.029	0.034	0.011	0	0	0	0	0
	250	0	0.013	0.016	0.029	0.033	0	0	0	0	0
	252	0	0	0.003	0	0.011	0	0	0	0	0

Table A.4 (Continued) Allele frequencies and sample sizes by population, when populations are divided by elevational band

Locus	Allele/n	pop1 350-600m	pop2 600-850m	pop3 850-1100m	pop4 1100-1350m	pop5 1350-1600m	pop6 1600-1800m	pop7 1800-2050m	pop8 2050-2300m	pop9 2300-2800m	pop10 2800-3292m
SSR10	**N**	14	164	234	147	75	15	8	9	9	24
	147	0	0	0.006	0	0.033	0.033	0.063	0.111	0	0
	149	0	0.034	0.028	0.034	0.033	0.1	0.125	0.222	0	0
	151	0	0.079	0.077	0.122	0.1	0.1	0.125	0.444	0.167	0.021
	153	0.036	0.088	0.113	0.129	0.12	0.233	0	0.056	0.111	0.042
	155	0.393	0.387	0.312	0.245	0.293	0.067	0.375	0.111	0.111	0.313
	157	0.321	0.216	0.241	0.204	0.187	0.2	0.188	0.056	0.278	0.208
	159	0.179	0.11	0.145	0.17	0.14	0.167	0.063	0	0.222	0.271
	161	0.071	0.073	0.038	0.061	0.06	0.1	0	0	0.111	0.125
	163	0	0.009	0.032	0.024	0.033	0	0.063	0	0	0.021
	165	0	0	0.006	0.01	0	0	0	0	0	0
	171	0	0.003	0	0	0	0	0	0	0	0

Table A.5. Heterozygosities over all populations for each microsatellite locus, when populations are divided by elevation band

Locus	Ht	Mean He	Mean Ho
SSR01	0.503	0.483	0.465
SSR02	0.832	0.800	0.773
SSR03	0.738	0.716	0.706
SSR04	0.498	0.440	0.328
SSR05	0.749	0.698	0.755
SSR06	0.597	0.550	0.429
SSR07	0.162	0.155	0.124
SSR08	0.208	0.201	0.189
SSR09	0.667	0.589	0.264
SSR10	0.834	0.786	0.618
Mean	0.579	0.542	0.465
SE		0.024	0.026

Table A.6 Least Square Means and Standard errors for height measurements (cm) at different ages among hybrid classes tested in Seed Planning Zones Quesnel (QL), East Kootenay (EK) and West Kootenay (WK). Hybrid classes are stratified as pure *Picea glauca* (1), *P. glauca*-like hybrids (2), *P. engelmannii*-like hybrids (3) and *P. engelmannii* (4); based on *INTROGRESS* hybrid index.

			Years after outplanting						
Region	Hybrid class		0	3	6	10	15	20	25
QL	1	lsmeans	21.375	37.535	86.813	165.051	333.298	540.173	752.720
		stderr	0.996	2.005	5.376	10.801	20.233	29.116	38.323
	2	lsmeans	23.397	41.616	93.841	177.908	337.692	526.632	726.272
		stderr	0.128	0.261	0.703	1.429	2.689	3.877	4.949
	3	lsmeans	22.670	40.352	89.220	166.151	316.338	494.789	693.513
		stderr	0.083	0.170	0.459	0.931	1.753	2.526	3.238
	4	lsmeans	21.442	38.817	84.028	151.937	292.666	466.725	655.341
		stderr	0.306	0.619	1.664	3.357	6.305	9.100	11.687
EK	1	lsmeans	--	45.592	--	191.792	--	538.776	--
		stderr	--	1.820	--	8.460	--	21.450	--
	2	lsmeans	--	42.425	--	170.482	--	509.725	--
		stderr	--	0.560	--	2.640	--	6.780	--
	3	lsmeans	--	37.626	--	156.604	--	459.322	--
		stderr	--	0.370	--	1.730	--	4.410	--
	4	lsmeans	--	36.499	--	150.300	--	436.636	--
		stderr	--	0.670	--	3.110	--	7.950	--

Table A.6 (Continued) Least Square Means and Standard errors for height measurements (cm) at different ages among hybrid classes tested in Seed Planning Zones Quesnel (QL), East Kootenay (EK) and West Kootenay (WK). Hybrid classes are stratified as pure *Picea glauca* (1), *P. glauca*-like hybrids (2), *P. engelmannii*-like hybrids (3) and *P. engelmannii* (4); based on *INTROGRESS* hybrid index

			Years after outplanting						
Region	Hybrid class		0	3	6	10	15	20	25
WK Hall/Duhamel	2	lsmeans	--	58.911	104.476	169.732	--	--	--
		stderr	--	4.304	10.220	24.070	--	--	--
	3	lsmeans	--	61.305	106.828	155.437	--	--	--
		stderr	--	0.355	0.743	1.426	--	--	--
	4	lsmeans	--	63.190	109.271	158.925	--	--	--
		stderr	--	0.582	1.230	2.436	--	--	--
WK Cortiana	2	lsmeans	--	53.700	101.200	170.200	--	--	--
		stderr	--	3.690	6.599	12.716	--	--	--
	3	lsmeans	--	50.013	90.751	158.879	--	--	--
		stderr	--	0.448	0.817	1.591	--	--	--
	4	lsmeans	--	49.322	88.974	154.540	--	--	--
		stderr	--	0.718	1.296	2.525	--	--	--

Table A.7 Least Square Means and Standard errors for height measurements (cm) at different ages among hybrid classes tested in Seed Planning Zones Quesnel (QL), East Kootenay (EK) and West Kootenay (WK). Hybrid classes are stratified as pure *Picea engelmannii*, F1 hybrids, advanced generation hybrids (Fn) and pure *P. glauca*; based on *NewHybrids* assignment.

			Years after outplanting						
Region	Hybrid class		0	3	6	10	15	20	25
QL	Engelmann	lsmean	21.86262804	38.99805655	85.67957487	155.4954235	297.1701047	472.193256	665.0421672
		stderr	0.14807058	0.299958014	0.808896898	1.634884732	3.078839873	4.448252533	5.685685574
	F1 hybrids	lsmean	24.79166667	43.22400908	98.75981261	190.0674487	357.5377429	545.2306717	767.7552158
		stderr	0.497131091	1.031092795	2.789383005	5.640888727	10.63500665	15.46209765	19.74170556
	Fn hybrids	lsmean	23.01723096	41.00118571	91.30519544	171.8720451	326.98934	509.9308798	709.6008129
		stderr	0.077155077	0.157976823	0.426708699	0.864598494	1.629793553	2.353898093	3.013088245
WK Hall/ Duhamel	Engelmann	lsmeans	--	62.07929104	107.7560132	157.1630072	--	--	--
		stderr	--	0.329587819	0.691642208	1.347430051	--	--	--
	F1 hybrids	lsmeans	--	54.73510994	89.89942695	135.2608433	--	--	--
		stderr	--	3.569098202	7.258935088	13.32690465	--	--	--
	Fn hybrids	lsmeans	--	60.40434583	106.5728547	153.3936788	--	--	--
		stderr	--	0.770636209	1.594697453	3.05843328	--	--	--
EK	Engelmann	lsmeans	--	36.61769257	--	151.8286283	--	444.0254009	--
		stderr	--	0.389129221	--	1.813096177	--	4.638395373	--
	F1 hybrids	lsmeans	--	37.73775866	--	151.4337404	--	494.354494	--
		stderr	--	3.436872289	--	15.9480566	--	40.63500249	--
	Fn hybrids	lsmeans	--	40.86007195	--	167.4251068	--	494.6057124	--
		stderr	--	0.414832929	--	1.923322652	--	4.951641351	--
	white	lsmeans	--	45.76581248	--	182.7917158	--	521.1339116	--
		stderr	--	2.060678048	--	9.56193718	--	24.36318245	--

Table A.8 Results of the univariate regressions of hybrid index on geographical and climate variables in each Seed Planning Zone. Significant regressions are highlighted (P<0.05).

	QUESNEL		MOUNT ROBSON		EAST KOOTENAY		WEST KOOTENAY	
Variable	**R^2**	**p>F**	**R^2**	**p>F**	**R^2**	**p>F**	**R^2**	**p>F**
Elevation	0.1439	<.0001	0.4739	<.0001	0.422	<.0001	0.1947	0.013
Latitude	0.0369	0.1599	0.3453	<.0001	0.1098	0.0175	0.0185	0.4662
Longitude	0.2569	<.0001	0.3192	<.0001	0	0.9751	0.0079	0.6339
Mean annual temperature (MAT)	0.1904	0.0009	0.5041	<.0001	0.2478	0.0002	0.1251	0.0509
Mean warmest month temperature (MWMT)	0.1305	0.0067	0.32	<.0001	0.2262	0.0004	0.0876	0.1059
Mean coldest month temperature (MCMT)	0.1779	0.0013	0.3955	<.0001	0.0056	0.602	0.0672	0.1591
Continentality (TD)	0.0491	0.1039	0.0132	0.4272	0.3746	<.0001	0.0344	0.3176
Mean annual precipitation (MAP)	0.1971	0.0007	0.3937	<.0001	0.203	0.0009	0.0153	0.5079
Mean summer precipitation (MSP)	0.1213	0.0092	0.1685	0.0031	0.0791	0.0456	0	0.981
Annual heat:moisture index (AHM)	0.2396	0.0001	0.5387	<.0001	0.2415	0.0003	0.0627	0.1741
Summer heat: moisture index (SHM)	0.1576	0.0027	0.2839	<.0001	0.1209	0.0124	0.0164	0.4928
Degree days below 0°C (D0)	0.2009	0.0006	0.5415	<.0001	0.134	0.0082	0.1232	0.0529
Degree days above 5°C (D5)	0.1525	0.0032	0.3654	<.0001	0.308	<.0001	0.1176	0.0589
Degree days below 18°C (D18)	0.1871	0.001	0.5075	<.0001	0.2459	0.0002	0.1265	0.0496
Degree days above 18°C (DD18)	0	0.979	0	0.9	0.0652	0.0706	0.0637	0.1707
Number of frost-free days (NFFD)	0.1174	0.0105	0.4509	<.0001	0.1818	<.0001	0.0914	0.0983
Julian date in which FFP begins (bFFP)	0.1203	0.0095	0.2228	0.0005	0.1047	0.0206	0.0814	0.1197
Julian date in which FFP ends (eFFP)	0.0766	0.0407	0.272	0.0001	0.1359	0.0078	0.0501	0.226
Frost-free period (FFP)	0.1061	0.0152	0.2509	0.0002	0.123	0.0116	0.0718	0.145
Precipitation as snow (PAS)	0.244	0.0001	0.5086	<.0001	0.2535	0.0002	0.0528	0.2139
Extreme minimum temperature over 30 years (EMT)	0.0289	0.2149	0.5128	<.0001	0.0401	0.159	0.0537	0.2096
Hargreaves reference evaporation (Eref)	0.2052	0.0005	0.2682	0.0001	0.2487	0.0002	0.1066	0.0731
Hargreaves climatic moisture deficit (CMD)	0.1882	0.0009	0.2964	<.0001	0.1606	0.0036	0.046	0.2464

Table A.9 Area under the curve of the receiver operating characteristic (AUC), model sensitivity (Sens), model specificity (Spec), and the number of palaeoecological records used for validation (N) for *Picea engelmannii* and *P. glauca* model projections for each time period within each GCM.

	Picea engelmannii				*Picea glauca*			
Period	N	AUC	Sens	Spec	N	AUC	Sens	Spec
Modern	6258	0.86	0.61	0.85	7168	0.89	0.61	0.84
CCM1								
6000	21	0.49	0.08	0.9	46	0.64	0.35	0.77
11000	15	0.58	0.17	0.9	7	0.37	0.08	0.76
14000	2	0.63	0.17	0.87	1	0.4	0.05	0.87
16000	3	0.78	0.49	0.86	1	0.98	0.79	0.88
21000	3	0.46	0.13	0.83	0	--	--	--
GFDL								
6000	21	0.63	0.25	0.88	46	0.71	0.44	0.82
9000	14	0.7	0.49	0.85	45	0.58	0.29	0.82
16000	3	0.4	0.08	0.85	1	0.45	0.1	0.86
21000	3	0.36	0.08	0.76	0	--	--	--

Table A.10 Allele frequencies and sample sizes for each of the nine populations of the *Picea glauca* x *P. engelmannii* hybrid zone based on 86 SNP markers. Populations ID are shown in Table 4.1.

Locus	Allele/n	FN	PG	QL	MR	EK	WK	E1	E2	E3
100_316_NS	**N**	22	10	216	187	187	108	8	13	16
	1	0	0.2	0.164	0.168	0.115	0.106	0.125	0.538	0.719
	3	1	0.8	0.836	0.832	0.885	0.894	0.875	0.462	0.281
103_455_NS	**N**	22	11	216	187	187	110	8	13	17
	1	1	0.955	0.887	0.882	0.853	0.923	0.813	1	1
	4	0	0.045	0.113	0.118	0.147	0.077	0.188	0	0
112_443_NS	**N**	22	11	216	187	187	110	8	13	17
	1	0.977	1	0.933	0.917	0.88	0.927	1	0.654	0.647
	3	0.023	0	0.067	0.083	0.12	0.073	0	0.346	0.353
114_144_S	**N**	22	11	214	185	183	110	8	13	17
	1	1	1	0.956	0.949	0.992	0.986	0.875	0.923	0.882
	3	0	0	0.044	0.051	0.008	0.014	0.125	0.077	0.118
114_248_S	**N**	22	11	216	187	184	108	8	13	17
	2	0.636	0.727	0.641	0.604	0.595	0.569	0.875	0.692	0.647
	3	0.364	0.273	0.359	0.396	0.405	0.431	0.125	0.308	0.353
11_348_S	**N**	20	9	216	187	187	110	8	13	17
	1	0.7	0.333	0.292	0.243	0.289	0.205	0.25	0	0.029
	3	0.3	0.667	0.708	0.757	0.711	0.795	0.75	1	0.971
124_495_S	**N**	22	11	216	187	187	110	8	13	17
	2	0.205	0.091	0.241	0.257	0.23	0.255	0.313	0.462	0.324
	4	0.795	0.909	0.759	0.743	0.77	0.745	0.688	0.538	0.676
124_656_S	**N**	22	10	215	187	187	110	8	11	17
	1	0	0	0.114	0.134	0.078	0.155	0.188	0.227	0.059
	3	1	1	0.886	0.866	0.922	0.845	0.813	0.773	0.941

Table A.10 (Continued) Allele frequencies and sample sizes for each of the nine populations of the *Picea glauca* x *P. engelmannii* hybrid zone based on 86 SNP markers. Populations ID are shown in Table 4.1.

Locus	Allele/n	FN	PG	QL	MR	EK	WK	E1	E2	E3
125_312_S	**N**	22	11	216	187	187	110	8	13	17
	2	0	0.136	0.123	0.102	0.126	0.164	0.188	0.038	0.118
	4	1	0.864	0.877	0.898	0.874	0.836	0.813	0.962	0.882
127_273_S	**N**	20	11	216	187	187	110	8	13	16
	2	0.675	0.682	0.394	0.393	0.481	0.377	0.188	0.077	0
	4	0.325	0.318	0.606	0.607	0.519	0.623	0.813	0.923	1
132_78_S	**N**	22	11	216	187	187	110	8	13	17
	1	0.114	0.5	0.204	0.27	0.171	0.182	0.125	0.077	0
	3	0.886	0.5	0.796	0.73	0.829	0.818	0.875	0.923	1
133_39_S	**N**	22	10	214	186	187	110	8	13	17
	1	0.773	0.65	0.523	0.532	0.441	0.468	0.375	0.846	0.794
	3	0.227	0.35	0.477	0.468	0.559	0.532	0.625	0.154	0.206
133_418_S	**N**	22	11	215	187	187	110	8	13	17
	2	1	1	0.935	0.947	0.928	0.955	0.938	1	0.971
	4	0	0	0.065	0.053	0.072	0.045	0.063	0	0.029
133_553_NS	**N**	22	11	216	187	187	110	8	13	17
	2	0.636	0.364	0.433	0.393	0.471	0.332	0.5	0.115	0.176
	4	0.364	0.636	0.567	0.607	0.529	0.668	0.5	0.885	0.824
135_122_NS	**N**	22	11	216	187	187	110	8	13	17
	1	0.136	0.227	0.132	0.096	0.08	0.086	0.188	0.192	0.176
	3	0.864	0.773	0.868	0.904	0.92	0.914	0.813	0.808	0.824
136_421_S	**N**	22	11	216	185	186	110	8	13	16
	2	1	1	0.921	0.924	0.973	0.95	0.875	0.962	0.938
	4	0	0	0.079	0.076	0.027	0.05	0.125	0.038	0.063

Table A.10 (Continued) Allele frequencies and sample sizes for each of the nine populations of the *Picea glauca* x *P. engelmannii* hybrid zone based on 86 SNP markers. Populations ID are shown in Table 4.1.

Locus	Allele/n	FN	PG	QL	MR	EK	WK	E1	E2	E3
13_496_NS	**N**	22	11	216	187	186	110	8	13	17
	2	0.864	0.727	0.324	0.267	0.231	0.073	0.063	0	0
	4	0.136	0.273	0.676	0.733	0.769	0.927	0.938	1	1
141_349_S	**N**	22	11	216	187	187	110	8	13	17
	1	0.591	0.5	0.572	0.559	0.674	0.65	0.813	0.923	1
	3	0.409	0.5	0.428	0.441	0.326	0.35	0.188	0.077	0
144_441_S	**N**	22	11	216	187	187	110	8	13	17
	1	0.977	0.955	0.972	0.984	0.989	0.968	1	1	1
	3	0.023	0.045	0.028	0.016	0.011	0.032	0	0	0
14_301_NS	**N**	22	11	216	187	187	110	8	13	17
	3	0	0	0.016	0.005	0	0.027	0	0.038	0.029
	4	1	1	0.984	0.995	1	0.973	1	0.962	0.971
162_199_S	**N**	22	11	216	187	187	110	8	13	17
	1	0.295	0.318	0.174	0.131	0.184	0.173	0.188	0.5	0.382
	4	0.705	0.682	0.826	0.869	0.816	0.827	0.813	0.5	0.618
164_465_S	**N**	22	11	216	187	187	110	8	13	17
	2	1	0.955	0.977	0.973	0.984	0.986	1	1	1
	4	0	0.045	0.023	0.027	0.016	0.014	0	0	0
169_375_NS	**N**	22	11	196	169	174	94	8	8	10
	1	0.5	0.636	0.462	0.453	0.489	0.394	0.375	0.625	0.7
	2	0.5	0.364	0.538	0.547	0.511	0.606	0.625	0.375	0.3
179_114_S	**N**	22	11	216	185	187	110	8	13	17
	2	0	0.091	0.058	0.111	0.027	0.059	0	0.038	0.059
	4	1	0.909	0.942	0.889	0.973	0.941	1	0.962	0.941

Table A.10 (Continued) Allele frequencies and sample sizes for each of the nine populations of the *Picea glauca* x *P. engelmannii* hybrid zone based on 86 SNP markers. Populations ID are shown in Table 4.1.

Locus	Allele/n	FN	PG	QL	MR	EK	WK	E1	E2	E3
179_319_NS	**N**	22	11	216	187	187	110	8	13	17
	2	0	0.136	0.014	0.021	0.003	0	0	0	0
	4	1	0.864	0.986	0.979	0.997	1	1	1	1
179_699_S	**N**	22	11	215	187	187	110	8	13	17
	1	1	0.864	0.956	0.949	0.987	0.977	0.938	1	1
	3	0	0.136	0.044	0.051	0.013	0.023	0.063	0	0
191_162_S	**N**	21	10	216	187	187	110	8	13	17
	2	0.667	0.65	0.78	0.848	0.917	0.927	0.938	1	1
	4	0.333	0.35	0.22	0.152	0.083	0.073	0.063	0	0
194_470_S	**N**	22	10	216	187	187	110	7	12	17
	1	0	0.05	0.002	0.016	0	0	0	0	0
	4	1	0.95	0.998	0.984	1	1	1	1	1
195_356_NS	**N**	22	11	216	187	187	110	8	13	17
	2	0	0.182	0.306	0.369	0.388	0.482	0.563	0.192	0.059
	3	1	0.818	0.694	0.631	0.612	0.518	0.438	0.808	0.941
198_447_S	**N**	22	11	214	181	186	107	7	13	17
	2	1	0.818	0.671	0.66	0.68	0.612	0.714	0.308	0.294
	4	0	0.182	0.329	0.34	0.32	0.388	0.286	0.692	0.706
19_567_S	**N**	22	11	216	187	187	110	8	13	17
	1	0	0.091	0.044	0.062	0.11	0.1	0.063	0.038	0
	3	1	0.909	0.956	0.939	0.89	0.9	0.938	0.962	1
205_292_S	**N**	22	11	216	187	187	110	8	13	17
	1	0.818	0.591	0.5	0.492	0.412	0.445	0.125	0.731	0.5
	2	0.182	0.409	0.5	0.508	0.588	0.555	0.875	0.269	0.5

Table A.10 (Continued) Allele frequencies and sample sizes for each of the nine populations of the *Picea glauca* x *P. engelmannii* hybrid zone based on 86 SNP markers. Populations ID are shown in Table 4.1.

Locus	Allele/n	FN	PG	QL	MR	EK	WK	E1	E2	E3
206_435_NS	**N**	22	11	216	187	187	110	8	13	17
	1	0.364	0.682	0.887	0.909	0.842	0.927	1	1	1
	4	0.636	0.318	0.113	0.091	0.158	0.073	0	0	0
209_523_S	**N**	22	11	207	185	183	107	8	13	17
	2	0.545	0.455	0.466	0.438	0.451	0.327	0.125	0.192	0.294
	3	0.455	0.545	0.534	0.562	0.549	0.673	0.875	0.808	0.706
20_374_NS	**N**	22	11	216	187	187	109	8	13	17
	2	1	0.955	0.965	0.933	0.952	0.959	0.938	1	1
	3	0	0.045	0.035	0.067	0.048	0.041	0.063	0	0
213_153_S	**N**	21	11	215	184	181	110	8	13	17
	2	0.143	0.409	0.456	0.342	0.395	0.441	0.563	0.615	0.882
	4	0.857	0.591	0.544	0.658	0.605	0.559	0.438	0.385	0.118
213_330_S	**N**	22	11	215	187	187	110	8	13	17
	2	1	0.955	0.974	0.955	0.997	0.977	1	1	1
	4	0	0.045	0.026	0.045	0.003	0.023	0	0	0
213_468_NS	**N**	22	11	214	187	184	110	6	13	17
	2	0.864	0.591	0.547	0.655	0.601	0.559	0.417	0.385	0.118
	3	0.136	0.409	0.453	0.345	0.399	0.441	0.583	0.615	0.882
213_72_S	**N**	22	11	216	187	187	110	8	13	17
	3	0	0.045	0.025	0.045	0.003	0.023	0	0	0
	4	1	0.955	0.975	0.955	0.997	0.977	1	1	1
214_180_S	**N**	22	11	216	187	183	110	8	13	17
	2	1	0.773	0.949	0.93	0.937	0.959	1	1	0.971
	4	0	0.227	0.051	0.07	0.063	0.041	0	0	0.029

Table A.10 (Continued) Allele frequencies and sample sizes for each of the nine populations of the *Picea glauca* x *P. engelmannii* hybrid zone based on 86 SNP markers. Populations ID are shown in Table 4.1.

Locus	Allele/n	FN	PG	QL	MR	EK	WK	E1	E2	E3
214_558_S	**N**	22	11	216	187	187	110	8	13	17
	1	0	0.227	0.053	0.07	0.072	0.041	0	0	0.029
	3	1	0.773	0.947	0.93	0.928	0.959	1	1	0.971
215_132_S	**N**	22	11	216	187	187	109	8	12	17
	1	0.909	0.636	0.593	0.457	0.551	0.509	0.5	0.667	0.765
	3	0.091	0.364	0.407	0.543	0.449	0.491	0.5	0.333	0.235
222_305_S	**N**	22	11	213	186	185	107	8	13	17
	1	0	0.045	0.103	0.177	0.13	0.173	0	0	0
	3	1	0.955	0.897	0.823	0.87	0.827	1	1	1
222_370_S	**N**	22	11	216	186	187	110	8	13	17
	1	0	0.182	0.171	0.156	0.203	0.177	0.125	0.308	0.412
	3	1	0.818	0.829	0.844	0.797	0.823	0.875	0.692	0.588
234_171_S	**N**	19	10	203	179	179	102	8	13	15
	1	0.5	0.35	0.362	0.327	0.341	0.373	0.313	0.192	0.167
	3	0.5	0.65	0.638	0.673	0.659	0.627	0.688	0.808	0.833
242_241_S	**N**	22	11	216	187	185	109	7	13	17
	2	0.409	0.318	0.306	0.257	0.222	0.239	0.214	0	0
	4	0.591	0.682	0.694	0.743	0.778	0.761	0.786	1	1
244_118_NS	**N**	22	11	215	187	186	109	8	13	17
	1	0.886	0.591	0.621	0.636	0.718	0.546	0.438	0.154	0.206
	3	0.114	0.409	0.379	0.364	0.282	0.454	0.563	0.846	0.794
245_170_NS	**N**	21	10	212	185	186	109	8	13	17
	2	0.786	0.65	0.434	0.422	0.341	0.266	0.375	0.385	0.265
	3	0.214	0.35	0.566	0.578	0.659	0.734	0.625	0.615	0.735

Table A.10 (Continued) Allele frequencies and sample sizes for each of the nine populations of the *Picea glauca* x *P. engelmannii* hybrid zone based on 86 SNP markers. Populations ID are shown in Table 4.1.

Locus	Allele/n	FN	PG	QL	MR	EK	WK	E1	E2	E3
245_281_S	**N**	22	11	216	187	187	110	8	13	17
	3	0.114	0.273	0.47	0.495	0.578	0.582	0.563	0.269	0.412
	4	0.886	0.727	0.53	0.505	0.422	0.418	0.438	0.731	0.588
245_98_NS	**N**	22	11	216	185	186	110	8	13	17
	2	0.455	0.636	0.662	0.605	0.707	0.75	0.625	0.846	0.824
	4	0.545	0.364	0.338	0.395	0.293	0.25	0.375	0.154	0.176
249_648_S	**N**	22	11	216	187	187	110	8	12	17
	2	1	0.909	0.838	0.807	0.848	0.868	0.875	1	0.971
	4	0	0.091	0.162	0.193	0.152	0.132	0.125	0	0.029
252_200_NS	**N**	22	11	216	187	187	110	8	13	17
	2	0.545	0.591	0.414	0.479	0.414	0.436	0.25	0.654	0.529
	3	0.455	0.409	0.586	0.521	0.586	0.564	0.75	0.346	0.471
259_736_NS	**N**	22	11	216	187	187	110	8	13	17
	1	1	0.955	0.947	0.947	0.976	0.991	0.938	1	1
	3	0	0.045	0.053	0.053	0.024	0.009	0.063	0	0
260_264_S	**N**	22	11	215	186	186	107	8	13	17
	2	0	0.045	0.053	0.054	0.024	0.009	0.063	0	0
	4	1	0.955	0.947	0.946	0.976	0.991	0.938	1	1
260_84_S	**N**	22	11	216	187	187	110	8	13	17
	2	0	0.045	0.053	0.053	0.024	0.009	0.063	0	0
	4	1	0.955	0.947	0.947	0.976	0.991	0.938	1	1
273507_S	**N**	21	11	216	187	187	110	8	13	17
	2	0.357	0.455	0.535	0.567	0.513	0.518	0.5	0.808	0.853
	4	0.643	0.545	0.465	0.433	0.487	0.482	0.5	0.192	0.147

Table A.10 (Continued) Allele frequencies and sample sizes for each of the nine populations of the *Picea glauca* x *P. engelmannii* hybrid zone based on 86 SNP markers. Populations ID are shown in Table 4.1.

Locus	Allele/n	FN	PG	QL	MR	EK	WK	E1	E2	E3
27_420_S	**N**	22	11	216	187	187	109	5	9	11
	1	1	0.909	0.88	0.885	0.89	0.881	0.5	1	0.818
	4	0	0.091	0.12	0.115	0.11	0.119	0.5	0	0.182
27_711_S	**N**	21	10	216	187	186	107	8	13	17
	2	0.5	0.6	0.676	0.679	0.634	0.748	0.813	0.923	0.971
	4	0.5	0.4	0.324	0.321	0.366	0.252	0.188	0.077	0.029
27_99_S	**N**	22	11	215	187	186	108	8	13	17
	2	0	0.091	0.095	0.123	0.108	0.097	0.313	0.5	0.471
	4	1	0.909	0.905	0.877	0.892	0.903	0.688	0.5	0.529
288_302_NS	**N**	22	11	215	187	187	110	8	13	17
	3	0.955	0.636	0.477	0.358	0.369	0.223	0.313	0.692	0.794
	4	0.045	0.364	0.523	0.642	0.631	0.777	0.688	0.308	0.206
288_628_NS	**N**	22	11	216	187	187	110	8	13	17
	1	0.955	0.591	0.442	0.334	0.31	0.2	0.313	0.654	0.765
	3	0.045	0.409	0.558	0.666	0.69	0.8	0.688	0.346	0.235
295_78_S	**N**	22	11	216	187	187	110	8	13	17
	1	0.091	0.182	0.306	0.209	0.08	0.145	0	0	0
	3	0.909	0.818	0.694	0.791	0.92	0.855	1	1	1
29_177_S	**N**	22	11	216	187	187	110	8	13	17
	2	1	0.909	0.877	0.874	0.939	0.909	0.875	1	1
	4	0	0.091	0.123	0.126	0.062	0.091	0.125	0	0
29_592_S	**N**	22	11	216	187	185	110	8	12	14
	3	0.955	0.818	0.831	0.816	0.881	0.85	0.813	0.917	0.821
	4	0.045	0.182	0.169	0.184	0.119	0.15	0.188	0.083	0.179

Table A.10 (Continued) Allele frequencies and sample sizes for each of the nine populations of the *Picea glauca* x *P. engelmannii* hybrid zone based on 86 SNP markers. Populations ID are shown in Table 4.1.

Locus	Allele/n	FN	PG	QL	MR	EK	WK	E1	E2	E3
30_423_S	**N**	21	11	209	178	185	109	8	12	17
	1	0.31	0.136	0.222	0.208	0.265	0.193	0	0.125	0.059
	3	0.69	0.864	0.778	0.792	0.735	0.807	1	0.875	0.941
41_150_NS	**N**	21	10	214	184	181	108	7	13	16
	2	0.167	0.35	0.315	0.326	0.307	0.384	0.571	0.385	0.25
	3	0.833	0.65	0.685	0.674	0.693	0.616	0.429	0.615	0.75
42_150_NS	**N**	16	8	196	170	172	106	7	13	16
	3	0.875	0.75	0.663	0.494	0.494	0.429	0.429	0.077	0.063
	4	0.125	0.25	0.337	0.506	0.506	0.571	0.571	0.923	0.938
45_1067_NS	**N**	22	11	215	187	187	110	8	13	17
	1	0.977	0.636	0.616	0.543	0.46	0.295	0.125	0.154	0.029
	3	0.023	0.364	0.384	0.457	0.54	0.705	0.875	0.846	0.971
46_623_NS	**N**	0	0	215	187	187	110	0	0	0
	3	0	0	0.04	0.056	0.027	0.073	0	0	0
	4	0	0	0.96	0.944	0.973	0.927	0	0	0
50_135_S	**N**	22	11	216	187	187	110	8	13	17
	2	0.432	0.364	0.308	0.316	0.281	0.259	0.25	0.192	0.147
	4	0.568	0.636	0.692	0.684	0.719	0.741	0.75	0.808	0.853
50_405_S	**N**	22	11	216	186	187	110	6	12	17
	1	0.159	0.364	0.444	0.347	0.433	0.514	0.583	0.417	0.588
	3	0.841	0.636	0.556	0.653	0.567	0.486	0.417	0.583	0.412
51_36_S	**N**	22	11	215	186	185	109	8	13	17
	3	0.977	0.818	0.723	0.785	0.805	0.803	0.875	0.654	0.765
	4	0.023	0.182	0.277	0.215	0.195	0.197	0.125	0.346	0.235

Table A.10 (Continued) Allele frequencies and sample sizes for each of the nine populations of the *Picea glauca* x *P. engelmannii* hybrid zone based on 86 SNP markers. Populations ID are shown in Table 4.1.

Locus	Allele/n	FN	PG	QL	MR	EK	WK	E1	E2	E3
51_409_NS	**N**	20	10	216	187	183	110	8	13	17
	1	0.05	0.15	0.294	0.225	0.199	0.195	0.125	0.346	0.265
	4	0.95	0.85	0.706	0.775	0.801	0.805	0.875	0.654	0.735
56_206_S	**N**	22	11	216	186	185	110	8	13	15
	1	0.295	0.364	0.398	0.317	0.414	0.405	0.438	0.538	0.667
	3	0.705	0.636	0.602	0.683	0.586	0.595	0.563	0.462	0.333
5_1408_NS	**N**	22	11	216	186	187	110	8	13	17
	1	0.023	0.091	0.125	0.121	0.128	0.186	0.125	0.038	0
	3	0.977	0.909	0.875	0.879	0.872	0.814	0.875	0.962	1
68_286_S	**N**	22	11	212	184	184	109	7	13	17
	2	1	0.955	0.226	0.274	0.163	0.234	1	1	1
	4	0	0.045	0.774	0.726	0.837	0.766	0	0	0
69_753_S	**N**	6	7	212	182	181	106	6	2	0
	1	1	0.071	0.427	0.393	0.401	0.472	0	0.5	0
	3	0	0.929	0.573	0.607	0.599	0.528	1	0.5	0
71_365_NS	**N**	22	11	216	187	187	110	8	13	17
	1	1	0.818	0.947	0.963	0.93	0.932	1	1	1
	4	0	0.182	0.053	0.037	0.07	0.068	0	0	0
84_370_NS	**N**	22	11	216	187	187	109	8	13	17
	3	0	0.091	0.132	0.166	0.094	0.234	0.125	0.115	0.059
	4	1	0.909	0.868	0.834	0.906	0.766	0.875	0.885	0.941
85_279_S	**N**	22	11	216	186	187	110	8	13	17
	1	0.864	0.545	0.572	0.503	0.61	0.55	0.25	0.038	0
	3	0.136	0.455	0.428	0.497	0.39	0.45	0.75	0.962	1

Table A.10 (Continued) Allele frequencies and sample sizes for each of the nine populations of the *Picea glauca* x *P. engelmannii* hybrid zone based on 86 SNP markers. Populations ID are shown in Table 4.1.

Locus	Allele/n	FN	PG	QL	MR	EK	WK	E1	E2	E3
86_438_S	**N**	17	10	216	187	187	110	8	13	17
	2	0.118	0.3	0.481	0.567	0.612	0.632	0.75	0.808	0.882
	3	0.882	0.7	0.519	0.433	0.388	0.368	0.25	0.192	0.118
89_300_NS	**N**	22	11	216	187	187	110	8	13	17
	1	0	0.091	0.132	0.163	0.094	0.236	0.125	0.115	0.059
	2	1	0.909	0.868	0.837	0.906	0.764	0.875	0.885	0.941
89_37_NS	**N**	22	11	216	187	187	110	8	13	17
	1	0	0.091	0.132	0.163	0.094	0.236	0.125	0.115	0.059
	3	1	0.909	0.868	0.837	0.906	0.764	0.875	0.885	0.941
97_489_S	**N**	22	11	216	187	187	110	8	13	17
	2	1	1	0.931	0.933	0.936	0.932	0.875	1	0.971
	4	0	0	0.069	0.067	0.064	0.068	0.125	0	0.029
SS_CO483349-contig3-358	**N**	22	11	202	179	185	109	8	13	15
	2	0.545	0.636	0.733	0.816	0.811	0.867	0.938	0.615	0.667
	4	0.455	0.364	0.267	0.184	0.189	0.133	0.063	0.385	0.333
SS_CO483349-contig3-496	**N**	22	11	215	187	187	110	7	13	17
	1	0.136	0.227	0.267	0.35	0.345	0.355	0.357	0.115	0.059
	3	0.864	0.773	0.733	0.65	0.655	0.645	0.643	0.885	0.941

Figure A.11 Analysis of population structure in the *Picea glauca* x *P. engelmannii* hybrid zone. *TESS* cluster memberships from K=2 to K=6.

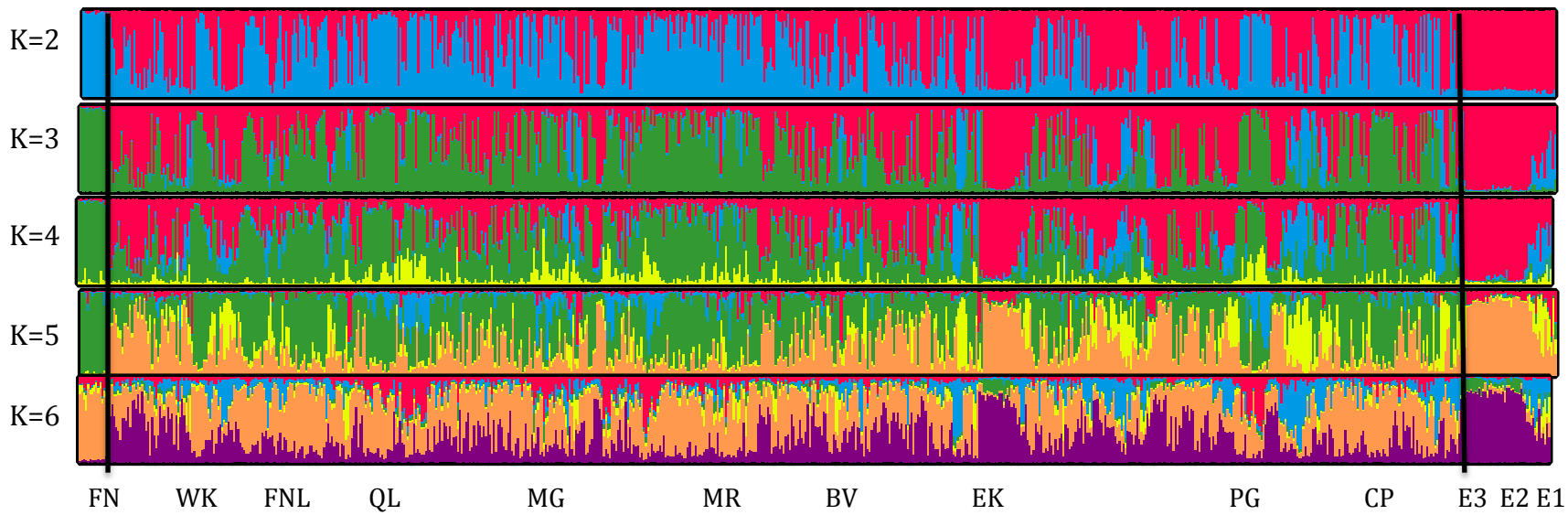

Figure A.12 Analysis of population structure in the *Picea glauca* x *P. engelmannii* hybrid zone using ten microsatellite markers. A) Delta K, for 20 *STRUCTURE* runs with K ranging from 1 to 10, Evanno et al. criterion indicates that there are two clusters in the data set (K=2). B) DIC for 30 *TESS* runs with Kmax ranging from 2 to 14.

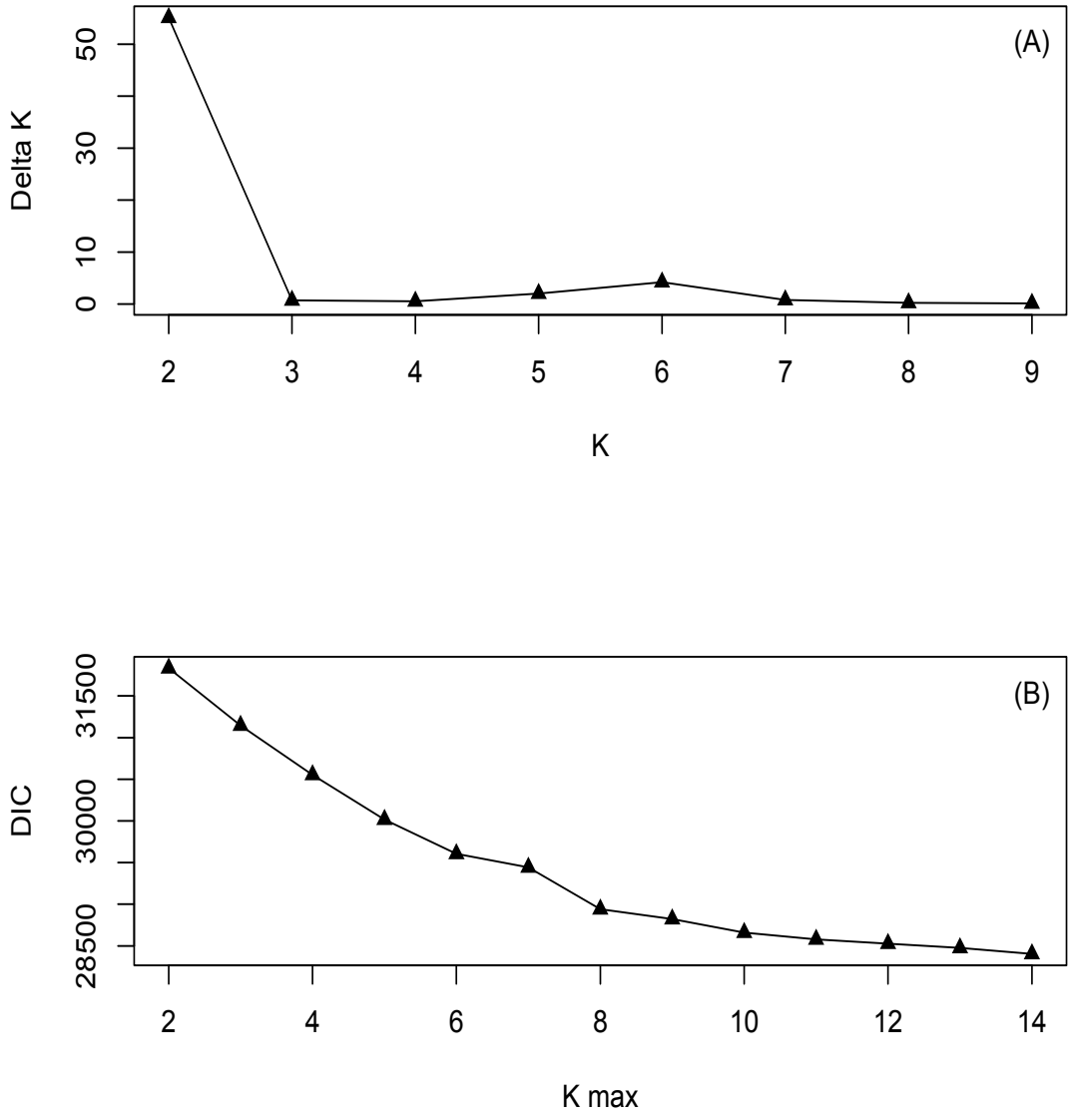

Figure A.13 Hybrid classes in the *Picea glauca* x *P. engelmannii* hybrid zone based on ten microsatellite markers. Individual hybrid index was divided into 11 classes with an interval of 0.1, where 0 is pure *P. glauca* and 1 is pure *P. engelmannii*. Only sympatric individuals are shown, allopatric reference individuals (pure *P. glauca* and *P. engelmannii*) used to calculate hybrid index were not included.

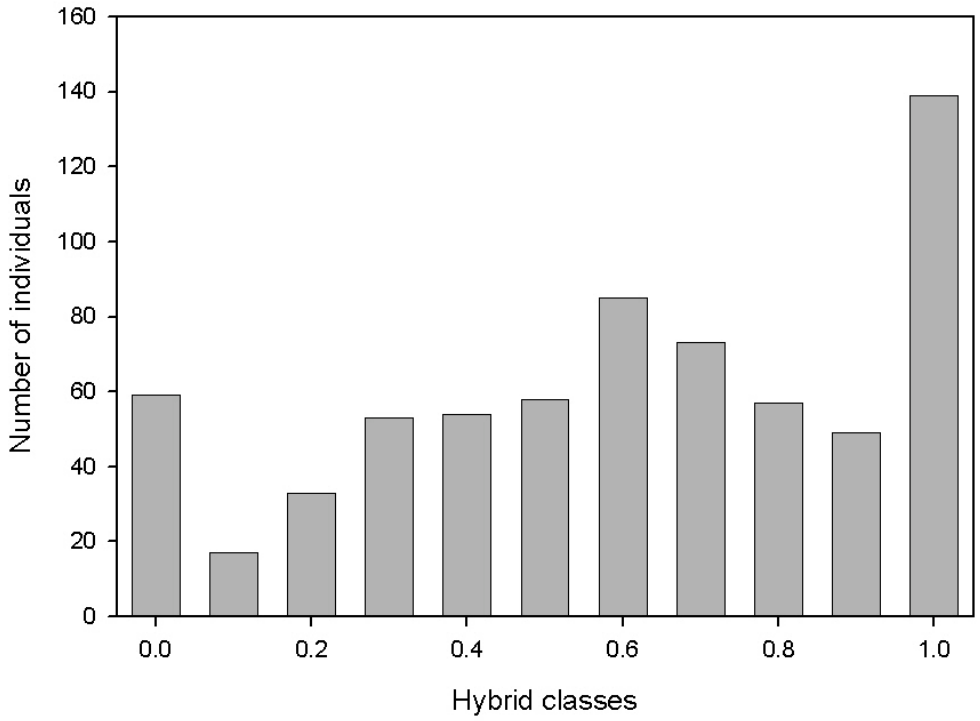

Figure A.14 Analysis of population structure in the *Picea glauca* x *P. engelmannii* hybrid zone using SNP markers. A) Delta K, for 20 *STRUCTURE* runs with K ranging from 1 to 16, Evanno et al criterion indicates that there are two clusters in the data set (K=2). B) DIC for 20 *TESS* runs with Kmax ranging from 2 to 14.

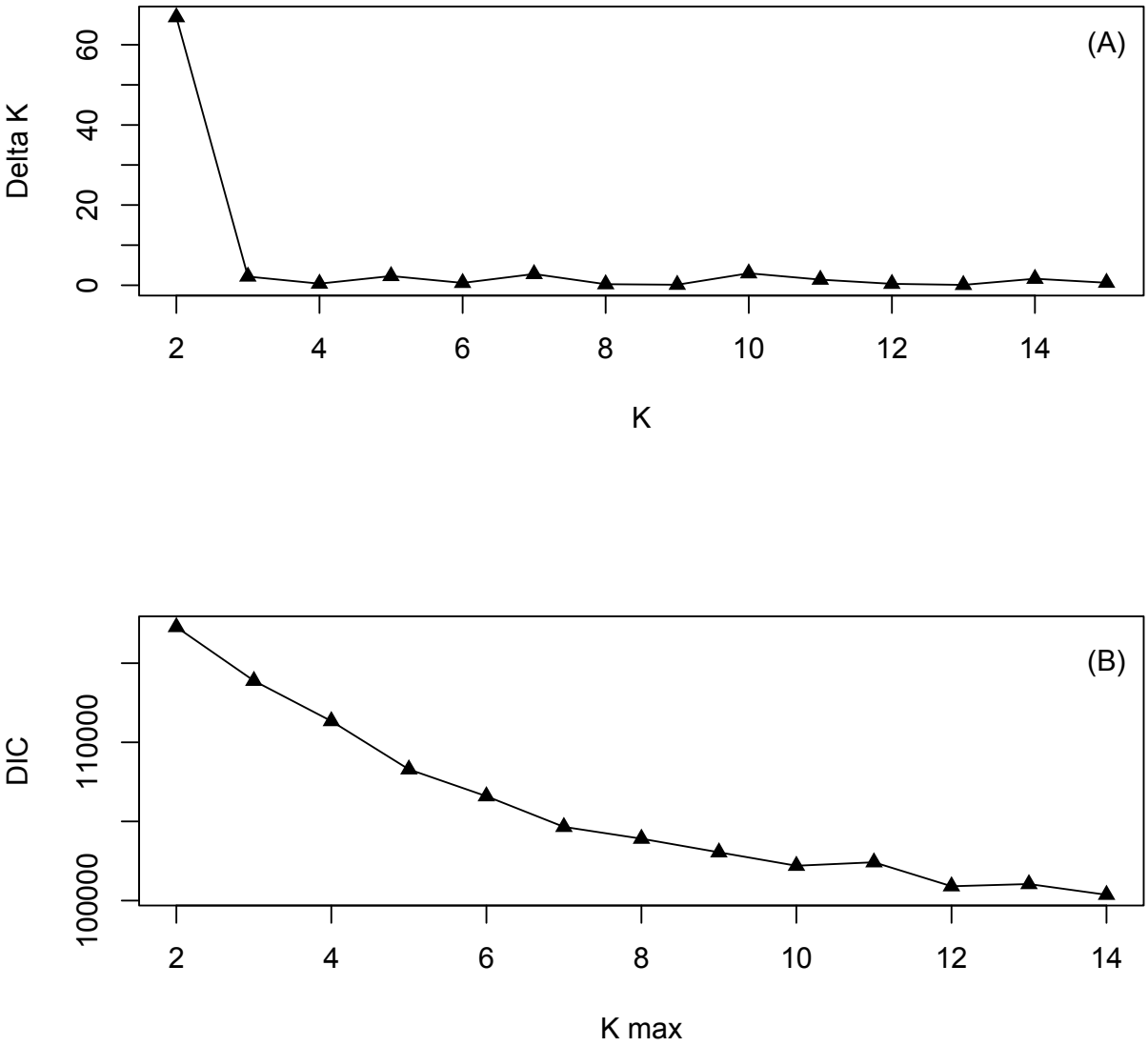

Figure A.15 Differences between *Picea glauca*, *P. engelmannii* and hybrids based on 86 SNP allele frequencies generated with *SAS* Principal Component Analysis. Circles represent *P. engelmannii*; crosses, *P. glauca*, and triangles, hybrid individuals.