Ecological and quantitative genetics of *Populus tremuloides* in western Canada

by

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Abstract

Aspen is a widespread forest tree of high economic and ecological importance in western Canada. The species has also been subject to tree improvement efforts over the past two decades to increase productivity of the forested land base. Successful selection and breeding programs rely on both accurate estimates of the expected genetic gain from selection for commercial traits as well as correlated responses of other traits that may be important for fitness. This thesis investigates geographic patterns of genetic variation observed in a reciprocal transplant experiment with 43 provenances and five sites across western Canada. In a second series of experiments, geographically restricted to Alberta, genetic parameters for growth and adaptive traits are assessed in ten progeny trials containing more than 30,000 trees with known pedigrees.

The reciprocal transplant experiment revealed strong patterns of local suboptimality, with increases in productivity as a result of experimental long-distance transfers in northwest direction. For example, provenances moved 1,600 km northwest from Minnesota to central Alberta (a shift of 7° latitude to the north) produced almost twice the biomass of local sources. Increased growth was not associated with lower survival rates. Bud break in provenances transferred northwest generally occurred slightly later than in local sources, suggesting decreased risk of spring frost injury. Leaf abscission was later in provenances transferred in northwest direction, but they appeared to be very frost hardy, well ahead of very rare early fall frost events.

A potential explanation for suboptimality is the longevity of aspen clones, where populations could be adapted to climates present during post-glacial recolonization. This hypothesis was explored with habitat reconstructions to the last glacial maximum, which indicated that western Canadian populations likely originated from eastern refugia. We conclude that observed suboptimality likely represents an adaptational lag and benefits in productivity outweigh potential risks

associated with long-distance northward transfer of aspen planting stock under both current and projected future climate conditions.

Progeny trials geographically limited to Alberta seed sources and planting sites revealed high within-population variation in both growth and adaptive traits that was not strongly associated with climatic or geographic variables. Heritabilities for growth and adaptive traits were low to moderate, but progeny trials revealed strong genetic correlations between growth and phenology, with tall trees and high survival being associated with early budbreak and late leaf abscission, which mirrors the results from the provenance trial series across western Canada. While genetic gains in growth traits will be due to expanding the growing season, the increased risk of frost damage in spring and fall does not appear a critical issue, particularly under projected climate warming.

Preface

A version of Chapter 2 has been published as: Schreiber, S.G., Ding, C., Hamann, A., Hacke, U.G., Thomas, B.R., & Brouard, J.S., 2013. Frost hardiness versus growth performance in trembling aspen: an experimental test of assisted migration. *Journal of Applied Ecology* **50**: 939–949. SGS and CD contributed equally to this paper as specified in the acknowledgements. AH, SGS, and CD conceived and designed the study. CD led the field measurements with SGS's assistance. Laboratory work on frost hardiness was executed by CD. The data analysis was a 50% joint effort between CD and SGS. SGS wrote the first draft of the paper with CD's assistance. All coauthors contributed to revisions.

A version of Chapter 3 has been submitted as: Ding, C., Schreiber, S.G., Roberts, D.R., Hamann, A. & Brouard, J.S., 2015. Post-glacial biogeography of trembling aspen inferred from genetic structure, genetic diversity, and habitat models. *Journal of Biogeography* (manuscript number JBI-14-0562). AH, CD, and JSB conceived and designed the study. CD conducted the analysis of genetic structure and genetic diversity. SGS and DRR contributed the species distribution modeling. AH and JSB contributed data. CD wrote the first draft of the paper, and all coauthors contributed to revisions.

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Dedication

To my parents.

Thank you for your dedication in raising and educating me.

Thank you for doing all you could to make my life full of opportunities.

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Chapter 1 - Introduction

1.1 General background

Ford (1971) described the field of ecological genetics as the study of "adjustments and adaptations of wild populations to their natural environment". In ecological genetics, two concepts are distinguished, acclimation and adaptation. Adaptation is an evolutionary process that results in genetically distinct populations. In contrast, acclimation (also sometimes referred to as plasticity) is a phenotypic response to different environments that does not involve genetic changes. Typically, acclimation refers to a phenotypic response to environmental change over time, whereas plasticity refers to the ability of an organism to acclimate to different biotic and abiotic environmental conditions (Nicotra et al. 2010; Pigliucci 2005). The degree of plasticity contributes to the fitness of the genotype and might therefore be under genetic selection itself.

Widespread tree species are usually composed of many locally adapted sub-populations, which are described by a variety of terms, including clines, ecotypes, varieties, or subspecies. A cline is defined as a continuous variation of genotypes along an environmental gradient and typically refers to observations of a single trait. In widespread tree species, this is a common type of genetic differentiation caused by gene flow. For example, in *Pinus contorta* provenances from the western United States show altitudinal clines in growth potential, with populations with the highest growth coming from the lowest elevation (Rehfeldt 1988). Another example for a cline in an adaptive trait is growth cessation in

Tsuga heterophylla, which is 4.5 days earlier per degree of latitude between 38° N and 60° N (Kuser 1980). Clines of traits in trees typically occur in species that span a large latitudinal or altitudinal range.

An ecotype is a population adapted to a particular habitat or ecological niche, which is not gradually differentiated (White et al. 2007). This can be caused by limited gene flow among disjunct populations. Ecotypes typically involve genetic differentiation in several traits. For example, in *Tsuga heterophylla* three ecotypes are found in common garden experiments: Coastal, Cascade Mountains and the Rocky Mountains. The bud set of the Coastal ecotypes are about 30 days earlier than that of the other disjunct ecotypes, and the survival of these ecotypes differed (Kuser 1980). Subtle differentiations in adaptive traits are found among ecotypes, which are not easily distinguishable in natural forests.

Varieties, or subspecies in forestry, are defined as distinct populations of species that not only show discrepancies in physiology, phenology, or growth, but also visible morphological differences in common garden environments. Subspecies are more frequently used in forestry while varieties are often used in agriculture. A good example is *Pseudotsuga menziesii* that has two main varieties: the coastal (*Pseudotsuga menziesii* var. *menziesii*) Douglas-fir and the Rocky Mountain (or interior) Douglas-fir (*Pseudotsuga menziesii* var. *glauca*). The variety *menziesii* have higher growth rate and tree size when mature, while the variety *glauca* express higher frost tolerance and hardiness due to the drier and harsher local winter climate (Hermann and Lavender 1990). ‘Variety’ as a term is less often used in forest genetics than ‘subspecies’.

Subspecies are distinct subdivisions which are geographically isolated within species. Subspecies can mutually hybridize and are not reproductively isolated through biological mechanisms. For example, *Pinus contorta* consists of subspecies divided by the differences of traits such as the morphology of cones, seeds and needles. These subspecies include shore pine (*Pinus contorta* subsp. *contorta*), Bolander pine (*Pinus contorta* subsp. *bolanderi*), tamarack pine (*Pinus contorta* subsp. *murrayana*), and interior lodgepole pine (*Pinus contorta* subsp. *latifolia*) (Wheeler et al. 1983). However, varieties and subspecies can be used interchangeably in *Pinus contorta* for taxonomic and practical purposes.

Species are distinguished from subspecies by a higher degree of reproductive isolation. Usually, different species have biological reproductive barriers, but there are exceptions. For example, in hybrid-zones, the interior spruce complex includes two sympatric species, white spruce and Engelmann spruce (*Picea glauca* and *Picea Engelmannii*), that extensively hybridize (Daubenmire 1974). In forest resources management, these two species are typically treated as one species complex.

1.2 Adaptive traits in forest trees

A number of adaptive traits are commonly assessed in trees, including spring phenological traits, which are often measured by the date of bud break or leaf unfolding (Vitasse et al. 2009b), timing of the loss of cold hardiness (Aitken and Adams 1997) or timing of dormancy release (Beck et al. 1995). Dormancy release results in increased respiration rates and photosynthetic efficiency (Beck et al. 1995). Another important phenological adaptive trait is the timing of bud set or

leaf senescence, marking the end of the growth cessation (Rohde et al. 2011; St Clair et al. 2005). Leaf senescence can be measured by leaf coloration and abscission (Fracheboud et al. 2009; Ibanez et al. 2010b). As well, the timing of frost hardiness onset is a critical component of fall phenology (Aitken and Adams 1997; O'Neill et al. 2001). Besides these phenological traits that can be relatively easily assessed, many other physiological and anatomical traits relate d to drought resistance, salt tolerance, pest and disease resistance, herbivore defenses, etc. have adaptive value.

1.2.1 Spring phenology

The adaptive traits listed above are often complex traits that arise from several underlying physiological mechanisms that may be independently inherited. For instance, bud break of trees is the initiation of shoot growth in spring at the apical meristem and the formation of new leaves after the release from winter dormancy (van Volkenhrugh and Taylor 1996). The timing of bud break in spring is usually controlled by two major mechanisms: a chilling requirement and a heat sum requirement (Korner and Basler 2010). Temperate trees have moderate to strong chilling requirement before heat accumulation begins (Polgar et al. 2014). Boreal trees such as *Populus tremuloides* break bud mainly in response to accumulated heat (Li et al. 2010). In some cases, such as the genus *Fagus*, timing of bud break is primarily controlled by daylight length and is to a lesser degree modified by thermal conditions (Korner and Basler 2010).

1.2.2 Fall phenology

Similarly, the timing of bud set is a complex physiological process. During bud set, trees respond to the external shortened daylight length by the endogenous phytohormones, such as cytokinins and gibberellic acid (Horvath et al. 2003). In *Populus tremula*, genetic variation in phytochrome genes have been associated with latitudinal clines in bud set (Ingvarsson et al. 2006). Genes related to abscisic acid production have been shown to play roles in regulating bud set in poplar trees (Rohde et al. 2002). Bud set timing is often correlated with growth, representing a tradeoff between utilizing the growing season for photosynthesis and timely recycling of nutrients before fall frosts (Bohlenius et al. 2006).

Although leaf senescence is not affected by the same photoperiod as bud set, we consider both leaf senescence and bud set as autumn phenology. The fall color and leaf fall are easier to observe than terminal bud set. Multiple pigments cause leaf coloration: the anthocyanins, carotenoids and chlorophyll. Carotenoids degrade while anthocyanin accumulates during fall color (Keskitalo et al. 2005). Chlorophyll drastically decreases in plastids and vacuoles of mesophylls until the cytoplasm is gone (Keskitalo et al. 2005). In *Populus*, fall color shows often yellow colors and occasional red, which appears before leaf abscission. However, species such as *Ulmus americana* only express yellow and brown colors. High night temperature may delay fall coloration given the same photoperiods.

The underlying physiological mechanisms that control the timing of leaf coloration and leaf senescence are phytochromes, a group of pigments that sense the discrepancies of light quality and night length. For example, two forms of

Phytochrome A vary between P_r and P_{fr} when exposed under red or far-red radiation (Gurevitch et al. 2002). Under red-light radiation, P_r changes to P_{fr} to regulate gene expression and thereby change the morphological features of plants, such as stem, hypocotyl or shoot elongation, seed germination, flowering, etc. In temperate plants, the ratio between P_{fr} and P_r is used as a measure daylength, as P_{fr} back-converts to P_r during darkness. This cue is sometimes combined with additional temperature and moisture cues to initiate fall phenology, such as budset, leaf abscission, and dormancy (Gurevitch et al. 2002).

Appropriate timing of leaf coloration and leaf abscission is an important mechanism to recycle plant resources and nutrients (Tanino et al. 2010). Leaf protein relocation happens during abscission, which helps plants to overwinter, because nitrogen transfers to the bark reserves (Cooke and Weih 2005), and carbon is lost when the leaf falls. Thus, this trade-off of seasonal nitrogen cycling and carbohydrate production is likely to occur, and so the optimal timing of leaf senescence is important for acclimation and ultimately for adaptation in fall.

1.2.3 Frost hardiness

Frost hardiness, the cold acclimation process, has several different physiological mechanisms that make the plant resistant to low temperatures. First, cryoprotection within cells is due to polysaccharides and proteins that serve as ice anti-nucleation agents, and allow for supercooling of cell water (Wisniewski et al. 2009; Zwiazek et al. 2001). Secondly, the high protein and polysaccharide concentrations lead to a viscous protoplast that can gradually vitrify (resembling

the molecular state of glass), preventing damage of plasma membranes by ice crystals (Franks 1985). Thirdly, structure proteins, lipoproteins and functional proteins stabilize cell membranes, which also contributes to reduce frost damage (Morin et al. 2007; Wisniewski et al. 2009). Fourth, under cold stress, stomata close and intercellular tissues dehydrate. Afterwards, metabolism and transpiration reduce, which leads to the inhibition of growth (Gusta and Wisniewski 2013; Yadav 2010).

1.2.4 Drought resistance

Drought resistance can be a function of multiple factors related to wood anatomical and physiological traits, including vessel diameter, cavitation resistance, leaf area, stomata control, etc. Schreiber et al. (2011) found that in *Populus tremuloides* and hybrid poplars, narrow vessel diameters would reduce the risks of embolisms induced by drought. Schreiber et al. (2011) also demonstrated that *Populus tremuloides* was more drought tolerant than hybrid poplars due to their smaller vessel diameter and better water-use efficiency.

In addition to drought resistance mechanisms, resilience mechanisms that facilitate recovery after a disturbance also exist in aspen. *Populus tremuloides* can allocate high soluble sugar storage in root to facilitate the osmoregulation and osmoprotection and to increase root survival (Galvez et al. 2011). Carbon optimization of uptake under water loss is one explanation of this (Raven 2002). Under water stress, translocation of carbon to the root system has been observed to function. By prioritizing the root survival and function, recovery by root

suckers is possible, which is a common resilience mechanism after drought stress (Worrall et al. 2010).

1.2.5 Other adaptive traits

Genetic variation also exists in adaptive traits of other specific biotic or abiotic effects. Rust resistance in white pines, for example, has significant natural variation in multiple populations from Eurasia to North America (King et al. 2010). For herbivore defense in trees, phenolic glycosides (salicinoids) and condensed tannins are the compounds that allow *Populus tremuloides* to tolerate herbivore damage (Lindroth and St. Clair 2013). The genetic variation and phenotypic plasticity of the herbivore defense traits are high in natural aspen forests (Lindroth and St. Clair 2013). However, this thesis will focus on adaptation to abiotic factors, specifically those relevant for adaptation to local climate conditions.

1.3 Geographic patterns of genetic variation in adaptive traits

Ecological genetics studies often quantify the within and among population variation of growth and adaptive traits, and these studies are often based on provenance trials where seeds are collected from multiple populations at different locations (provenances) and planted under a common environment to reveal the genetic differences. Typical adaptive traits that are assessed in common garden trials include the timing of bud flush, bud set, leaf coloration, leaf abscission, flowering and fruiting, and the onset and absolute degree of frost hardiness.

Genetic differences in the timing of bud break of trees such as *Populus* were studied with provenance common gardens. Soolanayakanahally et al. (2013) examined 35 provenances of *Populus balsamifera* from Alaska to Newfoundland and Labrador, spanning a latitudinal range from 45 °N to 70°N; and they found negative correlations among bud flush and latitude (r = -0.7 to -0.5), which was about 1 day earlier per degree of latitude for a total of 15 days difference among the opposite ends of the cline. Also, in *Populus trichocarpa*, the timing of bud break and leaf flushing differed by approximately 16-18 days among the most northern and southern genotypes (44° N to 60° N latitude) with one to two days earlier per degree latitude to the north, though the relationship is not proved with R –squared values (McKown et al. 2013). For *Populus tremuloides*, the clinal variation of spring phenology has been studied with provenance trials (Li et al. 2010), and slopes across western Canada were approximately -0.5 days per degree latitude. However, latitudinal variation in the date of bud break is not always found. For example, Mimura and Aitken (2007) found no latitudinal clines for bud flush phenology in *Picea sitchensis* among seventeen populations from Alaska to California. Similarly, no trends in bud flush were found in the Swedish Aspen collection along the transect from the 56° N to the 66° N latitude (Luquez et al. 2008).

Bud break generally expresses clines along elevation. Along an altitudinal cline, the bud flush timing of *Quercus petraea* in Europe was positively correlated with altitude (Alberto et al. 2011). The cline was 0.5 to 0.7 days later bud flush per 100 m increase in elevation over a range of 0-1800 m altitude with a P-value between 0.01 and 0.05 (Alberto et al. 2011). In conifers, an opposite trend is shown. For

example, in *Abies amabilis* and *Abies lasiocarpa*, Worrall (1983) found altitudinal clines from 0-1,500 m in western North America, and the slope was about -0.5 to -0.8 days per 100 m of an elevation increase, indicating an earlier bud break for sources from higher elevation in four common gardens with P-values <0.05.

In fall phenology, clinal patterns are generally strong. In European aspen (*Populus tremula*), Ingvarsson et al. (2006) reported that bud set was 4.4-day earlier per degree latitude further north over 10° latitude range in Sweden (R^2 = 0.9) (Ingvarsson et al. 2006). Similar studies with an aspen collection from Sweden showed approximately three to four days earlier per degree latitude increase of strong bud set clines to the north (R^2 = 0.63~0.68) (Luquez et al. 2008). In addition, in Canada and Alaska, Soolanayakanahally (2013) found the date of bud set being four days earlier per degree of latitude further north, with r^2 values from 0.6 to 0.9.

Another trait that is commonly assessed as an indicator for the onset of dormancy in hardwoods is leaf coloration or abscission. The timing of leaf coloration is highly correlated with latitude. Soolanayakanahally (2013) found a two days lagging per degree of leaf coloration phenology from the northern to the eastern provenances of *Populus balsamifera*, of which the leaf coloration lasted for one and a half to two months. Fracheboud et al. (2009) found that the onset of leaf senescence was ~3.9 days earlier per degree latitude for the northern *Populus tremula* provenances from 56°N to 66°N after August 29 (R^2=0.45, P<0.001) and the bud set had higher latitudinal correlation (R^2=0.82, P=0.001) than leaf senescence.

Frost hardiness traits also have strong geographical clines, and the onset of cold hardiness is typically well correlated with bud phenology or leaf senescence. And the critical temperatures of 50% lethal damage (LT50) of trees under artificial freezing test are used to distinguish the hardiness variation among populations or provenances (Larcher 2003), though sometimes 50% damage may not be achieved in all experiments. There are many studies of hardiness and onset of hardiness in trees. In *Alnus sinuata*, Benowicz et al. (1999) found that LT 50 in January reached to a range of -50°C to -20°C showing low difference among populations than the fall frost injury. Aitken and Adams (1996) demonstrated that in coastal and cascades populations of *Pseudotsuga menziesii*, frost hardiness of needles and buds in November was higher for Cascade populations, which was about 3~20% less vulnerable to frost than the coastal populations. However, the population divergence of tissue damage was small in January, which was about 0~ 9% for buds, stems and needles. In *Tsuga heterophylla*, Oregon populations showed higher fall frost injury (44~52%) and spring frost damage of needles (45~48%) than British Columbia populations (north) of which the injury was 21~29% in fall and 28~40% in spring respectively under freezing temperature from -6 °C to -14 °C (Hannerz et al. 1999). In *Pinus sylvestris*, a boreal conifer species, the maximum hardiness of needles vary among populations that is the damage temperature of the northern provenance reached to -40°C , while all populations were -30°C in Finland (Hurme et al. 1997). For altitudinal clines, Rehfeldt (1988) summarized provenance test results of 173 *Pinus contorta* populations in Rocky Mountains from 1,300 m to 2,900 m and found that 0.0009 decrease of frost injury index per meter altitude when population are from higher origins. In *Picea Sichensis* , another coastal conifer tree, Mimura and Aitken (2007) reported that strong latitudinal clines of cold injury index in a range wide

collection area at the Pacific coast from Alaska to British Columbia, which is 7% more hardened per 100 km from south to north (R^2=0.58, P<0.0001).

Frost hardening, an acclimation process, starts as early as late August in northern boreal forests during which population variation is high. For example, in interior *Pseudotsuga menziesii* populations, the LT50 of seedling twigs drops from -10 °C to -40 °C after mid-August till December (Rehfeldt 1979). In *Pinus sylvestris*, Hurme et al (1997) tested the hardiness of needles and stems started from August at -5~-8°C, but the northern population was about 11 days earlier to reach the artificial freezing temperature of -10 to -30°C than other populations. More hardiness at the same testing freezing temperature treatment (e.g., fewer cell lysis) means earlier timing of hardening in artificial freezing test. Benowicz et al. (2000) found that in *Alnus sinuata*, when the least hardened population reached LT50 of -18°C in fall, they were 35~44 days later than the most hardened (north) populations. Benowicz et al.(2000) also discovered that the bud break in spring and frost injuries (%) in last fall were correlated (r=0.87, P<0.05). However, the fall frost hardiness is usually related to fall phenology (Howe et al. 2003).

In summary, in less than half the studies reviewed above, spring phenology showed strong clines either latitudinal or altitudinal. However, more than two-thirds of the studies showed pronounced clines of bud set and all studies of fall hardiness and leaf senescence cited here demonstrated geographic cline patterns. To take advantage of the growing season length, especially in areas of high latitude and elevation, trees start shoot growth as soon as the required heat sum is reached. Growth cessation traits show an earlier onset of dormancy in northern populations and at higher elevations. The short season with late budbreak, early

growth cessation and frost hardening reduces frost damage risk. This adaptation results in intra-specific genetic variations of growth cessation traits.

1.4 Growth versus adaptive traits

In natural populations of plants, investments in growth versus fitness often represent tradeoffs. MacArthur (1972) proposed that habitat ranges are shaped in terms of the adaptive capacity of cold survival in the north or high altitudes and their ability to compete with more adaptive species in the south or lower altitudes. This range limit theory could be explained by adaptive tradeoffs (Koehler et al. 2012), which forms a strategy of choices between the growth capacity adaption and survival adaptation. Capacity adaptation is the maximum utilization of the growing season leading to earlier bud break or late senescence. The opposite and conservative strategy of avoiding stresses such as frost is survival adaptation (Bennie et al. 2010).

The tradeoffs between adaptive traits and growth demonstrate critical physiological interaction of multiple traits, and these trade-offs are caused by optimizing or maximizing the fitness of a plant under long-term local climates (Bennie et al. 2010). In tree species, seasonal growth and cold adaptive phenology determine the duration of primary growth and total amount of shoot elongation (Howe et al. 2003; Leinonen and Hanninen 2002). In addition to trade-offs (investment in resources to avoid risks that could have otherwise been allocated to growth) positive associations may exist (traits that minimize risks without compromising growth). For example, Schreiber et al. (2011) found that trees with smaller vessel diameters had better drought resistance due to lower drought-

induced embolisms that also benefitted growth. Growth and fitness tend to be positively related in natural populations that are well adapted to their environments.

In tree improvement programs, breeders have to consider both growth and adaptive that may have strong genetic correlations. By selecting for one trait (e.g. increased productivity or wood density), breeders may inadvertently also change adaptive traits (e.g. extending the growing season or changing hydraulic traits). Strong genetic correlations may indicate finely tuned trade-offs between growth and adaptive traits that tree breeders should not ignore. To select superior genotypes for growth traits and study potential trade-offs with adaptive traits, tree breeders traditionally rely on information from common garden experiments.

1.5 Common garden experiments

Common garden experiments can be laboratory, greenhouse or field trials where multiple genotypes of plants are evaluated in an experimental design where environmental variation is minimized or controlled through blocking and randomization. This genetic variation can be separated from environmental variation, and significant difference among plant populations, families or clones can be attributed to genetic differences. The first well-known common garden was for Yarrow (*Achillea millefoluim*) in a reciprocal transplant experiment a special case of multiple trials, where all populations originating from multiple different environments are also tested across the same planting environments (Clausen et al. 1940). Common garden experiments have been a widely used approach to

distinguish genetic and environmental effects in phenotypic variation of trees (White et al. 2007).

In forestry, common garden trials of open-pollinated bulk seed collections are usually referred to as provenance trials, which are long-term field trials where height and diameter growth of provenances are measured and analyzed for a substantial period of the rotation. They often involve multiple trials across a wide range of planting environments where the response of provenances to different environmental condition is tested (Agren and Schemske 2012; Ishizuka and Goto 2012; Leinonen et al. 2011). As well, the reaction norm of genotypes is estimated along environmental gradients such as temperature and moisture (Franks et al. 2013). These studies are valuable for understanding adaptation of populations to local environmental conditions, and to infer how different genotypes may respond to climate change.

Common garden experiments can also be carried out in controlled environments. For example, physiological traits are typically measured in nursery, greenhouse or growth chamber settings that have more homogeneous environmental conditions (Howe et al. 1995; Rohde et al. 2011). By manipulating the growing environments, genetic differences are revealed, though they are rarely observed in field trials. For example, freezing treatments can be carried out in the lab to examine the cold hardiness, where the equivalent frost damages are unlikely to happen in natural settings. Thus, common gardens in laboratory or nursery settings are a useful addition to information from long-term field trials.

Common garden trials, where the pedigree of the tested plants is known are usually referred to as progeny trials. Progeny trials can be open pollinated half-sib families, where the mother is known, but the father is not. Full-sib progeny trials are the result of controlled crosses with both parents known, and clonal trials test multiple ramets from a single ancestor. Given pedigree information, genetic parameters of traits can be estimated (Howe et al. 2003), and genetic gains from selections can be predicted. Progeny trials are classic tools for studying quantitative genetics of important traits to determine the adaptation capacity of populations.

Provenance trials where populations from a wide range of locations and environments are planted, and progeny trials where pedigree information is available, can be combined and are sometimes referred to as progeny-provenance trials (Alberto et al. 2013; Grady et al. 2011; Matyas 1996; Vitasse et al. 2009a). Provenance-progeny trials most often just track the female parent from wide-ranging collections of open-pollinated seed (Kiss and Yanchuk 1991). Provenance-progeny trials do not always have an adequate amount of genetic structure to reliably estimate genetic parameters (Hodge and Dvorak 2004; Pinyopusarerk et al. 1996).

1.6 Suboptimal fitness and growth

Common garden field experiments often reveal apparent suboptimality of local provenances, meaning that provenances introduced from elsewhere appear to consistently outperform seed sources that are collected nearby. Several factors for this apparent suboptimality have been commonly proposed: (1) adaptational lag,

(2) tradeoff between fitness and growth, (3) asymmetrical gene flow, (4) metapopulation dynamics and founder population effects.

Adaptational lag can be caused by environmental changes that is faster than an adaptive evolutionary response (Aitken et al. 2008; Grady et al. 2011). In long-lived trees, suboptimality after an environmental shift may persist for a long time. This issue is particularly relevant in the context of observed and projected climate change. In British Columbia, forest researchers have initiated the Assisted Migration Adaptation Trial (AMAT), a series of dozens of long-term common garden field trials for sixteen commercial tree species to understand the climatic and latitudinal tolerance of tree populations and select the best-adapted seed sources for reforestation under projected future environmental conditions (Marris 2009; Ukrainetz and O'Neill 2009).

A trade-off between fitness and growth may cause apparent suboptimality. For example, a plant defense mechanism to herbivores involves tradeoff of resources allocation between defense and growth (Koricheva 2002). Thus, optimal fitness may not necessarily mean maximum growth observed in common gardens. Similarly, investments to protect a plant against rare environmental extremes could lead to apparent suboptimality. During the duration of the provenance trial, such rare extreme events may have never occurred, so that fast growing provenances that may not invest into frost or drought protection may appear best adapted. However, it is important to realize that genotypes with the best growth may not be the genotypes with the highest fitness.

Asymmetric gene flow may cause suboptimality. For example, small tree populations at high elevation might be pollinated by larger populations from lower elevation (Savolainen et al. 2011; Savolainen et al. 2007). Generally, asymmetric gene flow between populations from the periphery of a species range and the center would lead to reduced fitness and suboptimality in the peripheral populations (GarciaRamos and Kirkpatrick 1997).

Another possible cause for maladaptation observed to local environments is long-distance dispersal events that lead to small founder populations on the periphery of the species range. Inbreeding depression within small founder populations would further exacerbate a poor fitness (Sezen et al. 2005) and suboptimal growth (Fenster and Galloway 2000; Williams and Savolainen 1996).

1.7 *Populus tremuloides*

The species studied in this thesis, trembling aspen (*Populus tremuloides* Michx), is an early successional species with a natural range including the boreal forest of North America, the eastern United States, and the western mountain ranges from Mexico to Alaska. Aspen can regenerate both via sexual and asexual reproduction and may also form triploids that are not fertile (Mock et al. 2012). Mock et al. (2012) reported 16% (4/25) triploids and 84% diploids (21/25) for an Alberta Foothills population compared to 100% diploids (12/12) for a north central Alberta population. The species regenerates quickly from root suckers, often resulting in single species stands after disturbances (Perala 1990). Clones of aspen can persist for very long times and grow to large sizes by root suckering. The largest and oldest documented aspen clone, 'Pando' has been reported as the

largest organism on land, which occupies 43 ha and weighs 6 million kilograms with 47, 000 individual stems (Dickmann 2002) .

Seedling regeneration is relatively rare in the stand regeneration of stands after disturbance, with aspen normally sprouting from roots (Long and Mock 2012). *Populus tremuloides* has small cottony seeds that lack endosperm and dispersal distances regularly exceed 5 to 10 km (Wyckoff and Zasada 2002). However, seedling establishment requires moist, mineral-organic substrate and sufficient light (Schott et al. 2014).

Over the last two decades, aspen has become one of the most important commercial forestry species throughout the region due to the development of oriented strand board (OSB) production (Ondro 1991). In Alberta, aspen represents 40% of the total forest harvests in 2010 (Gylander et al. 2012), and tree improvement programs have been developed to maximize the yield in short rotation forestry systems. In Alberta, two breeding regions were delineated to develop locally adapted and highly productive planting stock via tree breeding programs that target these two regions (Gylander et al. 2012; Li 1995).

Selection and breeding require sufficient genetic control of observed variation for the traits of commercial interest. This is measured as narrow sense and broad sense heritability. Previous studies demonstrated moderate to marginal high broad sense heritability in the traits of commercial interests (e.g., growth and fibre properties), as well as morphological, physiological traits. Some of these studies take advantage of the clonal structure of aspen in natural stands that can be

delineated from their fall phenology. Such 'putative' clonal structure can be used to estimate genetic parameters for traits.

Barnes (1975) studied 31 putative clones in Michigan and estimated broad-sense heritability for height and diameter at breast height that were 0.43 to 0.45 and 0.28 to 0.36 respectively. Mitton and Grant (1980) studied 106 putative clones in the Front Range of Colorado and estimated the broad-sense heritability growth rate that was less than 0.33. Yanchuk et al. (1984) estimated broad-sense heritability of wood density and fibre length that were between 0.35 to 0.43 based on 15 clones sampled in Blue Ridge, in central Alberta. Yanchuk et al. (1988) found low broad sense heritability (0.13) for secondary plant compounds relevant for pulp quality in aspen. Thomas et al. (1998c) tested 29 male clones in Alberta based a growth chamber experiment and estimated the broad-sense heritability of seedling height ranged from 0.74 to 0.77. Gylander et al. (2012) found the broad-sense heritability of height growth ranged from 0.36 to 0.64 based on 242 clones from Alberta, which were tested in 13 clonal trials after five to eight growing seasons in the field. St Clair et al. (2010) tested 417 ramets from 18 clones at age 27, and found the broad sense heritability ranging from 0.27 to 0.36 for height and DBH growth traits.

There are limited studies that have estimated heritability of key adaptive traits, such as spring and fall phenology and frost hardiness in aspen. However, some information is available from related poplar species. Genetic controls of adaptive traits range from moderate to strong, which depends on specific traits. Heritability (H^2) of bud break in *Populus balsamifera* ranges from 0.21 to 0.47 (Farmer 1993). For *Populus trichocarpa* ×*deltoides,* heritability (H^2) of bud break ranges from

0.48 to 0.80 (Howe et al. 2000). Bud set has a different heritability: in *Populus balsamifera*, H^2 was 0.65 (±0.15) (Riemenschneider et al. 1992) and in *Populus trichocarpa* it reaches to 0.81 (±0.03) (Riemenschneider et al. 1994). Regarding *Populus trichocarpa* ×*deltoides*, H^2 ranges from 0.48 to 0.72 (Howe et al. 2000). In *Populus nigra*, Rohde et al. (2011) studied onset of growth cessation (bud set) and the duration of bud set, and they found the onset trait (bud set) had higher H^2 (0.5-0.9) than the duration trait (0.3-0.7). And yet, Fabbrini et al. (2012) tested 162 progenies of *Populus nigra* from Italy (40° N and 45° N) at three common garden sites. They found H^2 ranged from 0.4 to 0.8 for bud set onset, while H^2 of the duration trait was from 0.2 to 0.7. And they discovered a strong $G \times E$ effect, which was from 0.1 to 0.2 of total phenotypic variances. For frost hardiness, $H^2$of *Populus trichocarpa* ×*deltoides* was 0.27 (Howe et al. 2000).

Recent work on *Populus tremuloides* has also investigated geographic differentiation of populations in western Canada in growth traits based on a wide-ranging provenance trial series with collections ranging from Minnesota to northern British Columbia (Gylander et al. 2012; Schreiber et al. 2013). These studies have identified a possible adaptational lag but the cause that leads to the suboptimal growth remains unclear. To increase productivity of aspen in Alberta, assisted migration of populations in the context of regular reforestation programs has been proposed (Gray et al. 2011). A north-west migration would improve height growth relative to local provenances in western Canada (Gray et al. 2011), but it is unknown whether such prescriptions would carry risks of maladaptation to rare climate extreme events, such as unseasonal frosts. Thus there is a knowledge gap for ecological and quantitative genetic studies that could help to assess the risk of assisted migration prescriptions to address climate change.

The Western Boreal Aspen Corporation (WBAC) established a provenance-progeny trial series in 1998 with source material from western Canada, as well as a progeny trial series established between 2005 and 2008 that test a much larger sample of Alberta's sources with full pedigree information. These trials are the basis for assessments of adaptive and growth traits, and the study of quantitative and ecological genetics of *Populus tremuloides* in this thesis.

1.8 Thesis structure

In this thesis I address three major questions that relate to apparent suboptimality of local aspen populations, with practical applications for tree breeding programs for aspen in western Canada. I ask: (1) Can we move seed sources north and west to increase aspen productivity without risks of maladaptation? (2) What are the causes of apparent sub-optimality? I hypothesize that due to the clonal nature of aspen local populations may be adapted to past environmental conditions and that from a practical reforestation perspective, local sources should not be considered best adapted; and (3) Can we select genotypes in tree improvement programs for better growth characteristics without increasing risks of maladaptation?

Previous studies have documented that movement of aspen seed sources in northwest direction leads to higher productivity relative to local seed sources. I will try to assess whether implementation of such seed movement in large scale reforestation programs carries risks of frost damage due to lack of synchronization with the growing season. Better growth could be the result of transferred provenances utilizing a longer growing season, with bud break earlier

and leaf senescence occurring later than in local provenances, thus increasing the vulnerability to late spring frosts and early fall frosts. In my first research chapter, I study geographic variation of adaptive traits in a wide-ranging progeny-provenance trial series with 43 sources and 5 test sites throughout western Canada and the eastern United States. I investigate how the timing of spring bud break, fall senescence and the onset frost hardiness relates to the available growing season and frost risks across a wide range of source and planting environments.

My aim for my second research chapter is to better understand the causes of apparent suboptimal growth of local aspen populations in western Canada. I make use of a recently developed species distribution model for aspen (Worrall et al. 2013) to reconstruct suitable habitat of the species to the last glacial maximum. I compare postglacial migration history with patterns of genetic variation and genetic differentiation in growth and adaptive traits observed in the wide ranging provenance-progeny trial, and I hypothesize that low productivity of aspen populations found in northeast British Columbia and northern Alberta could be due to these populations being the result of recolonization from glacial refugia in Alaska.

In my third research chapter I investigate if better growth characteristics without increasing risks of maladaptation can be accomplished by tree breeding. Here, I use ten progeny trials containing more than 30,000 trees with known pedigree structure, including 60 half-sib families, 100 full-sib families and 1,400 clones to estimate the breeding potential and genetic parameters for collections from Alberta. This chapter focuses on the genetic variation within-populations which is essential for tree improvement. Additive and non-additive genetic variance

components are estimated for two growth traits, height and diameter at breast height, and two adaptive traits, the timing of bud break and leaf abscission. I am specifically interested in genetic correlations among these traits to identify potential trade-offs between growth and adaptive traits, and to quantify if selection for height could lead to inadvertently selection for utilizing a longer growing season resulting in higher risks of frost damage.

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Chapter 2 - Frost hardiness versus growth performance in trembling aspen (*Populus tremuloides* Michx.): an experimental test of assisted migration

2.1 Summary

According to the range limit hypothesis, the distribution of many temperate species is restricted by a trade-off between their capacity to survive winter extremes in the north (or high elevation), and their ability to compete with better-adapted species in the south (or low elevation range limits). This trade-off has important implications in forestry, particularly in the context of managed seed movement under climate change. In this study, we aim to quantify trade-offs among growth, frost hardiness, and timing of leaf senescence and bud break in populations of trembling aspen Populus tremuloides Michx., which were observed in a large reciprocal transplant experiment across five planting sites in western Canada, including additional provenances from Minnesota. After 10 years, we found pronounced increases in productivity as a result of long-distance transfers in northwest direction. For example, provenances moved 1,600 km northwest from Minnesota to central Alberta (a shift of 7° latitude to the north) produced almost twice the biomass of local sources. Similarly, provenances moved 800 km from central Alberta to northeast British Columbia (4° latitude north) also produced twice the biomass of local sources. We further found that increased growth was not associated with lower survival rates. Bud break in provenances transferred northwest generally occurred slightly later than in local sources, suggesting decreased risk of spring frost injury. Leaf abscission was later in provenances transferred in northwest direction, but they appeared to be very frost

hardy, well ahead of very rare early fall frost events. This study demonstrated that assisted migration prescriptions have considerable potential to enhance forest productivity. In the case of aspen, even long-distance seed transfers in northwest direction were successful. We conclude that benefits in productivity outweigh potential risks associated with northward transfer of aspen planting stock under both current and projected future climate conditions.

2.2 Introduction

Populations of widespread forest trees are locally adapted to a variety of climate conditions. As climate changes, locally-adapted genotypes may become increasingly mismatched with their current environments. This trend potentially leads to reduced productivity and forest health (Gray et al. 2011). In this case, human-aided movement of planting stock to appropriate climate environments can reduce the risks. And large-scale reforestation programs offer an opportunity to implement such a climate change adaptation strategy cost-effectively (Gray et al. 2011; Pedlar et al. 2012). However, such prescriptions, especially those that aim to pro-actively match planting stock to anticipated future climates, run the risk of mal-adaptation under current conditions, especially for frost risks (Howe et al. 2003; Savolainen et al. 2007).

In North America, trembling aspen (*Populus tremuloides* Michx.) is one of the most widespread and genetically diverse trees in the boreal mixed-wood forests. Here, the trembling aspen covers an area of approximately 60 million hectares, providing significant habitats for mammals, birds and insects, as well as other plants (Stelfox 1995; Canadian Forest Service 2011). Aspen is also an important

commercial tree in western Canada, accounting for approximately 50% of the annual forest harvest, and is primarily processed into oriented strand board (OSB) and paper pulp. Recently, aspen has also been treated as raw materials for biofuels and other biomaterials (Balatinecz, Kretschmann and Leclercq 2001; Sannigrahi, Ragauskas and Tuskan 2010).

Given current and predicted climate change for western Canada (IPCC 2007; Mbogga, Hamann and Wang 2009), this important forest resources appear to be under considerable threat. Over the last two decades, a loss of forest productivity and a dieback of aspen and other species have been frequently documented along the southern edge of the boreal forest and further south in the United States (Hogg, Brandt and Michaelian 2008; Allen *et al.* 2010; Peng *et al.* 2011; Anderegg, Kane and Anderegg 2013). Michaelian *et al.* (2011) conducted a detailed survey covering an area of 11.5 million hectares in western Canada to assess the impact of drought-induced aspen dieback. They reported 45 megatonnes of dead above-ground biomass, which represented 20% of the total above-ground biomass (i.e., 226 megatonnes) in the surveyed area. A warmer and drier climate is the primary cause of the observed aspen decline, which is amplified by other factors such as forest pests, fire suppression policies, and other management practices (Marchetti, Worrall and Eager 2011; Anderegg, Kane and Anderegg 2013; Worrall *et al.* 2013).

Afforestation in the affected areas with different species or adapted planting stock of the same species is one solution to that issue of aspen dieback, which provides trees with a better match to current and anticipated climates. In a study of the lodgepole pine, Rehfeldt, Wykoff and Ying (2001) suggest that adapting to global

climate change requires a major redistribution of trees and genotypes across the landscape. They report, for example, that genotypes which are best suited to future climates in northeast British Columbia (latitude 60°) are currently located as far as 9° latitude to the south. Similar work for aspen indicates that relocating aspen planting material northwards by 1–2° latitude is required just to account for climate change observed over the last two decades (Gray *et al.* 2011).

Any movement of planting stocks, however, could increase the risk of freezing injuries if migrated genotypes are not synchronized with the local growing season (Aitken and Hannerz 2001). Frost hardening in late fall coincides with leaf senescence and de-hardening in spring coincides with bud break. Early spring growth is particularly susceptible to spring frosts, since tissues are actively growing and not lignified. Bud break is primarily a response to heat degree days and is initiated by a certain heat sum (Hunter and Lechowicz 1992; Li, Wang and Hamann 2010). In contrast, autumn leaf senescence in most broad-leaf species, including aspen, is triggered by photoperiod (Horvath *et al.* 2003; Keskitalo *et al.* 2005; Fracheboud *et al.* 2009). Notably, the day length, the trigger for the onset of cold hardiness, is decoupled from the actual selective environmental factor (i.e., frost events). This poses a special concern for transferred seeds, because even if the day length is the same at the planting sites, the timing of frost risks varies compared to the source geographical locations.

The distribution of many temperate trees is thought to be determined by their adaptive capacity to survive winter extremes in the north or at high elevations, and their ability to compete with better adapted species in the south or at low elevation range limits (MacArthur 1984; Woodward 1987). This is a consequence

of trade-offs between maximizing growth by fully utilizing the available growing season, and avoiding injuries or mortalities due to spring or autumn frosts (Loehle 1998; Aitken and Hannerz 2001; Leinonen and Hänninen 2002; Koehler, Center and Cavender-Bares 2012). According to Loehle (1998), frost protection requires significant structural investments in plants (e.g., increased lignification, thicker leaves and cell walls), physiologic responses (e.g. accumulation of lipids, sugars or membrane proteins), and conservative growth strategies (e.g. early autumn leaf senescence and late bud break).

This paper aims at developing recommendations for moving planting stocks, considering such trade-offs in growth and adaptive traits. Hence, the objectives of the present study are: (1) to assess the impact of moving aspen seed sources throughout western Canada on growth and survival in a large-scale reciprocal transplant experiment; (2) to investigate geographic patterns of genetic variation in adaptive traits, including the timing of bud break and leaf senescence, and the onset and degree of frost hardiness; (3) to quantify frost risk environments in early autumn and late spring, to which local and transferred aspen populations need to be adapted; and (4) to assess risks and potential benefits of seed movement throughout the western boreal forest. This information could in principle be used to develop sophisticated climate change adaptation strategies. For example, we could potentially lower risks (e.g. due to variable future climate), by sacrificing some growth potential (e.g. through a more conservative growth strategy). However, to keep the scope of the study manageable, we do not model performance and trade-offs under uncertain future environments. Instead, this paper aims at management recommendations that enhance health and productivity of planted aspen forests in response to recent climate change trends.

2.3 Material and Methods

2.3.1 Plant material and experimental design

The effects of moving planting stock to new growing environments were tested with a reciprocal transplant experiment, established by the industrial members of the Western Boreal Aspen Cooperative in 1998. The planting sites and collection locations were chosen to represent forest management areas of the participating Canadian forestry companies. And the management areas in turn reflect ecological zones with relatively homogeneous climatic and physio-geographic conditions (Selby and Santry 1996). The ecological regions include the southeastern boreal plains of Saskatchewan (SK), the Alberta foothills ecoregion (ABf), the central boreal plains of Alberta (cAB), the northern boreal plains of Alberta (nAB) and the taiga plains of British Columbia (BC). The regions were unequally represented with three to eleven collection locations, reflecting assumptions where productive genotypes for reforestation in western Canada may be found. Also five seedlots are included from the boreal shield ecoregion in Minnesota (MN) for testing in western Canada (Fig. 2.1, Table 2.1). Note that there is no corresponding test site for this MN region.

The experiment comprises five common garden trials, and all seed sources collected for this study were planted at all sites. In total, 43 half-sib families from six regions were tested. Each collection thus represents seeds from a single open-pollinated female tree. In the subsequent text, we refer to half-sib families from

this trial as provenances or collection locations. Trials were established with over-winter dormant stock in spring of 1998 in a style of randomized complete block designs with six replications per seed source, five trees per row plot, and surrounded by two rows of border trees. Thus, a total of 6450 seedlings (excluding border trees) were planted in the entire experiment with 1290 trees in each common garden.

Height and diameter at breast height (DBH) measurements for all 6450 trees were taken after one growing season in an outdoor nursery bed and nine growing seasons in the field in the fall of 2006. An additional height measurement was taken for the central Alberta site in 2008 after ten growing seasons in the field. Total above-ground biomass for trembling aspen was calculated according to the Canadian national biomass equations in kg dry weight (Ung, Bernier and Guo 2008). For all other physiological and phenological traits, we had to employ a sub-sampling strategy to keep the study feasible while trying to capture most of the genetic variation presented in this experiment. Phenology observations were carried out for all 1290 trees at the central Alberta test site. Cold hardiness measurements were carried out at the same site on six provenances from three regions (Minnesota, central Alberta and northeast British Columbia), sampling eight trees per provenance (indicated by numbers in Fig. 2.1). In total, 864 cold hardiness measurements were carried out during three sampling days, under six test temperatures.

2.3.2 Phenology measurements and daylength calculation

Timing of leaf senescence, expressed as day of year (DoY) was determined by an eight-level senescence scale according to Fracheboud *et al*. (2009). Scoring was carried out in autumn of 2010 on seven dates: 14, 18, 21, 23, 25, 28 September and 2 October. When a score of 5 (all leaves yellow) was reached only on a single day for individual tree, the senescence stage was determined by that time (DoY). If a score of 5 was recorded on multiple dates, the day of the phenological event was calculated as an average of DoYs. If a score of 5 was not directly recorded, the senescence stage was inferred by means of linear regression from the bracketing dates and scores.

The corresponding day length (*DL*) for the day of year (*DoY*) when a score of 5 was reached was calculated as a function of latitude and *DoY* according to Forsythe *et al*. (1995):

$$DL = 24 - \frac{24}{\pi}\cos^{-1}\left[\frac{\sin\frac{p\pi}{180} + \sin\frac{LAT\pi}{180}\sin\phi}{\cos\frac{LAT\pi}{180}\cos\phi}\right]$$

where p is the daylength coefficient approximate as zero and ϕ is the declination angle of the sun, calculated as:

$$\phi = \sin^{-1}[0.3979\cos\theta]$$

and where θ is the revolution angle, calculated as:

$$\theta = 0.2163 + 2\tan^{-1}[0.9671\tan[0.00860\times(\mathrm{DoY}-186)]]$$

Assuming that leaf senescence was primarily controlled by day length, which was well documented for temperate trees including poplars (Kriebel *et al.* 1976; Kriebel 1993; Horvath *et al.* 2003; Keskitalo *et al.* 2005; Fracheboud *et al.* 2009), we inferred differences in the day of leaf senescence for provenances based on the latitudes of the other four planting sites. These estimates are meant to broadly characterize the average dates of leaf senescence. We noted that there may be temperature-modulated year-to-year variations in the date of leaf abscission, but for the purpose of interpreting geographic patterns of adaptive genetic variation, these were not considered.

Bud break scores, calculated similarly as the leaf senescence data described above, were obtained from a previous study using the identical plant material from the central Alberta test site (Li, Wang and Hamann 2010). Here, we re-analysed this data in a different research context, inferring the average DoY of bud break (score 3: buds broken and leaves extending) for the six regions of our study design, using daily weather station data for the 1961–1990 normal period. The expected dates of bud break for each individual tree were calculated according to a model optimized for aspen in the boreal forest (Beaubien and Hamann 2011). Required heat sums for bud break were determined as the daily sum of average temperatures with a start date set as January 1_{st} and a threshold value set as 0°C. This summation continues up to the day at which a bud break score of 3 was reached, yielding a required heat sum statistic for the observed event. Based on the well-supported assumption that bud break is determined by a genetically controlled heat sum requirement (Lechowicz 1984; Hunter and Lechowicz 1992), an expected date of bud break could then be estimated for all provenances at all test sites.

2.3.3 Cold hardiness measurements

Cold hardiness was measured using the electrolyte leakage method (Zhang and Willison 1987; Morin *et al.* 2007), which quantifies frost damage by measuring the leakage of cell sap into the extracellular space due to ruptured plasma membranes. Plant materials were sampled at the central Alberta test site from six provenances within three regions (Minnesota, central Alberta and northeast British Columbia) representing the full geographical extent of the experiment. For each provenance, we randomly selected eight trees from which we sampled one current year twig per freezing treatment. The collection was repeated three times in autumn 2011: on 22 August, 12 September and 10 October. All twigs were cut into 5cm pieces and placed in 30ml high-density polyethylene bottles (Fisherbrand, Fisher Scientific). Adding 5ml of deionized water to the samples before freezing treatments were applied to ensure ice formation. The freezing treatments were 8°C (control), -5°C, -10°C, -20°C, -30°C on 22 August; 8°C (control), -10°C, -30°C, -50°C, -60°C on 12 September; and 8°C (control), -30°C, -60°C, -70°C, -80°C on 10 October. A programmable freezer (Model 85-3.1A, Scientemp Corp., Adrian, MI, USA) cooled the samples at a rate of 5°C per hour, holding the target temperature for one hour, before re-warming to 8°C. Each segment was subsequently cut into 5mm pieces, topped up with 20ml deionized water, stored for 20–24 hours at 8°C, and manually shaken three times during storage. The amount of electrolyte leakage was measured at room temperature (approximately 20°C) using a conductivity meter (Oakton Acorn CON 6 Meter, Oakton Instruments, Vernon Hills, IL, USA). Conductivity readings were taken

before (c1) and after (c2) all samples were boiled at 100°C for 50 min. Cell lysis (L) was calculated as:

$$L = \frac{REL - \overline{REL_C}}{100 - \overline{REL_C}} \times 100 \ ,$$

where REL is the relative amount of electrolyte leakage of sample undergoing freezing treatments calculated as (c1/c2) $\times 100$, and $\overline{REL_C}$ is the mean value of the control samples.

2.3.4 Statistical analysis

Statistical analyses were performed using the R program (R Development Core Team 2011), and graphics were prepared with the R package *ggplot2* (Wickham 2009). Growth traits and phenology traits were analysed in a two-step process. First, means of provenances were estimated with a mixed model with the *lmer()* function from the *lme4* package (Bates, Maechler and Bolker 2011). Blocks were treated as a random effect, provenance as a fixed effect, and row-plot means were considered the experimental units.

Second, regional means and standard errors of regional means were calculated in a second step from provenance means. Standard errors of regional means were used to calculate effect size statistics, i.e., the probability that a transferred provenance matched or exceeded local sources at each test site. We used the *pt()* function of the R, which integrated the area under a Student's *t*-distribution for small sample sizes. For example, for an observed regional mean of 5.0m height

based on three provenances with a standard error of 0.25, the probability of matching or exceeding a reference value of 4.7m was calculated as *pt*((5.0–4.7)/0.25, 3–1), resulting in an estimate of 82% probability.

For frost hardiness measurements, a null-hypothesis testing was carried out to detect significant differences in the degrees of cold hardiness. To take advantage of the randomized block experimental design, these data were also analysed using a mixed model implemented with the *lmer()* function in the R package *lme4* (Bates, Maechler and Bolker 2011). The fixed effects in this model were the selected regions MN, cAB, and BC; the random terms were block and provenance. Experiment-wise *P*-values were calculated using the Tukey's adjustment for multiple comparisons.

2.3.5 Climate data and frost risk assessment

To assess current frost risk environments to which plant populations were putatively adapted, we used daily weather station data for the 1961–1990 normal period from the National Climate Data and Information Archive for Canada (Environment Canada; http://www.climate.weatheroffice.gc.ca) and the Minnesota Climatology Working Group for historical climate data for Minnesota, United States (University of Minnesota; http://climate.umn.edu/doc/historical.htm) (Table 2.1). We derived the frost risks for three regions sampled in following steps: first, calculating means and standard deviations for daily minimum temperatures for each day of the year from 1961 to 1990; then estimate the probability of a frost event based on a normal distribution

characterized by daily means and standard deviations. Frost thresholds of interest (-5, -10, -20, -30 and -40°C) were first converted to *z*-scores, by subtracting the mean daily minimum temperatures and subsequently dividing by the daily standard deviations. The probability of a frost event equalling or exceeding a given frost threshold was then calculated for each day as an integral under the normal distribution, using the function *pnorm()* of the R program (R Development Core Team, 2011). The resulting time series were subsequently smoothed with a 7-day moving average, since the day-to-day variation simply reflects variability in daily climate data but not the true day-to-day variation in frost risks.

2.4 Results

2.4.1 Growth data

After nine growing seasons in the field we found pronounced increases in productivity of aspen trees as a result of long-distance seed transfers in a northwest direction. For example, provenances moved 1,600km northwest (i.e. 7° latitude north) from Minnesota to central Alberta were 34% taller. They had 84% more biomass than the local sources (Table 2.2, Table 2.6). Similarly, provenances moved approximately 800km northwest (i.e. 4° latitude north) from central Alberta to northeast British Columbia produced two times the biomass compared to the local and were 15% taller. The farthest seed transfer tested was from Minnesota to northeast British Columbia (2,300km northwest and 11° latitude north), still outperformed local sources by 17% in height and had more than twice the biomass. Increased performances as a result of northwest transfers

were not associated with reduced survival. Minnesota provenances had similar survival rates to the local sources at all sites. The next most southern group, the Alberta Foothills provenances, had better survival rates at all northern test sites. However, relative to other sources, at its own local planting site, the Foothills provenances ranked second-lowest. Similar to Minnesota provenances, survival rates of the Saskatchewan and central Alberta provenances were comparable to local sources when transferred to the northern Alberta or northeast British Columbia test sites (Table 2.2).

In contrast, transferring northern provenances southward consistently resulted in decreased productivity. The northeast British Columbia and the northern Alberta provenances were always ranked as the lowest and second lowest groups of provenances at southern planting sites. For example, the northeast British Columbia provenances were 16%, 28%, and 50% shorter in height than the local sources at the northern Alberta, the central Alberta, and the Alberta Foothills test site (Table 2.2). Northeast British Columbia provenances showed only 45% survival rate at the Alberta Foothills test site, which represented the warmest test site with a mean annual temperature of 2.6°C (Table 2.1). Yet, the northern Alberta provenances showed only a reduced height of 5% at the central Alberta site, and 8% at the Foothills test site.

2.4.2 Spring and autumn phenology

At the central Alberta test site, where phenology was recorded, the sequence of leaf senescence started with the most northern provenances (BC and nAB),

followed by mid-latitude provenances (i.e., cAB, ABf and SK), and ended with Minnesota provenances; the Minnesota trees turned yellow 10 days later than the first provenances from the north (Table 2.3). For the inferred dates of leaf senescence, assuming day light length as a trigger, we found no discernible differences in sequences or dates of leaf senescence. For the spring phenology, bud break occurred first among the northeast British Columbia provenances, while the central Alberta and Saskatchewan provenances consistently broke bud latest (Table 2.3). The inferred bud break dates for 1961–1990 normal climate period differed only by a few days, with provenances flushing first at the central Alberta site, followed by the Saskatchewan, Alberta Foothills, and northern Alberta sites, and last at the northeast British Columbia site.

The above observations are also reflected in a relatively strong correlation between height and leaf senescence at the central Alberta test site, where phenology measurements were carried out ($R^2 = 0.36$, $P < 0.0001$). This correlation is driven by the pattern of the early leaf senescence of the northern British Columbia trees and the late leaf senescence of the Minnesota provenances, when both provenances were transferred to the central Alberta test site (Fig. 2.2a). For bud break, no latitudinal differentiation was found (Fig. 2.2b). Sources from the northeast British Columbia had the lowest heat sum requirements and broke bud first, but there was more within- than among- regional variations in terms of the timing of bud break, which could not explain the variation in height ($R^2 = 0.07$). We found no correlation between height and the growing season, calculated as the day of leaf senescence minus the day of bud break ($R^2 = 0.002$, $P = 0.78$, data not shown).

2.4.3 Cold hardiness

The amount of freezing injury, measured as electrolyte leakage, revealed a general trend in which trees from Minnesota appear to be more vulnerable than trees from central Alberta and from northeast British Columbia; the onset of frost hardiness happened earliest in the northeast British Columbia trees (Fig. 2.3a). Our cell lysis data suggests clear regional differences with very little variation of frost hardiness within regions (Table 2.4, Fig. 2.5). During the August sample date, the -10 °C and -20°C treatments resulted in higher vulnerability of Minnesota trees. Regional differences were most pronounced at all freezing treatments in September, with a sequence of increasing vulnerability from British Columbia, Alberta to Minnesota. During the October sample date, all trees showed generally low degrees of cell lysis, even under the -80°C treatments. In contrast, trees from Minnesota were still the most vulnerable (Table 2.4, Fig. 2.3a).

The onset of frost hardiness was significantly correlated with leaf senescence (Fig. 2.3b). Trees from Minnesota were the least hardy and their leaves turned yellow the latest. BC provenances showed a high degree of hardiness and the leaves also were the first to turn uniformly yellow. The central Alberta provenances ranked between the Minnesota and BC provenances.

2.4.4 Phenology, hardiness and frost risks

Fig. 2.4 shows a joint representation of phenology and regional frost risks. The probability of frost curves indicates a progression from relatively mild winters in Minnesota, to more severe winters in Alberta and British Columbia. For example, the British Columbia site has a 30–40% chance of a -30°C or colder frost events in January; whereas the corresponding probability in Minnesota is about 10–15%. Nevertheless, the time period during which mild frost events of -5°C or colder (< -5°C) are likely at the three planting sites is remarkably similar, although the probability increases faster in autumn and decreases rapidly in spring at the northern test sites.

The phenology of local provenances further was substantially well acclimatised to the frost risks of their local environments. The central Alberta provenances appear to avoid any frost risk without sacrificing the available growing season at their local central Alberta planting site. The British Columbia provenances start their growing season early in spring, but are likely to avoid spring frost risks in the BC local plant site and in central Alberta. Regarding the dates of leaf senescence, Minnesota provenances are the latest to turn yellow in fall. However, by mid-September, during the risk period of the -5°C frost at all planting sites, the Minnesota trees are well-hardened against even the -10°C frosts (Table 2.3). By mid-October, when -10°C frost risks start to appear with very low probabilities, Minnesota provenances are similarly hardened against -30°C to -80°C treatments. Thus, overall cell lysis values indicate that British Columbia and Alberta provenances are always more hardened at any given time than the Minnesota provenances.

2.5 Discussion

The trade-offs of growth optimization versus survival optimization are normally expected to be critical for temperate trees (Leinonen and Hänninen 2002). The ability of frost tolerance and avoidance strongly influences the fitness of trees from high-latitudinal ecosystems. However, trees from milder temperate forests are favoured by natural selection for higher growth rates and competitive fitness (Loehle 1998). Northward transplantation of trees is generally expected to increase risks of frost damage in autumn, due to delayed growth cessation (Howe *et al.* 1995). We found such a trend in this study that showed a delayed timing of leaf senescence and less degree of cold hardiness within Minnesota and Alberta provenances, when these trees were migrated to northern sites. However, this did not compromise survival; and the onset timings of dormancy and frost hardiness suggest that few severe risks are involved with moderate northward transfers of planting material.

Spring phenology was quite similar across all provenances observed at the central Alberta test site, except for the northern Alberta provenances. Provenances from very high latitudes or very high elevation are commonly adapted to take advantage of a short period of favourable temperatures even when the photoperiods are relatively longer than the southern sites. But the growing season available is shorter than in the southern sites. These northern provenances tend to be cautious in using such relatively short growing seasons to avoid the lethal harsh winter, which starts earlier than in the southern sites (Beuker 1994; Aitken and Hannerz 2001). In our case, this means that a northward movement of more southern tree stocks in contrast would typically lead to slightly delayed onset of

growth relative to local provenances. Yet, they can still survive the harsh winter conditions during their early ages.

We also found that the inferred dates of bud break and leaf senescence (for the regions BC, nAB, ABf, SK, MN), were not drastically different from the observed dates in the common garden site (cAB). This may result from two reasons: First, although the risk of severe frost increases from southeast to northwest, there are virtually no striking differences in the frost free period from Minnesota to northeast British Columbia (Fig. 2.4). Secondly, the date of leaf senescence coincides exactly with the inflection point of the day length curve near the fall equinox, 22 September(Fig. 2.6). Thus, although the day length trigger is not dependent on temperature, the day light trigger will work more or less appropriately under latitudinal transfers, because the day length does not vary with latitude around the date of the southern equinox. The daylight on the equinox coincides with the observed leaf senescence timing. The true critical day length that initiates senescence must be somewhat earlier than the date, during which we observed leaf senescence, so small shifts may still occur in the timing of senescence under long distance transfers. However, such timing deviation is quite small in absolute terms (~1 to 2 days). For example, the leaves of the Minnesota provenances turned yellow six days later than the local trees, when moved over 7° of latitude to the central Alberta planting site (Table 2.3).

It is striking that moving aspen as far as 2,300 km northwest from Minnesota to northeast British Columbia did not result in higher mortality rates or inferior growth during the ten growing seasons. In fact, trees from Minnesota outperformed all local trees at Saskatchewan, Alberta and northeast British

Columbia test sites. It seems that moving aspen as far as 2,300 km northwest was not enough to reach the cold tolerance limit of aspen from Minnesota. Yet, there are discernible differences in frost hardiness from southeast to northwest, suggesting trade-offs between growth (i.e., Minnesota provenances) and survival and cold resistance (northeast British Columbia provenances). However, when looking at the corresponding risk environments, investments of resources in cold resistance appear non-optimal for current climate conditions, i.e. too conservative to use the end period of the growing season before the frost (Fig. 2.3b). All provenances appear to be sufficiently hardy enough very early to withstand extremely unlikely cold events, for example -30°C in mid-September.

Such observations are normally interpreted as adaptational lag, caused by environmental change that exceeds the speed of evolutionary change (Matyas 1990; Matyas and Yeatman 1992). It is not uncommon that provenances transferred around two degrees north show increases in growth relative to local sources (Namkoong 1969; Mangold and Libby 1978; Morgenstern 1996). What is remarkable in the study is the magnitude of the adaptational lag in aspen, which may be due to the unique life history and regeneration biology of aspen. Aspen predominantly reproduces through vegetative reproduction from the distal portion of the root system, resulting in clones that are the oldest and largest known organisms (Mitton and Grant 1996). Aspen seed are very small and lack endosperm, resulting in a narrow window of viability. Suitable conditions with adequate moisture, bare ground, and sufficient light are rarely met, limiting reproduction by seed (Peterson and Peterson 1992). Thus, adaptation through evolutionary processes is expected to be slow, which could explain the unusually large adaptational lag observed in this study.

A strong adaptational lag implies that a species should be more vulnerable to movement in one geographic or climatic direction than the opposite. We do, in fact, find indications that heat-tolerances may be exceeded and compromise survival in provenances that were transferred southward. A transfer of the most northern provenances from northeast British Columbia to the warmest test site in the Alberta foothills (ABf being 3.6°C warmer than BC, Table 2.1), yielded by far the lowest survival of any transfer tested in this experiment (45%, Table 2.3). The data indicate that northward rather than southward movement of trees would be associated with less risk, which also suggest that inaction in the face of climate change may result in higher mortality.

To support decisions on regional seed transfers, we use means and standard errors for height (Table 2.2) to calculate the probability of a transferred provenance to match or exceed the productivity of local sources, or to exceed pre-determined reference values of 10%, 20% or 30% gain over the local sources (Table 2.5). It should be noted that regional representation from BC is quite low with 3 provenances (or 90 genotypes per site). However, we only report probabilities for north or northwest transfers and exclude transfer recommendations for BC provenances. Transfers in the opposite directions yield probabilities near zero (data not shown).

When interpreting these probabilities, it is important to keep in mind that these probabilities are based on the ranking of provenances at single test sites. Nevertheless, while different planting sites may strongly influence absolute productivity, the relative ranking of provenances should not change. Only if

planting environments are so different from test sites that genotype by environment interactions become a major factor, the regional rankings could change. An example for a site that does not conform to the general pattern of this trial series may be the northern Alberta (nAB) test site in a dry ecoregion, where provenances transferred from more southern or eastern origins have a low probability of matching or exceeding local sources.

There may be other important trade-offs, where more northern sources sacrifice growth and instead invest in resistance mechanisms to biotic or abiotic risk factors that we have not considered. One possible risk factor, drought resistance, was excluded by a related study (Schreiber *et al.* 2011) that showed that the Minnesota provenances tested in this experiment also have small vessel diameters, which conferred adequate drought resistance across all genotypes tested in this experiment. Adaptations to biotic factors such as pests and diseases by northern provenances that are absent in southern sources also appear unlikely. Sources from warmer environments and milder winters would generally be expected to be more exposed, and therefore better adapted to pest and disease factors.

2.6 Conclusions

This study evaluated potential trade-offs and risks associated with seed transfer of aspen seedlings for reforestation in western Canada. Gray et al. (2011) suggested that in order to adapt to observed and predicted climate warming for western Canada, planting stock should be moved 2–3 degrees of latitude northward. Such a prescription could lead to increased frost damage and a mismatch in the timing of bud break and leaf senescence with the available growing season.

Experimental cold hardiness testing and phenology observations in a common garden experiment revealed that seed transfer to more northern locations results in delayed timing of leaf senescence, but the onset of dormancy and frost hardiness suggests that there should be no severe risks involved with northward transfers of planting material. Northward movement was also associated with a slightly delayed onset of growth of introduced genotypes relative to local provenances, and therefore pose no additional risks. We conclude that benefits in growth outweigh potential risks to survival associated with a northward movement of aspen populations in forestry operations. Even extreme long-distance northward movements had positive or neutral effects on growth and survival, while southward movement had clear negative consequences, highlighting the risk of inaction in the face of climate change. We therefore recommend that seed transfer guidelines in western Canada allow a moderate movement of aspen planting stock to account for adaptational lag. As for true long-distance transfers, and notably for the use of Minnesota sources in western Canada, we encourage forest companies and government agencies to pursue this option first on a relatively small operational scale. General recommendations of long-distance transfers should await results from this test series at rotation age, and concurrent experience from small-scale operational plantations.

2.7 References

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Table 2-1 Regional representation of seed collections, geographic information of test sites, source of weather station data, and average temperature values for the 1961–1990 climate normal period.

Region	Number of provenances	Test site coordinates			Weather stations[1]	Temperature (°C)[2]			
		Latitude	Longitude	Elevation		EMT	$T_{min}01$	$T_{max}07$	MAT
Minnesota (MN)	5	-	-	-	216612	-42.2	-20.2	27.2	4.9
Saskatchewan (SK)	8	53°20'N	105°36'W	480	4056240	-49.4	-26.1	24.2	0.6
Alberta Foothills (ABf)	11	52°44'N	114°47'W	970	3015520-3	-44.1	-18.2	21.8	2.6
Central Alberta (cAB)	11	54°53'N	113°18'W	570	3060321	-46.1	-20.6	22.3	1.8
Northern Alberta (nAB)	5	56°46'N	117°28'W	525	3075040	-48.9	-22.9	22.4	0.8
Northeast British Columbia (BC)	3	58°32'N	122°20'W	335	1192940	-47.2	-26.3	23.0	-1.0

[1]) Weather station IDs according to (http://www.climate.weatheroffice.gc.ca) for Canada and according to (http://www.noaa.gov/) for Minnesota.

[2]) All temperature values based on the 1961-1990 climate normal period. EMT, 30-year extreme minimum temperature; $T_{min}01$, mean monthly minimum temperature for January; $T_{max}07$, mean monthly maximum temperature for July; MAT, mean annual temperature.

Table 2-2 Height (m) and survival (%) of provenances grown in the reciprocal transplant experiment after nine growing seasons in the field. Test sites are ordered along a northwest gradient. Local sources are marked in bold, and standard errors are given in parenthesis.

	Test site				
Origin of seed source	SK	ABf	cAB	nAB	BC
Height at age nine (m)					
Minnesota (MN)	4.0 (0.06)	4.0 (0.23)	6.9 (0.23)	5.0 (0.11)	3.0 (0.08)
Saskatchewan (SK)	**3.3 (0.14)**	3.5 (0.09)	5.6 (0.08)	5.0 (0.16)	2.9 (0.07)
Alberta Foothills (ABf)	3.1 (0.12)	**3.1 (0.11)**	5.2 (0.15)	5.1 (0.16)	2.8 (0.14)
Central Alberta (cAB)	3.4 (0.11)	3.4 (0.11)	**5.2 (0.15)**	5.1 (0.11)	2.9 (0.09)
Northern Alberta (nAB)	3.1 (0.15)	2.9 (0.16)	5.0 (0.09)	**5.3 (0.06)**	3.6 (0.11)
Northeast British Columbia (BC)	2.5 (0.15)	1.6 (0.24)	3.7 (0.11)	4.4 (0.12)	**2.5 (0.11)**
Survival at age nine (%)					
Minnesota (MN)	61.6 (6.01)	76.4 (6.96)	91.8 (1.53)	92.8 (1.36)	87.8 (2.24)
Saskatchewan (SK)	**66.0 (4.72)**	89.0 (2.00)	94.5 (1.43)	95.9 (1.43)	92.4 (2.07)
Alberta Foothills (ABf)	67.3 (4.54)	**74.2 (3.95)**	88.9 (3.91)	90.7 (3.11)	80.9 (2.90)
Central Alberta (cAB)	72.4 (2.19)	79.5 (5.18)	**94.8 (1.68)**	95.4 (1.61)	87.5 (2.29)
Northern Alberta (nAB)	73.6 (2.40)	78.8 (1.62)	92 (3.13)	**92.2 (2.63)**	90.0 (1.34)
Northeast British Columbia (BC)	65.3 (3.53)	44.7 (4.41)	84.7 (2.33)	97.7 (2.33)	**77.7 (5.21)**

Table 2-3 The inferred average date of leaf senescence for four test sites based on a day length trigger measured at the cAB planting site in autumn 2011, and the average date of bud break for the 1961–1990 climate normal conditions inferred from heat sum requirements observed at the cAB planting site in spring of 2009. Test sites are ordered along northwest gradient. The response in the native environment are marked in bold, and standard errors are given in parenthesis

	If transferred to				
Origin of seed source	SK	ABf	cAB	nAB	BC
Leaf senescence (day of year)					
Minnesota (MN)	269 (0.3)	269 (0.3)	270 (0.3)	269 (0.2)	269 (0.2)
Saskatchewan (SK)	**262 (0.2)**	262 (0.2)	263 (0.2)	263 (0.2)	263 (0.2)
Alberta Foothills (ABf)	262 (0.3)	**262 (0.3)**	263 (0.3)	262 (0.2)	263 (0.2)
Central Alberta (cAB)	263 (0.2)	262 (0.2)	**264 (0.2)**	263 (0.2)	263 (0.2)
Northern Alberta (nAB)	259 (0.4)	259 (0.4)	260 (0.4)	**260 (0.3)**	261 (0.3)
Northeast British Columbia (BC)	259 (0.8)	258 (1.0)	260 (0.8)	260 (0.7)	**260 (0.8)**
Bud break (day of year)					
Minnesota (MN)	134 (0.7)	134 (0.7)	131 (0.7)	133 (0.7)	136 (0.7)
Saskatchewan (SK)	**140 (0.9)**	141 (1.1)	137 (1.0)	140 (1.0)	142 (1.0)
Alberta Foothills (ABf)	137 (0.8)	**137 (0.9)**	134 (0.9)	137 (0.9)	139 (0.9)
Central Alberta (cAB)	139 (1.0)	140 (1.2)	**137 (1.1)**	140 (1.1)	142 (1.1)
Northern Alberta (nAB)	134 (0.9)	135 (1.0)	132 (1.0)	**135 (1.0)**	137 (1.0)
Northeast British Columbia (BC)	129 (0.7)	128 (0.3)	126 (0.3)	128 (0.3)	**131 (0.3)**

Table 2-4 Differences in frost hardiness measured as percent cell lysis among the regions Minnesota (MN), central Alberta (cAB) and northwest British Columbia (BC), based on data shown in Supporting Information S1. Different letters in rows indicate significant differences at $P < 0.05$ and standard errors are given in parenthesis.

Freezing treatment	Cell lysis by region of origin (%)		
	MN	cAB	BC
August			
-5°C	1.4 (0.6) ^{A}	1.5 (0.5) ^{A}	0.4 (0.2) ^{A}
-10°C	49.6 (3.8) ^{A}	31.2 (3.6) ^{B}	22.7 (3.3) ^{B}
-20°C	52.7 (4.1) ^{A}	31.7 (3.9) ^{B}	23.0 (4.1) ^{B}
-30°C	62.4 (2.1) ^{A}	56.2 (1.8) ^{A}	55.3 (1.9) ^{A}
September			
-10°C	21.2 (1.8) ^{A}	10.9 (1.7) ^{AB}	8.0 (3.8) ^{B}
-30°C	58.7 (1.2) ^{A}	44.2 (2.7) ^{B}	29.1 (2.6) ^{C}
-50°C	58.8 (1.2) ^{A}	40.2 (4.4) ^{B}	21.0 (2.5) ^{C}
-60°C	66.0 (1.2) ^{A}	44.9 (2.9) ^{B}	32.1 (3.7) ^{C}
October			
-30°C	18.0 (2.4) ^{A}	8.1 (0.8) ^{B}	5.8 (1.1) ^{B}
-60°C	28.2 (3.5) ^{A}	13.3 (0.9) ^{B}	13.0 (1.6) ^{B}
-70°C	18.0 (1.7) ^{A}	10.4 (1.2) ^{AB}	9.9 (1.2) ^{B}
-80°C	18.8 (1.5) ^{A}	11.5 (0.8) ^{A}	12.1 (1.9) ^{A}

Table 2-5 Probability of a transferred provenance to match or exceed the productivity of local sources, based on the means and standard errors for height (Table 2). We only report probabilities for north or northwest transfers. Transfers in the opposite directions yield probabilities near zero.

		Probability of match or gain			
Seed sources from:	Transferred to:	Match	+10%	+20%	+30%
Minnesota (MN)	SK	>0.99	>0.99	0.73	<0.01
	ABf	0.99	0.97	0.85	0.45
	cAB	>0.99	>0.99	0.98	0.71
	nAB	0.03	<0.01	<0.01	<0.01
	BC	>0.99	0.98	0.50	0.02
Saskatchewan (SK)	ABf	>0.99	0.82	0.02	<0.01
	cAB	>0.99	0.09	<0.01	<0.01
	nAB	0.05	<0.01	<0.01	<0.01
	BC	>0.99	0.97	0.10	<0.01
Alberta Foothills (ABf)	cAB	0.50	<0.01	<0.01	<0.01
	nAB	0.12	<0.01	<0.01	<0.01
	BC	0.97	0.64	0.09	<0.01
Central Alberta (cAB)	nAB	0.05	<0.01	<0.01	<0.01
	BC	>0.99	0.94	0.15	<0.01
Northern Alberta (nAB)	BC	>0.99	>0.99	>0.99	0.98

Table 2-6 DBH (cm) and total dry mass (kg) of provenances grown in the reciprocal transplant experiment after nine growing seasons in the field. Test sites are ordered along northwest gradient. Local sources are marked in bold, and standard errors are given in parenthesis.

	If transferred to				
Origin of seed source	SK	ABf	cAB	nAB	BC
Leaf senescence (day of year)					
Minnesota (MN)	269 (0.3)	269 (0.3)	270 (0.3)	269 (0.2)	269 (0.2)
Saskatchewan (SK)	**262 (0.2)**	262 (0.2)	263 (0.2)	263 (0.2)	263 (0.2)
Alberta Foothills (ABf)	262 (0.3)	**262 (0.3)**	263 (0.3)	262 (0.2)	263 (0.2)
Central Alberta (cAB)	263 (0.2)	262 (0.2)	**264 (0.2)**	263 (0.2)	263 (0.2)
Northern Alberta (nAB)	259 (0.4)	259 (0.4)	260 (0.4)	**260 (0.3)**	261 (0.3)
Northeast British Columbia (BC)	259 (0.8)	258 (1.0)	260 (0.8)	260 (0.7)	**260 (0.8)**
Bud break (day of year)					
Minnesota (MN)	134 (0.7)	134 (0.7)	131 (0.7)	133 (0.7)	136 (0.7)
Saskatchewan (SK)	**140 (0.9)**	141 (1.1)	137 (1.0)	140 (1.0)	142 (1.0)
Alberta Foothills (ABf)	137 (0.8)	**137 (0.9)**	134 (0.9)	137 (0.9)	139 (0.9)
Central Alberta (cAB)	139 (1.0)	140 (1.2)	**137 (1.1)**	140 (1.1)	142 (1.1)
Northern Alberta (nAB)	134 (0.9)	135 (1.0)	132 (1.0)	**135 (1.0)**	137 (1.0)
Northeast British Columbia (BC)	129 (0.7)	128 (0.3)	126 (0.3)	128 (0.3)	**131 (0.3)**

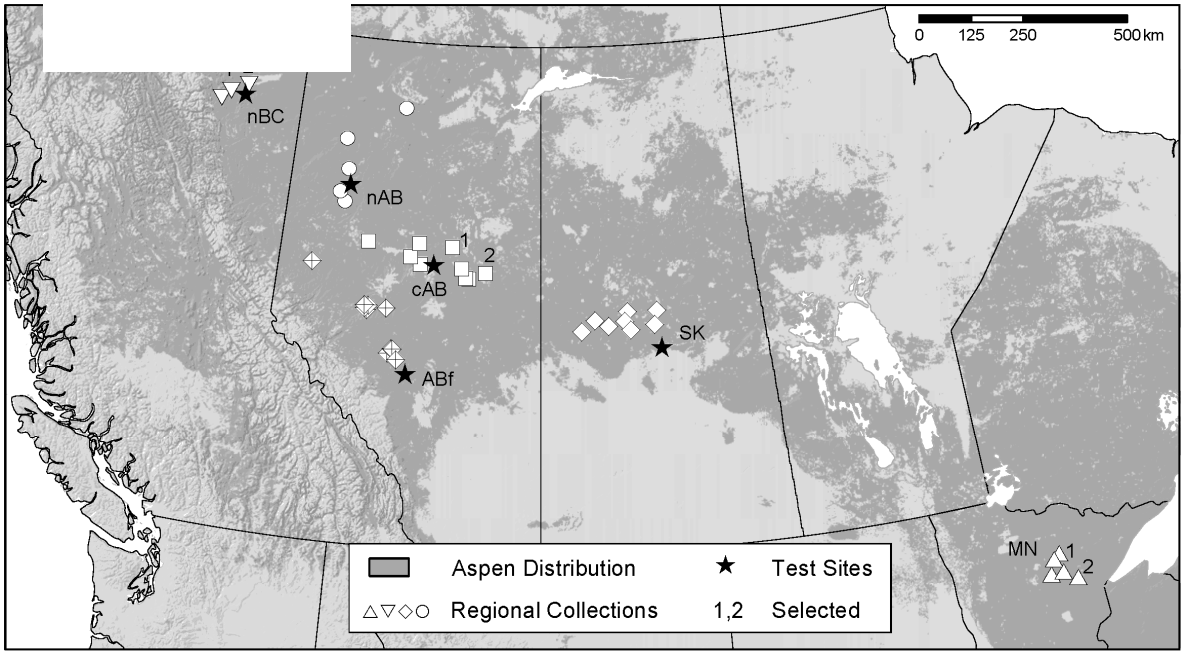

Figure 2-1 Collection locations and test sites of the provenance trial series. Genotypes selected for the physiological study are indicated by numbers and are, for example, referred to as MN1 or MN2 in subsequent figures.

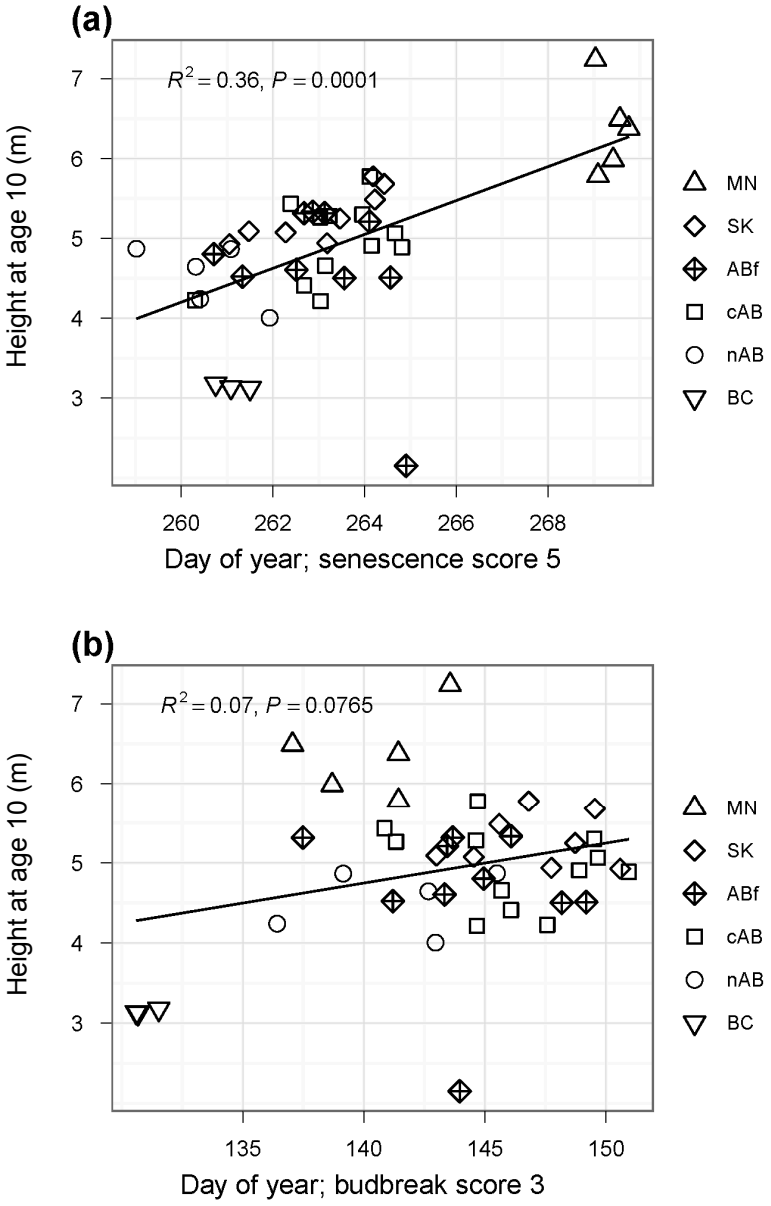

Figure 2-2 Regression of 11-year height and timing of leaf senescence (a) and bud break (b). Shapes represent regions ordered along a northwest gradient for each provenance.

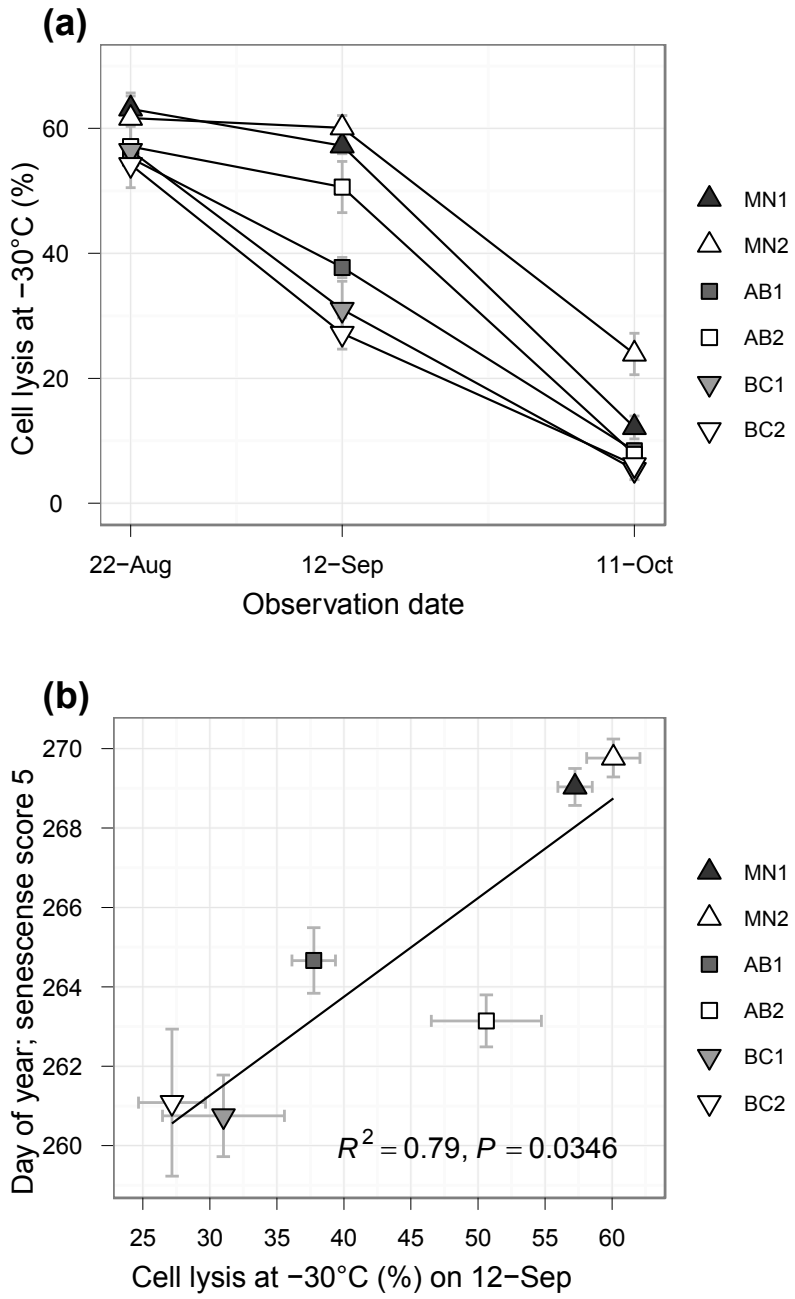

Figure 2-3 Cell lysis at -30°C for six different aspen provenances measured on three dates in late summer and autumn in 2011 (a). Regression of the day of year of leaf senescence and cell lysis at -30°C for 12 September (b). Symbols and shading represents regions and genotype within region ordered along northwest gradient.

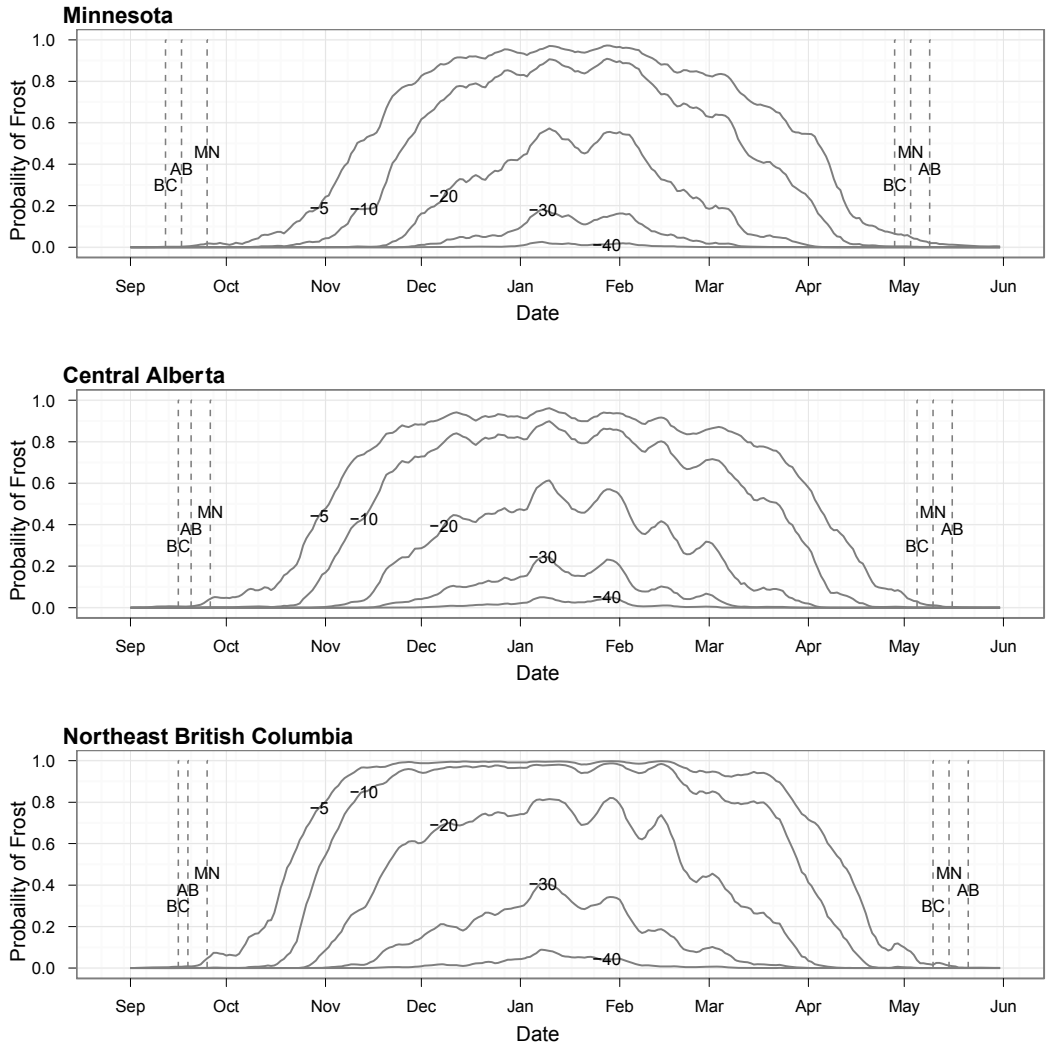

Figure 2-4 Probability of a frost event being equal or exceeding a certain threshold value for any given day between 1 September and 31 May at the Minnesota (top) central Alberta (middle), and northeast British Columbia (bottom) planting sites. The expected day of bud break calculated for 1961–1990 normal climate, and the expected day of leaf senescence for the latitude of planting sites is indicated by vertical lines for provenances from central Alberta, northeast British Columbia, and Minnesota.

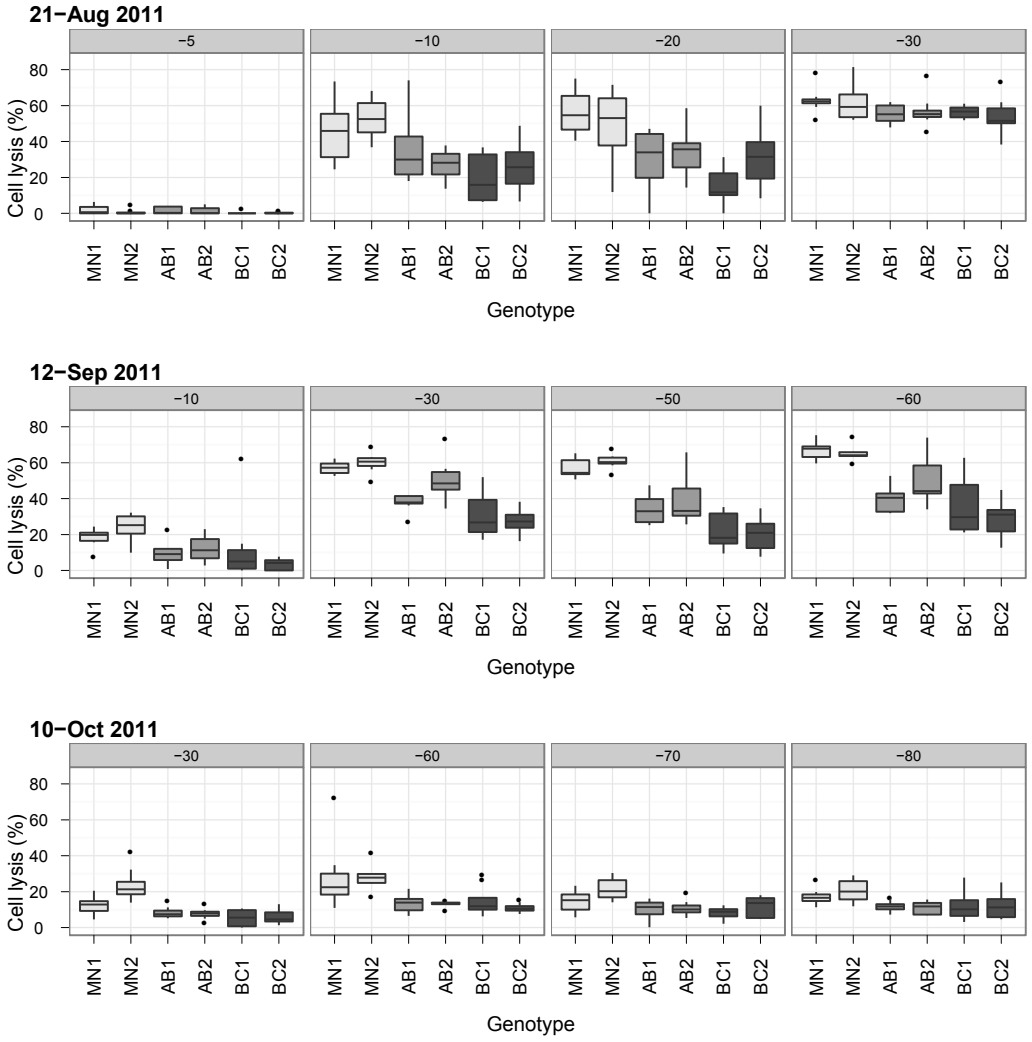

Figure 2-5 Frost hardiness as indicated by for six aspen provenances measured on 21-August (top), 12-September (middle) and 10-October (bottom) in response to different artificial freezing treatments.

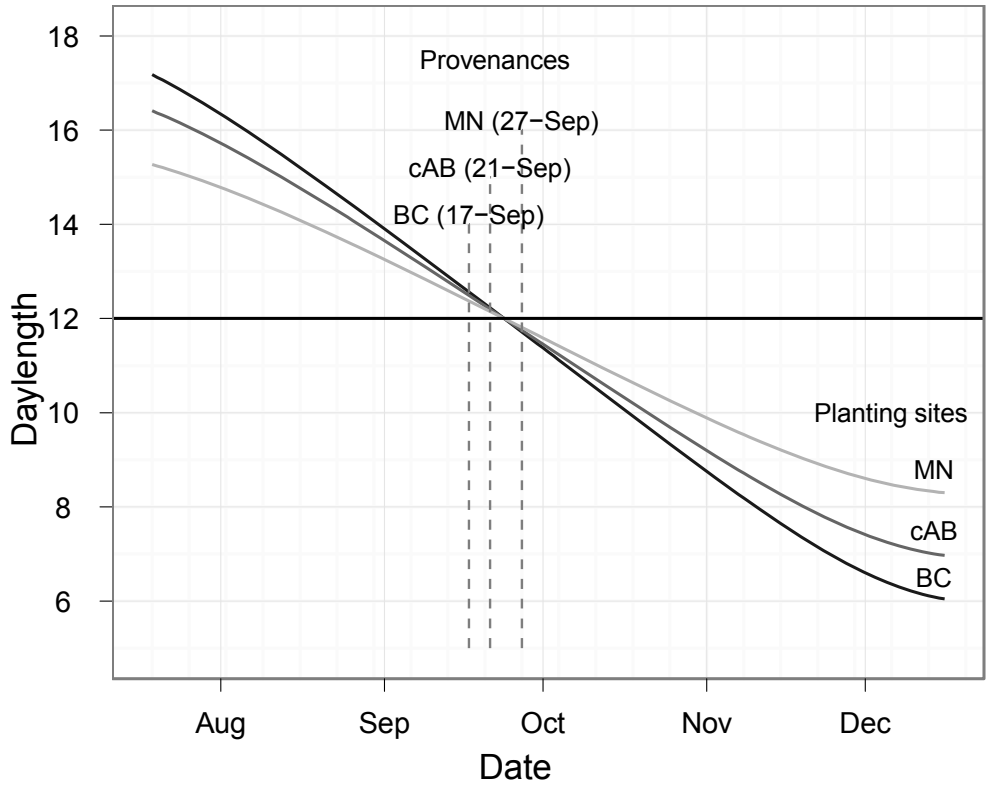

Figure 2-6 Changes of daylength for the latitudes of the regions Minnesota (MN), central Alberta (cAB), and northeast British Columbia (BC). The dates of leaf senescence for the corresponding provenances, observed in a common garden at the central Albert test site, are indicated by vertical lines.

Chapter 3 - Post-glacial biogeography of trembling aspen inferred from genetic structure, genetic diversity, and habitat models

3.1 Summary

We use information on among- and within-population genetic differentiation, as well as bio-climatic envelope models to reconstruct the paleoclimatic and biogeography of *Populus tremuloides* Michx.. Our main goals were to identify plausible glacial refugia and potential recolonization paths since the last glacial maximum, and to potentially locate suitable habitats, where aspen clones may have survived multiple glaciations. We used RandomForest method to reconstruct paleoclimatic habitats for the periods 21,000, 14,000, 11,000 and 6,000 years before present. Genetic structures and diversity in quantitative traits were evaluated in common garden trials with 43 open-pollinated aspen familycollections ranging from Minnesota to northern British Columbia. Paleoclimatic habitat reconstructions indicated the recolonization of Canada and Alaska populations from refugia in the eastern United States. This was also supported by a southeast to northwest gradient of decreasing genetic variance in quantitative traits, which was likely due to repeated founder effects. Stable habitats where aspen clones may have survived multiple glaciations were predicted in Mexico and the eastern United States, but not in the west where some of the largest aspen clones have been documented. We reviewed published information on genetic structure in neutral genetic marker data and we concluded

that aspen recolonized areas covered by the Laurentian and Cordilleran ice sheets from the east, while recolonization from the southwest or Alaska was unlikely. We estimated that the largest documented aspen clones were therefore not ancient organisms that survived multiple glaciations which agreed with previous molecular genetic studies on aspen.

3.2 Introduction

Trembling aspen (*Populus tremuloides* Michx.) is arguably the most widely distributed, abundant and genetically diverse forest tree in North America, occupying many ecological site types from Mexico to Alaska in the west, and across Canada and the United States to the Atlantic ocean in the east (Little 1971; Mitton and Grant 1996; Peterson and Peterson 1992). Aspen is considered a pioneer species, yet can persist in newly colonized environments for thousands of years through clonal reproduction. Due to its unique life history, large range, and wide ecological amplitude, aspen is an interesting study object for questions concerning ecological genetics, physiology, and biogeography.

Aspen can reproduce in marginal habitats and survive disturbance events by root suckering (Barnes 1966). Seed production is commonly observed but seedling establishment is relatively infrequent, especially in the semiarid areas of western North America (Kemperman and Barnes 1976; Mitton and Grant 1996). In moister habitat of the northern Rocky Mountains and eastern North America, seedling establishment occurs more frequently (Kemperman and Barnes 1976;

Landhäusser et al. 2010). Once an individual is established, it will send out lateral roots from which hundreds of ramets can originate. The clone increases in size as each ramet also contributes distally to the expanding root system from which new stems can be formed (Barnes 1966). The largest aspen clone to date (known as "Pando") covers 43 ha and comprises 47,000 stems with an estimated biomass of 6,000 t (DeWoody et al. 2008; Grant et al. 1992; Kemperman and Barnes 1976). In the eastern United States, the average clone size has been estimated to be approximately 0.04 ha, with exceptional individuals reaching 14 ha (Barnes 1966, 1969; Zahner and Crawford 1965). In central Canada, average clone sizes were reported around 0.08 ha, with the largest clones reaching 1.5 ha (Steneker 1973).

Several genetic studies have also shown that trembling aspen shows exceptionally high levels of genetic diversity, but little among-population genetic differentiation in neutral genetic markers, such as isozymes, microsatellites or other molecular markers (Callahan et al. 2013; Cheliak and Dancik 1982; Cole 2005; De Woody et al. 2009; DeWoody et al. 2008; Hyun et al. 1987; Lund et al. 1992; Mock et al. 2008; Namroud et al. 2005; Wyman et al. 2003; Yeh et al. 1995). When comparing levels of genetic diversity using isozymes, some western populations (e.g. Alberta) had exceptionally high genetic diversity with an expected heterozygosity (H_e) of 0.42 (Cheliak and Dancik 1982), while other aspen populations in Alberta showed an average H_e of 0.29 (Jelinski and Cheliak 1992).

In contrast, electrophoretic surveys of aspen in eastern populations (e.g. Minnesota and Ontario) were lower with H_e rates of 0.22 and 0.25, respectively (Hyun et al. 1987; Lund et al. 1992). In a recent range-wide study of genetic structure and diversity based on microsatellite markers, Callahan et al. (2013)

identified a clear geographic differentiation into a genetically more diverse northern cluster (Alaska, Canada, northeastern US) and a less diverse southwestern cluster (western US and Mexico). However, due to different rates and mechanisms of mutations in isozyme versus microsatellite marker systems the outlined results may not be contradictory (Arnaud-Haond et al. 2007; De Woody et al. 2009).

A different approach to investigate genetic structure and diversity is to assess genetic differences of quantitative traits in common garden experiments. Common garden studies can help identify distinct ecotypes and provide information on local adaptation. Regional common garden trials have shown strong within-population genetic variation and moderate to high heritabilities for growth and adaptive traits in aspen (Kanaga et al. 2008; Lindroth et al. 2007; St. Clair et al. 2010; Thomas et al. 1998a, b). In a reciprocal transplant experiment, Schreiber et al. (2013) showed evidence for strong suboptimality in adaptive traits.

Suboptimality in quantitative traits could potentially be explained by considering aspen's clonal life history, with populations being adapted to fossil climate conditions (Brouard 2004). In fact, it has been speculated that aspen clones may be millions of years old and have survived dozens or hundreds of glacial cycles (Barnes 1966; Kemperman and Barnes 1976; Mitton and Grant 1996). Although precise dating of aspen clones remains an elusive task, recent studies have drawn some boundaries. Ally et al. (2008) found that the upper boundary for the age of aspen clones at two study sites in British Columbia is approximately 4,000 and 10,000 years, which corresponds well with the timing of glacial retreat at those two sites. Relating clone size with age, however, proved not possible.

Speculations about very large clones that may have persisted through repeated glacial cycles are also not supported by Mock et al. (2008), who studied the largest known clone "Pando" and concluded that it does not exhibit enough somatic mutations to be more than several thousand years old.

Another valuable approach to address questions concerning biogeography and species migration are species distribution models. These models use observed species range data in combination with environmental predictors (typically climate) to generate statistical relationships, which can be used to project probabilities of species presence from new environmental data (Elith and Leathwick 2009). Although more typically used as a risk assessment tool for future climate change (e.g. Thomas et al. 2004), they are also employed to reconstruct biogeographical histories of species (e.g. Gugger et al. 2011; Gugger et al. 2013; Rissler and Apodaca 2007; Roberts and Hamann 2012b). Model reconstructions of species' past ranges and glacial refugia can provide insight into phylogeographic processes that can determine patterns of genetic diversity on the present-day landscape.

Here, we apply this approach to investigate (1) whether geographic patterns in neutral genetic markers correspond to different glacial refugia, (2) whether areas of high genetic diversity could be explained by merging of refugial populations during the recolonization history of aspen, (3) whether patterns of suboptimality could be explained by persistence of clones after recolonization, and (4) whether habitat reconstructions support the possibility of very old clones that may have persisted through multiple glaciation cycles.

3.3 Methodology

3.3.1 Climate data

Climate data were generated according to Hamann et al. (2013), available for downloading at *http://tinyurl.com/ClimateNA.* We use a 1961-1990 climate normal baseline dataset generated with the Parameter-elevation Regressions on Independent Slopes Model (PRISM) for monthly average minimum temperature, monthly average maximum temperature and monthly precipitation (Daly et al. 2008). From 36 monthly variables, six biologically relevant climate variables were derived that account for most of the variance in climate data while avoiding multicollinearity: the number of growing degree days above 5°C, mean maximum temperature of the warmest month, temperature difference between mean January and mean July temperatures, mean annual precipitation, April to September growing season precipitation, and November to February winter precipitation. The procedure of selecting these climate variables is described in more detail in Worrall et al. (2013), Supplement 1. The algorithms to estimate biologically relevant variables from monthly temperature and precipitation surfaces are explained in detail by Rehfeldt (2006). To represent paleoclimatic conditions, we overlayed the 1961-1990 baseline climate with temperature and precipitation anomalies for 6,000, 11,000, 14,000 and 21,000 years before present, generated by the Community Climate Model (CCM1) developed by the National Center for Atmospheric Research (NCAR) (Kutzbach et al. 1998). Subsequently the same derived variables were generated as above.

3.3.2 *Species distribution modeling*

Past aspen habitat was reconstructed using a species distribution model for aspen based on more than 300,000 presence/absence data points from forest inventory plots, ecology plots and herbarium accessions throughout North America (Worrall et al. 2013). We used a regression tree ensemble classifier to relate climate variables to aspen census data, implemented by the *randomForest* package (Liaw and Wiener 2002) for the R programming environment (R Development Core Team 2013). Model hindcasts were validated against 9,568 records of combined fossil pollen, macrofossil, and neotoma midden data, drawn from the Neotoma Paleoecology Database (www.neotomadb.com) for the time periods considered, using the area under the curve (AUC) of the receiver operating characteristic (Fawcett 2006). The AUC for the model based on this independent validation was 0.67, with mean sensitivity and specificity values of 0.56 and 0.63, respectively, suggesting moderately good model fit and well-balanced errors of omission and commission. For more details on standard cross-validations and completely independent validations against fossil and fossil pollen records, see Roberts and Hamann (2012a). Because the model estimates a probability of species presence for a given geographic location, we multiplied projected aspen presence for the 1961-1990 baseline period with projected aspen probability of presence for 21,000 years BP to identify areas on the landscape where aspen had a high probability of surviving multiple glaciations.

3.3.3 *Common garden experiments*

In this paper we also reanalyze data from a large-scale common garden trial in a different context. The trial is a randomized complete block design with 43 provenances planted in 5-tree row plots, in 6 blocks, at each of 5 sites. Provenances are open-pollinated single-tree seed collections from six ecological regions (Fig.3.1; for further details refer to Schreiber et al. 2013). The measured traits were tree height, timing of bud break and timing of leaf senescence. Tree height was measured for 6,450 trees after nine growing seasons in the field in autumn of 2006 for all five test sites. Phenological measurements, i.e. timing of bud break and timing of leaf senescence were taken on 1,290 trees at the central Alberta test site.

To visualize multitrait genetic differentiation of the 43 seed sources, as well as the multivariate differences in the climate conditions of the seed source locations, we use principal component analysis implemented with the *FactoMineR* package (Husson et al. 2013) for the R programming environment (R Development Core Team 2013). For the genetic ordination, traits summarized into principal components were height at five sites plus bud break and leaf abscission measured at one site (7 variables). For the climatic ordination, nine variables were used to describe the multivariate climate space more completely (Fig. 3.2).

3.3.4 *Within-population variance*

The common garden trial was primarily meant as a provenance experiment to investigate genetic differentiation in adaptive traits among populations. However, it can also be used to estimate regional within-population phenotypic variation by

calculating residual variance components. Since all provenances experience the same environmental conditions at a given test site, differences in the residual phenotypic variance components can be attributed to different levels of genetic variance (including dominance and epistatic genetic effects that we cannot quantify). We therefore refer to differences in the residual variance components as differences in within-population genetic variation hereafter. Strictly speaking, they are differences in within-population phenotypic variation with the environmental variance component held constant (although we cannot quantify its absolute value). To estimate variance components, we use a random-term linear mixed effects model implemented with PROC MIXED of the SAS statistical software package:

$$Y_{ijk} = \mu + P_i + S_j + P \times S_{ij} + B(S)_{jk} + P \times B(S)_{ijk} + e_{ijk}\,, \quad (1)$$

where Y_{ijk} is the phenotypic observation of a trait made for the *i*-th provenance (*P*) grown in the *j*-th test site (*S*), in the *k*-th block (*B*) within test site (*S*). A genotype × environment effect is given by the interaction between provenance and test site ($P \times S$) as well as provenance and block within test site $\left(P \times B(S)\right)$. Bud break and leaf senescence were only measured at one test site (central Alberta), and in this case the test site effect does not apply and the block within site effect becomes a simple block effect.

3.4 Results

3.4.1 *Genetic differentiation and adaptation*

Principal component analyses for climate conditions at seed source locations and multitrait measurements in common garden trials are shown in Fig. 3.2. The vectors represent component loadings, which are the correlations of the principal components with the original variables. The strength of the correlation is indicated by the vector length, and the direction indicates which seed sources have high values for the original variables. Climate conditions of seed source locations show a number of distinct groups (Fig. 3.2a). Minnesota sample site climates are characterized by warm and long summers (MWMT, DD>5), Saskatchewan sources have the longest and harshest winter conditions (DD<0, opposite MCMT), the Alberta Foothills sources have the strongest maritime influence with mild winters (MCMT, opposite DD<0 and TD) and high precipitation (MAT, MSP), whereas the boreal forest locations (cAB, nAB, BC) are characterized by cool summers and short growing seasons, as well as dry growing season conditions (opposite MWMT, MAP, DD>5).

Regarding genetic structure of populations (Fig. 3.2b), only two groups of samples are clearly differentiated from the other groups based on the measured traits. In this figure, symbols represent provenance collections, and vectors represent height measurements at five test sites (arrow labels BC, nAB, cAB, ABf, SK), plus bud break and leaf senescence measurements at one site (cAB). Provenances from British Columbia (BC) are characterized by poor relative performance at most test sites, particularly under the mild climates of the Alberta Foothills test site (opposite to most height vectors, particularly ABf). BC provenances are also characterized by early bud break. The other group that is

clearly separated is the Minnesota (MN) provenances, which grow well at most test sites, particularly the central Alberta site (cAB). They are also characterized by late leaf senescence. The remaining groups of samples are not genetically differentiated in the measured traits, although the climate conditions of their origins are quite distinct, particularly for the Saskatchewan (SK) and Alberta Foothills (ABf) source climates (*cf*. Fig. 3.2a).

3.4.2 *Regional within-population genetic variation*

Residual variance components of growth and adaptive traits by region of origin reveal the Alberta Foothills and Minnesota as the most genetically diverse regions in quantitative traits (Table 3.1). If we ignore the sub-boreal Foothills location, a trend toward decreasing genetic diversity across aspen's main boreal distribution from southeastern Minnesota to Northwestern British Columbia is apparent in all measured traits (*cf*. Fig. 3.1). The gradient is most pronounced for height measurements (0.94 in MN to 0.61 in BC), and height measurements also have the highest accuracy of within-population diversity estimates, because they were evaluated at five sites. With respect to timing of bud break, all western Canadian provenances are fairly homogenous only contrasting with the Minnesota provenances with much higher within-population diversity. The Alberta Foothills region has the highest residual variance for the timing of leaf senescence followed by Minnesota.

3.4.3 *Paleoclimatic habitat reconstructions*

Our historical projections of aspen habitat for 6,000, 11,000, 14,000 and 21,000 years before present (BP) show three potential glacial refugia in which aspen may have found suitable habitat during the last glacial maximum (Fig. 3.3). The predicted 21,000 years BP refugia are found in present-day Alaska, although small and with a low probability of presence, and in the southwestern and eastern United States (Fig. 3.3a). Our maps highlight a potential contact zone located in the prairie provinces of western Canada in which populations from these three refugia may have merged after the retreat of the Wisconsin glaciers at around 11,000 years BP (Fig 3.3c). The largest predicted glacial refugium was in the eastern United States, which may have contributed the highest within population gene flow and genetic variation during recolonization of the North American continent.

Fig. 3.4 shows a higher resolution image of the same projections of aspen habitat for the Fish Lake National Forest in south central Utah, where the largest confirmed aspen clone "Pando" has been documented. The model predicts suitable habitat to emerge at the earliest at 14,000 years BP, and no suitable habitat is predicted in the vicinity of today's location of the clone at the last glacial maximum at 21,000 years BP. At a larger scale, the overlap of suitable aspen habitat between the present and the last glacial maximum was obtained by multiplying probabilities of presence between the model outputs for the 1961-1990 baseline period and for 21,000 years BP (Fig. 3.5). The analysis reveals only a few locations in which aspen populations had a moderate or high probability of surviving multiple glaciations. These areas are located in eastern United States (southeastern Ohio) and the Sierra Madre mountain range in northeastern Mexico.

3.5 Discussion

3.5.1 Potential glacial refugia

Paleoclimatic habitat reconstructions suggest three potential glacial refugia for trembling aspen from which recolonization may have occurred. The eastern United States represent by far the largest refugium with the highest probabilities of presence, followed by the low elevation areas of the southwestern United States, and Alaska. Although the modeled Alaska refugium was very small with a low-probability of presence, the possibility of aspen recolonization from the north cannot be convincingly excluded based on habitat reconstructions alone. This leaves three conceivable recolonization scenarios for aspen: (1) recolonization almost exclusively from the southeast to northwest up into Alaska; (2) recolonization predominantly from the east but with contributions from either the southwestern or Alaskan refugia, and (3) simultaneous recolonization from all three glacial refugia with a potential contact zone in Alberta, Canada, potentially explaining high levels of genetic diversity documented for this region.

This study did not investigate genetic structure and diversity in neutral genetic markers, which are well suited to infer origins of populations from different glacial refugia. However, Callahan *et al*. (2013) analyzed genetic structure in neutral microsatellite markers in a rangewide study of the species. They found two main genetic clusters in today's populations, an isolated southwestern cluster and a northern cluster comprised of the Alaskan, Canadian and the eastern United States populations. Notably, Callahan et al. (2013) observed an "outlier" in the

southern cluster (a Yellowstone population), which according to the microsatellite data grouped into the northern cluster. The Yellowstone area was covered with ice at the last glacial maximum (Dyke et al. 2002) meaning that the area was recolonized after retreat of the glaciers.

3.5.2 *Recolonization from eastern refugia*

Our habitat reconstructions suggest that this Yellowstone population was indeed recolonized from the east. Although westward extending habitat from eastern refugia at 14,000 years BP did not reach all the way to the Yellowstone region, it does extend well into Montana (Fig. 3.3b). It seems therefore plausible that eastern population stretched all the way to the Rocky Mountain foothills at some point in time between 11,000 and 14,000 years BP, providing a complete southern front along the entire length of the Laurentian ice sheet for northward recolonization of the Canadian boreal and Alaska, with little opportunity for southwestern contributions.

The hypothesis of aspen recolonization exclusively from the east (1) is further supported by our finding of a southeast to northwest gradient of decreasing genetic variance in quantitative traits (Table 3.1). Such a gradient would be expected because of repeated founder effects during post-glacial migration northwards (Davis and Shaw 2001). Patterns of adaptational lag in quantitative traits also fits well with this migration history. Aspen provenances from northeastern British Columbia are the least well-adapted populations in terms of growth, survival, phenology and frost hardiness compared to populations from

Alberta and Minnesota (Schreiber et al. 2013). Decreasing genetic diversity in combination with aspen's clonal life history slow the process of adaptation to new environmental conditions, with current populations essentially being adapted to fossil climates (Brouard 2004).

3.5.3 *Alternate recolonization patterns*

We should note, however, that a southward expansion from a genetically low diverse and isolated refugial population in Alaska may also explain the observed high degree in suboptimality in the British Columbia populations. However, data reported by Callahan *et al.* (2013) does not support the existence of glacial refugia for aspen in Alaska, with an absence of genetically differentiated populations in this region. Alaska populations have not been sampled in our study, and only few locations were sampled by Callahan, but a southward recolonization hypothesis (2) seems unlikely based on molecular genetic information and habitat reconstructions.

The post-glacial migration scenario (3) with populations from three refugia making contact in Alberta and potentially explaining high levels of genetic diversity observed by (Cheliak and Dancik 1982) also seems implausible. Interestingly, we also found high levels of genetic diversity in quantitative traits in the Alberta Foothills region (Table 3.1). One explanation may be that under more favorable environmental conditions in the foothills, sexual reproduction and successful seedling establishment is more common (Landhäusser et al. 2010), and

becomes a driver for generating and maintaining genetic diversity through recombination.

3.5.4 *Stable habitat and ancient clones*

The largest and potentially oldest aspen clone to date, known as “Pando”, occupies 43 ha in the Fish Lake National Forest in south central Utah. The age of this clone has been subject to speculation that it could be several millions of years old and having survived multiple glaciations (Barnes 1966; Kemperman and Barnes 1976; Mitton and Grant 1996). However, paleoclimatic habitat projections suggest 14,000 years BP as the earliest date for the emergence of suitable habitat, which is in agreement with molecular studies suggesting that Pando may in fact be of relatively young age (Mock et al. 2008). Our model predicts only a few regions in northern Mexico and a few small patches in the eastern United States from which no exceptionally large clones have been documented. At this point, there is no strong indication for the existence of ancient aspen organisms that may have survived multiple glaciations.

3.6 References

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Table 3-1 Measured adaptive traits and residual variance components summarized by region. Standard errors (SE) in parentheses. Height measurements were taken at all five test sites. Bud break and leaf senescence was measured only at the central Alberta test site

	Within-population variance components		
Region	Height	Bud break	Leaf senescence
BC Northeast	0.61 (0.06)	8.4 (1.7)	5.2 (2.7)
Northern AB	0.71 (0.04)	8.9 (1.3)	6.6 (1.4)
AB Foothills	0.87 (0.03)	9.2 (1.0)	10.3 (1.4)
Central AB	0.81 (0.03)	8.8 (0.8)	7.5 (0.8)
Saskatchewan	0.80 (0.04)	8.9 (0.9)	6.2 (0.7)
Minnesota	0.94 (0.06)	13.1 (1.5)	8.3 (1.2)

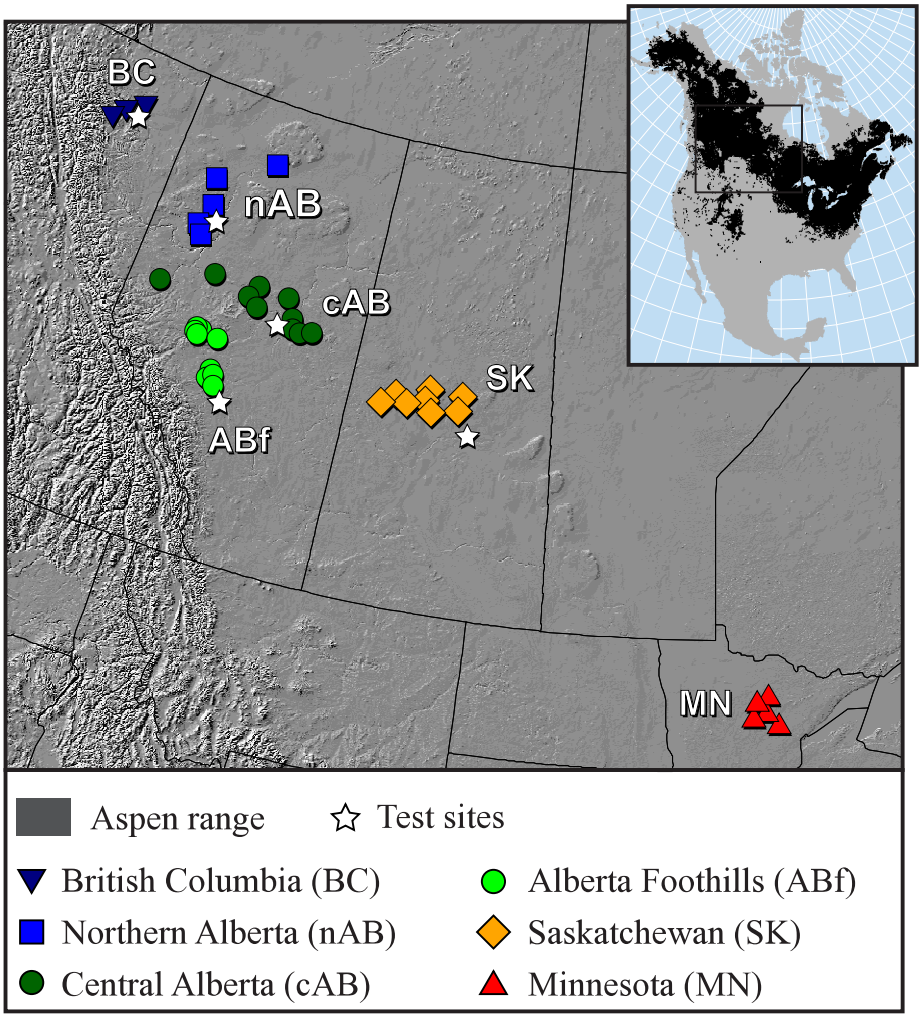

Figure 3-1 Collection locations and test sites of the aspen provenance trial series.

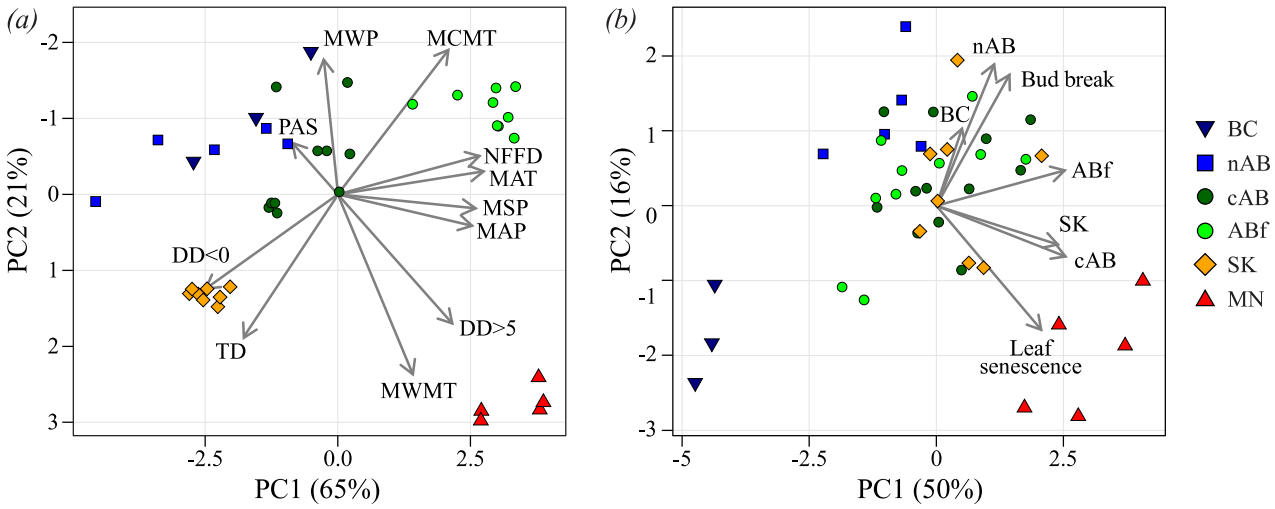

Figure 3-2 Principal component analyses for climate conditions at seed source locations (a) and multitrait measurements in common garden trials (b). Vector labels represent the input variables. Symbols in (a) represent the geographic location of provenances; symbols in (b) represent the provenance collections. Vector labels in (a): PAS = precipitation as snow (mm), MWP = mean winter precipitation (°C), MCMT = mean coldest month temperature (°C), NFFD = number of frost free days, MAT = mean annual temperature (°C), MSP = mean summer precipitation (mm), MAP = mean annual precipitation (mm), DD>5 = degree-days above 5°C (growing degree-days), MWMT = mean warmest month temperature (°C), TD = temperature difference between MCMT and MWMT (or continentality, °C), DD<0 = degree-days below 0°C (chilling degree-days); Vector labels in (b): BC = height at British Columbia test site, nAB = height at northern Alberta test site, ABf = height at Alberta Foothills test site, SK = height at Saskatchewan test site, cAB = height at central Alberta test site, Bud break = timing of bud break at central Alberta test site, Leaf senescence = timing of leaf senescence at central Alberta test site.

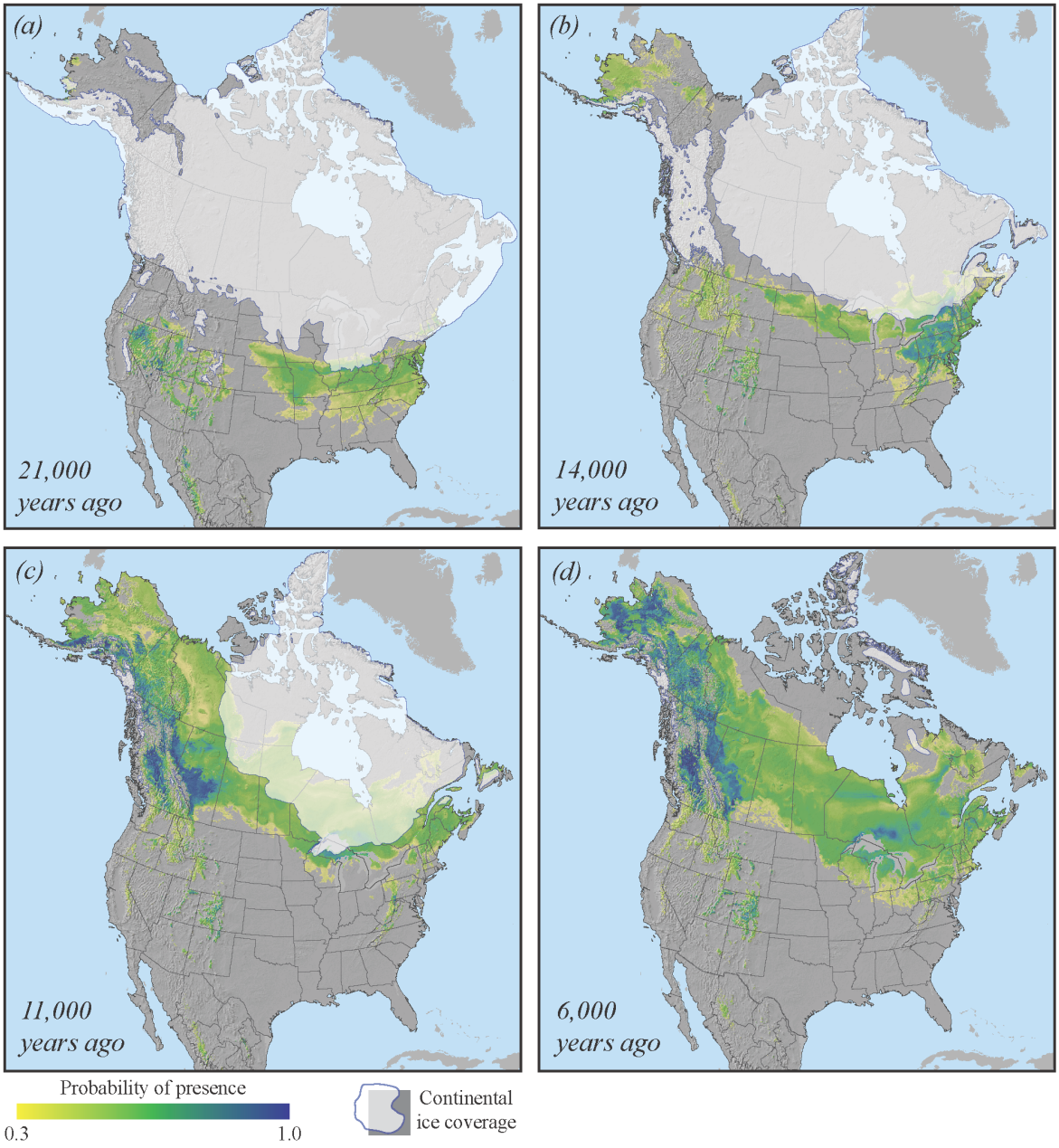

Figure 3-3 Paleoclimatic habitat projections for trembling aspen (a) present day, (b) 14,000 years before present (BF), (c) 11,000 years BF and (d) 6,000 years BF.

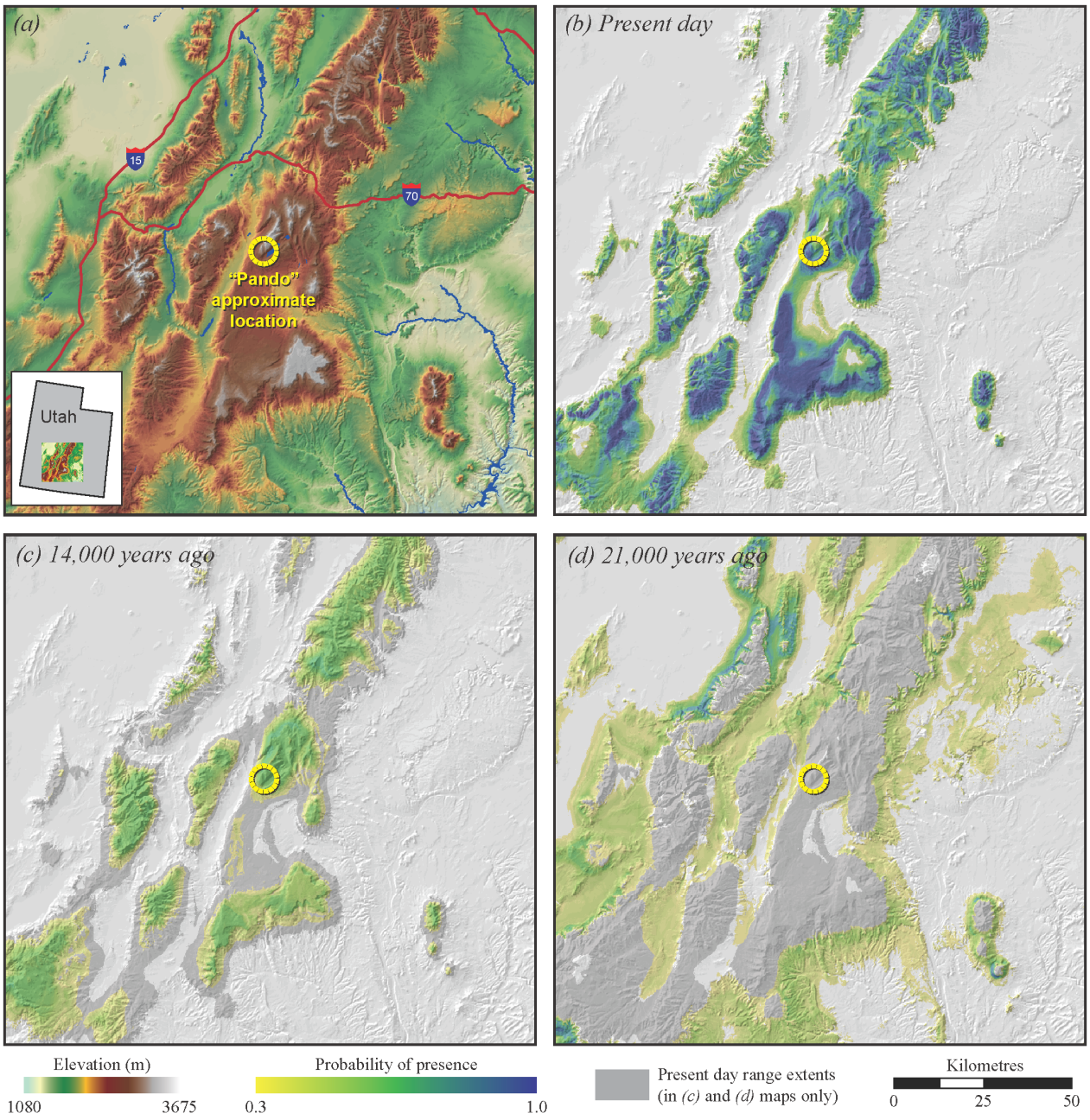

Figure 3-4 (a) Topographic map of south-central Utah highlighting the approximate location of the aspen clone "Pando". Paleoclimatic habitat projections for trembling aspen in south-central Utah (b) present day, (c) 14,000 years BF (before present), (d) 21,000 years BF.

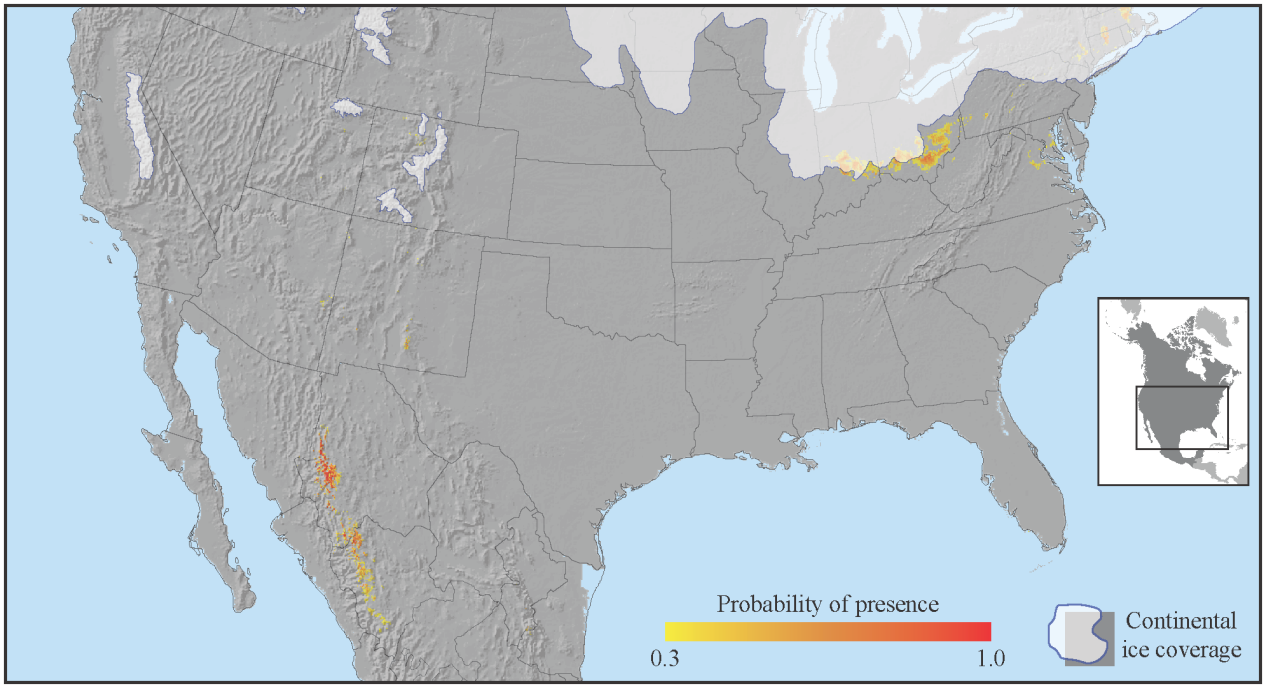

Figure 3-5 Probabilities of geographic locations in which aspen clones may have persisted through multiple glaciations. Data points were derived by multiplying the probability of presence estimates of the 1961-1990 reference climate with the 21,000 years before present period.

Chapter 4 - Genetic parameters of growth and adaptive traits for aspen (*Populus tremuloides*): implications for tree breeding

4.1 Summary

Aspen is a widespread commercial forest tree of high economic importance in western Canada and has been subject to tree improvement efforts over the past two decades to increase productivity of the forested land base. Successful selection and breeding programs rely on both accurate estimates of the potential for genetic gain for commercial traits as well as correlated responses of other traits that may be important for fitness. Here, we estimate genetic parameters of growth and adaptive traits in 10 progeny trials containing more than 30,000 trees with known pedigree structure based on a partial factorial mating design that includes 60 half-sib families, 100 full-sib families and 1,400 clones. Narrow-sense as well as broad-sense heritabilities were generally low with values around 0.2 and standard errors of approximately 0.1. Two phenology traits, bud break and leaf abscission, had moderate broad- and narrow-sense heritabilities around 0.4 with standard errors of 0.1. For all measured traits, additive genetic variation was most important and dominance and epistatic variance components were small or zero. Moderate to strong genetic correlations were found between growth and phenology (r=-0.3 and 0.7) with tall trees being associated with early budbreak and late leaf abscission. Survival was not compromised, and in fact positively associated with early bud break or late leaf abscission, indicating that utilization of the growing season was more important than avoidance of early fall frosts or

late spring frosts across all test sites in this experiment. We conclude that selection for juvenile growth in aspen trees promises only small genetic gains. Strong additive genetic correlations between growth and phenology indicates that much of the genetic gain at the early stage of stand development will be due to expanding the growing season, which may increase the risk of frost damage in spring and fall, but those issues may have been mitigated by observed climate warming trends.

4.2 Introduction

Trembling aspen (*Populus tremuloides* Michx), is an early successional species with a broad natural range including the boreal forest of North America, the eastern United States, and the western mountain ranges from Mexico to Alaska. Aspen can regenerate both via sexual and asexual reproduction. The species regenerates quickly from root suckers, often resulting in single species stands after disturbances (Perala 1990). Clones of aspen can persist for very long times and grow to large sizes by root suckering (Long and Mock 2012).

Over the last two decades, aspen has become one of the most important commercial forestry species in western Canada due to the development of oriented strand board (OSB) production (Ondro 1991). In Alberta, aspen represents 40% of the total forest harvests in 2010 (Gylander et al. 2012), and tree improvement programs have been developed to maximize the yield in short rotation forestry systems. In Alberta, three breeding regions were initially delineated to develop locally adapted and highly productive planting stock via

tree breeding programs (Li 1995). The present tree improvement program targets two of these regions, which are also subject to the present study (Gylander et al. 2012).

Selection and breeding require sufficient genetic control of observed variation for the traits of commercial interest. Additive genetic variance components and narrow-sense heritabilities are of interest for normal recurrent selection. In aspen, dominance and epistatic genetic variance components are of interest as well for clonal selection, because the species can be clonally propagated to generate reforestation stock (Barnes 1975). Broad-sense heritability of height and diameter at breast height (DBH) has previously been estimated in clonal trials and ranges from 0.36 to 0.64 (Gylander et al. 2012). In a similar experiment, St Clair et al. (2010) reported broad-sense heritability ranging from 0.23 to 0.35. Estimates of narrow-sense heritabilities, relevant for breeding programs, are usually not available because the additive and non-additive genetic effects are confounded in genetic parameter estimation from clonal trials.

Also lacking for trembling aspen are estimates of heritability and genetic correlations of adaptive traits that are important in tree breeding programs to avoid mal-adaptation and minimize the risk of mortality in plantations (Ibanez et al. 2010a; Ingvarsson et al. 2008; Rohde et al. 2011). For example, unseasonal frost events in spring and fall may damage buds and leaves, and eventually jeopardize productivity and survival (Hanninen 2006). In selection and breeding for tree growth, inadvertent response of other traits related to fitness may occur as a byproduct. Antagonistic pleitropy, where one gene controls multiple traits, at least one of which increases and others decrease fitness, may play a role in trade-

offs between traits that show high negative genetic correlations (Hedrick, 1999). Such antagonistic pleiotropy may result in unexpected responses to selection, when correlated response in adaptive traits compromises expected gains in productivity (McKown et al. 2014a). Pleiotropic loci of phenological traits were reported in *Populus trichocarpa* (McKown et al. 2014b), where trees selected for growth traits expressed delayed leaf senescence with weak frost tolerance.

Here, we investigate if better growth characteristics can be accomplished through tree breeding without increasing risks of maladaptation in aspen. We evaluate ten progeny trials containing more than 30,000 trees with known pedigree structure, including 60 half-sib families, 100 full-sib families and 1,400 clones to estimate the breeding potential and genetic parameters for collections from Alberta. This chapter focuses on the genetic variation within-populations which is essential for tree improvement. Additive and non-additive genetic variance components are estimated for two growth traits, height and diameter at breast height, and two adaptive traits, the timing of bud break and leaf abscission. Specifically, genetic correlations among these traits to identify potential trade-offs between growth and adaptive traits are investigated, and we ask if selection for better productivity could lead to inadvertent selection for utilizing a longer growing season resulting in higher risks of frost damage.

4.3 Materials and Methods

4.3.1 Study area and plant material

Aspen tree improvement in western Alberta is organized into a northern and a southern breeding region with different macroclimate conditions (Fig. 4.1). The tree improvement programs initially tested a large number of clones collected from natural stands, targeting plus trees with good form and without signs of pathogens and diseases from natural stands. From the initial series of clonal trials, 122 clones of superior growth were selected as male or female parents for a partial factorial mating design (to be detailed below). The offspring were planted in ten progeny trials, established between 2005 and 2008 by the Western Boreal Aspen Corporation (WBAC), an industrial collaborative that includes Ainsworth Engineered Canada LP, Daishowa Marubeni International Ltd. and Weyerhaeuser Canada Ltd. The origins of the parental material for the progeny trials are listed in Appendices 4.1. and 4.2.

There were 64 half-sib families as well as 100 full-sib families generated in a partial factorial mating design for two breeding regions (Appendices 4.3 and 4.4). Each male and female parent was represented by one or two full-sib families and each female parent was also pollinated with a polymix to generate half-sib families. The pedigree structures of northern and southern breeding regions were constructed separately. The first two trials, planted in 2005, were seedling trials planted in the southern breeding region, sharing the same half-sib and full-sib families without clonal structure. Trials 3 to 8 were planted in 2007 and utilized families from both breeding regions. Families were clonally replicated prior to planting, so that these trials have half-sib, full-sib, and clonal structure (i.e., multiple ramets of the same clone, with clones replicating multiple individuals of full-sib families). The trials 9 and 10 were established in 2008, sharing different clonal material but overlap in half-sib and full-sib families with trials 3 to 7.

Appendix 4.5 and 4.6 show how trials are connected through shared half-sib and full-sib families.

Seedling material for trials 1 and 2 were grown in a greenhouse in April to May 2004, hardened in September to October 2014, packed and cold-stored in a refrigerator in winter before planting in May 2005. Clonal planting stock for trials 3-10 were produced from root cuttings from seedlings of half-sib and full-sib families, grown and hardened in a nursery, packed and cold-stored in a refrigerator in winter before planting in May 2007 and 2008.

4.3.2 Experimental design and measurements

All trials were constructed using an alpha design (Williams et al. 2002). The use of alpha designs allowed for the flexible allocation of treatment and block numbers that is not available in many conventional randomized incomplete block designs. The location, exact experimental design, number of half-sib, full-sib families and clones for each trial are provided in Table 4.1. Over 30,000 individual trees were planted in this progeny trial series. Border trees surrounded each trial and all trials except 7 and 10 were fenced to prevent browsing. Mulch layers or brush blanket mats were used to control competing vegetation, and spacing was 3×3m.

Height measurements were carried out multiple times between 2005 and 2013. Bud break scores were obtained based on a repeated scoring method according to Li et al. (2010). Score 0 was recorded for dormant buds, score 1 indicates swollen buds, score 2 indicates broken bud scales, score 3 was given for the emergence of green leaves, score 4 indicates leave extensions, score 5 indicates more than two

leaves emerged, and score 6 indicates fully unfolded leaves. Scores were recorded for each individual tree on April 12, 20, 22, 24, 26, May 1, 3, 9, 11, 13, 15, 17, 19 in 2010 at trial 2. At trial 3, scores were recorded on April 18, 21, 25, 27 and May 2, 10, 12, 14, 16, 18, 20 in 2010. In trial 8, scores were recorded on April 11, 18, 23, 26, May 3, 9, 10, 13, 15, 17, 19, 21 in 2010.

For leaf senescence scores , scoring was based on an eight-level senescence scale according to Fracheboud et al. (2009). Score 0 represents uniformly green leaves, score 1 indicates more dark than pale green leaves, score 2 indicates a majority of pale green leaves, score 3 indicates more green than yellow leaves, score 4 indicates a majority of yellow leaves, score 5 indicates only yellow leaves, score 6 indicates 20% brown leaves, score 7 indicates 50% leave abscission, and score 8 represents ≥90% leave abscission. Observation dates for fall phenology in trial 2 were September 1, 7, 21, 29 October 5, 20 in 2011. Scores in trial 3 were recorded on September 1, 6, 23, 30 and October 6, 21 in 2011. Trial 8 was assessed on August 31, 7, 21, 29 September and October 20, 2011.

The day of year of a penology event (DoY) was subsequently calculated for a critical score that showed the best separation among genotypes: score 3 for spring phenology (emergence of green leaves) and score 7 for fall phenology (50% leaf abscission). The date when the phenology reached the critical score of individual trees was determined by either the first record of the critical score, or by means of linear regression from the bracketing dates.

4.3.3 Statistical and quantitative genetic analyses

All quantitative genetic analysis was conducted with the ASReml-R package (Butler 2009). For the seedling trials (1 and 2) the analysis of variance components was according to the following general mixed linear model:

$$\mathbf{Y} = \mathbf{X}\beta + \mathbf{Z}_1 a + \mathbf{Z}_2 f + \mathbf{Z}_3 r + \mathbf{Z}_4 b + \mathbf{Z}_5 p + e \qquad (1)$$

where **Y** is the vector of observations of traits (tree height, day of year of bud break or leaf senescence etc.); β is a vector of fixed effects; a, f, r, b and p are vectors of additive genetic effects, full-sib family (cross) effects, replicate effects, block-within-replicate effects and plot effects, respectively; e is a vector of random residuals; **X** is the incidence matrix of the fixed effects relating β to observations **Y**; and $\mathbf{Z}_1$ to $\mathbf{Z}_5$ are the incidence matrices relating the random effects a, f, r, b and p to observations **Y**. We assume that the observation vector follows a normal distribution with the expected value of E(**Y**) = $\mathbf{X}\beta$ and with the covariance matrix of Var(**Y**) = **V**, i.e., $\mathbf{Y} \sim N(\mathbf{X}\beta, \mathbf{V})$. For our data, the β vector has only one element (the overall mean) and the vectors of five random effects and residuals are normally distributed as:

$$\begin{bmatrix} a \\ f \\ r \\ b \\ p \\ e \end{bmatrix} \sim N\left(\begin{bmatrix} \mathbf{0} \\ \mathbf{0} \\ \mathbf{0} \\ \mathbf{0} \\ \mathbf{0} \\ \mathbf{0} \end{bmatrix}, \begin{bmatrix} \mathbf{A}\sigma_A^2 & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \mathbf{I}_f\sigma_f^2 & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & \mathbf{I}_r\sigma_r^2 & \mathbf{0} & \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{I}_b\sigma_b^2 & \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{I}_{plot}\sigma_{plot}^2 & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{I}_e\sigma_e^2 \end{bmatrix} \right) \qquad (2)$$

where σ_A^2 is the additive genetic variance, $\mathbf{A}$ is the pedigree kinship matrix for describing the additive genetic relationships among individual trees, σ_f^2 represents

25% of the dominance genetic variance, $\sigma_r^2, \sigma_b^2, \sigma_{plot}^2$ and σ_e^2 are the variance components corresponding to the vectors of random effects *r, b, plot* and residuals *e*, respectively, $\mathbf{I}_t$ is the identity matrix of order *t* (*t* = *f, r, b, plot, e*). Thus, the total variance matrix can be partitioned into components due to the five vectors of random effects described above as well as the residuals,

$$\mathbf{V} = \sigma_A^2 \mathbf{Z}_1 \mathbf{A} \mathbf{Z}_1^{'} + \sigma_f^2 \mathbf{Z}_2 \mathbf{Z}_2^{'} + \sigma_r^2 \mathbf{Z}_3 \mathbf{Z}_3^{'} + \sigma_b^2 \mathbf{Z}_4 \mathbf{Z}_4^{'} + \sigma_{plot}^2 \mathbf{Z}_5 \mathbf{Z}_5^{'} + \sigma_e^2 \mathbf{I}_e \quad (3)$$

The best linear unbiased estimation (BLUE) of fixed effect (β) and best linear unbiased prediction (BLUP) of random effects (a, *f*, *r*, *b* and p) are solutions to the following mixed model equations,

$$\begin{bmatrix} \hat{\beta} \\ \hat{a} \\ \hat{f} \\ \hat{r} \\ \hat{b} \\ \hat{p} \end{bmatrix} = \begin{bmatrix} \mathbf{1'1} & \mathbf{1'Z}_1 & \mathbf{1'Z}_2 & \mathbf{1'Z}_3 & \mathbf{1'Z}_4 & \mathbf{1'Z}_5 \\ \mathbf{Z}_1^{'}\mathbf{1} & \mathbf{Z}_1^{'}\mathbf{Z}_1 + \mathbf{A}^{-1}\frac{\sigma_e^2}{\sigma_A^2} & \mathbf{Z}_1^{'}\mathbf{Z}_2 & \mathbf{Z}_1^{'}\mathbf{Z}_3 & \mathbf{Z}_1^{'}\mathbf{Z}_4 & \mathbf{Z}_1^{'}\mathbf{Z}_5 \\ \mathbf{Z}_2^{'}\mathbf{1} & \mathbf{Z}_2^{'}\mathbf{Z}_1 & \mathbf{Z}_2^{'}\mathbf{Z}_2 + \mathbf{I}_f\frac{\sigma_e^2}{\sigma_f^2} & \mathbf{Z}_2^{'}\mathbf{Z}_3 & \mathbf{Z}_2^{'}\mathbf{Z}_4 & \mathbf{Z}_2^{'}\mathbf{Z}_5 \\ \mathbf{Z}_3^{'}\mathbf{1} & \mathbf{Z}_3^{'}\mathbf{Z}_1 & \mathbf{Z}_3^{'}\mathbf{Z}_2 & \mathbf{Z}_3^{'}\mathbf{Z}_3 + \mathbf{I}_r\frac{\sigma_e^2}{\sigma_r^2} & \mathbf{Z}_3^{'}\mathbf{Z}_4 & \mathbf{Z}_3^{'}\mathbf{Z}_5 \\ \mathbf{Z}_4^{'}\mathbf{1} & \mathbf{Z}_4^{'}\mathbf{Z}_1 & \mathbf{Z}_4^{'}\mathbf{Z}_2 & \mathbf{Z}_4^{'}\mathbf{Z}_3 & \mathbf{Z}_4^{'}\mathbf{Z}_4 + \mathbf{I}_b\frac{\sigma_e^2}{\sigma_f^b} & \mathbf{Z}_4^{'}\mathbf{Z}_5 \\ \mathbf{Z}_5^{'}\mathbf{1} & \mathbf{Z}_5^{'}\mathbf{Z}_1 & \mathbf{Z}_5^{'}\mathbf{Z}_2 & \mathbf{Z}_5^{'}\mathbf{Z}_3 & \mathbf{Z}_5^{'}\mathbf{Z}_4 & \mathbf{Z}_5^{'}\mathbf{Z}_5 + \mathbf{I}_{plot}\frac{\sigma_e^2}{\sigma_{plot}^2} \end{bmatrix}^{-1} \begin{bmatrix} \mathbf{1'Y} \\ \mathbf{Z}_1^{'}\mathbf{Y} \\ \mathbf{Z}_2^{'}\mathbf{Y} \\ \mathbf{Z}_3^{'}\mathbf{Y} \\ \mathbf{Z}_4^{'}\mathbf{Y} \\ \mathbf{Z}_5^{'}\mathbf{Y} \end{bmatrix} \quad (4)$$

R code for model implementation is provided in Appendix G.

For the trials with clonal single tree plot trials, we modified model (1) into following linear mixed model:

$$\mathbf{Y} = \mathbf{X}\beta + \mathbf{Z}_1 a + \mathbf{Z}_2 f + \mathbf{Z}_3 c + \mathbf{Z}_4 b + \mathbf{Z}_5 r + e \quad (5)$$

where the model remains the same as (1) except the plot effect is removed and the effects of clones within full-sib family (c) are added. The c factor accounts for the epistasis and ¾ of the dominance (Araujo et al. 2012; Foster and Shaw 1988). The vectors of five random effects and residuals are normally distributed as:

$$\begin{bmatrix} a \\ f \\ c \\ b \\ r \\ e \end{bmatrix} \sim N\left(\begin{bmatrix} \mathbf{0} \\ \mathbf{0} \\ \mathbf{0} \\ \mathbf{0} \\ \mathbf{0} \\ \mathbf{0} \end{bmatrix}, \begin{bmatrix} \mathbf{A}\sigma_A^2 & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \mathbf{I}_f\sigma_f^2 & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & \mathbf{I}_c\sigma_c^2 & \mathbf{0} & \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{I}_b\sigma_b^2 & \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{I}_r\sigma_r^2 & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{I}_e\sigma_e^2 \end{bmatrix} \right) \quad (6)$$

where the parameters are defined as the previous function, and $\mathbf{I}_c$ is the identity matrix of which order equals to the levels of clones within full-sib families. R code for model implementation is provided in Appendix H.

Narrow-sense and broad-send heritabilities were calculated as follows:

$$h_i^2 = (\sigma_A^2)/(\sigma_P^2) = \frac{\sigma_A^2}{\sigma_A^2 + \sigma_{NA}^2 + \sigma_e^2} \quad (7)$$

where $\sigma_A{}^2$ is the additive genetic variance component; $\sigma_P{}^2$ is the phenotypic variance component represented by the sum of $\sigma_A{}^2$, $\sigma_{NA}{}^2$ and σ_e^2; $\sigma_{NA}{}^2$ is the variance of non-additive genetic effects; the residual error is σ_e^2 (Costa e Silva et al. 2004). The broad-sense heritability was estimated as:

$$H_i^2 = (\sigma_G^2)/(\sigma_P^2) = \frac{\sigma_A^2 + \sigma_{NA}^2}{\sigma_A^2 + \sigma_{NA}^2 + \sigma_e^2} \quad (8)$$

The standard errors of the heritability were calculated with the delta method (Lynch and Walsh 1998).

Additive genetic correlation in seedling trials and genetic correlation in clonal trials (r_G), phenotypic correlation (r_P) are estimated based on individual trees. Least square means for clones within each replicate is calculated as the surrogate

of individual tree measurement. The linear model of tree growth and leaf phenology for single site sites is:

$$y_{ijlmn} = t_n + r_{in} + g_{nim} + e_{nimj} \quad (9)$$

where t_n represents the n-th trait effect, r_{ni} is the i-th replicate effect of n_th trait, g_{nim} is the additive genetic effect of m-th genotype of n-th trait in i-th replicate in the seedling trial, while in the clonal trails, g_{nim} is the genetic effect. In the seedling trial, the genotypes are seedlings nested in replicates, while in the clonal trial, genotypes are clones evenly assigned in each replicate. For all trials e_{nimj} is the residual of j-th tree of im-th genotype for trait n. The fixed effects of the mixed model are similar as the previous model, although the trait effect is added as a fixed factor. The genetic correlation ($r_{G_{ij}}$) was in the form as

$$r_{G_{ij}} = \frac{\hat{\sigma}_{G_{ij}}}{\hat{\sigma}_{G_i}\hat{\sigma}_{G_j}} \quad (10)$$

where $\hat{\sigma}_{P_{ij}}$ is the estimated phenotypic covariance between trait i and j; $\hat{\sigma}_{P_i}$ is the estimated phenotypic standard deviation of trait i. R code for estimation of phenotypic and genetic correlations is provided in Appendix I.

Based on BLUPs, the breeding value reliability of half-sib parents and individual clones were calculated as follows:

$$R_i = 1 - \frac{PEV}{\hat{\sigma}_i^2} = 1 - \frac{se_i^2}{\hat{\sigma}_A^2} \quad (11)$$

where R_i is the reliability of the breeding value of the i^{th} parent, where PEV is the prediction error variance that equals to the standard error square of the predicted breeding value; and $\hat{\sigma}_A^2$ is the estimated additive genetic variance component.

The correlation of breeding values among sites were calculated as the pearson's correlation coefficients of halfsibs (breeding values) and clones (genetic values) between trials with *chart.correlation* of the R package *PerformanceAnalytics*. Bootstrapping of correlation coefficients of survival was carried out with R the *boot* package (R Development Core Team, 2013). The G×E effect is explored with the Type-B genetic correlation for tree height (Appendix J).

4.4 Results

4.4.1 Genetic parameters of growth traits

Genetic parameters were estimated at a relatively young age. Trees were between 5 and 8 years old at the time of evaluation with the oldest seedling trials having an average height of 3-4m and most clonal trials reaching average heights of 1-2m (Table 4.1). Dominance and epistatic variance components were small, less than 10% of the phenotypic variance component, so that most broad sense heritabilities were only marginally higher than narrow-sense heritabilities (Table 4.2). The highest heritabilites were estimated for a relatively small seedling trial 1 with values around 0.5. For all other trials, heritabilities were quite low (or unreliable) with narrow sense heritabilities typically ranging from 0.1 to 0.2, and broad sense

heritabilities typically ranging from 0.2 to 0.3. Heritabilities at all assessment ages are shown in Fig. 4.2. There appears to be a slight decrease of heritability estimates from initial establishment (which represents heritabilities under homogenous nursery conditions) to age four, with particularly large drops in trials 2 and 8. After age four, heritabilities appear to stabilize or increase slightly.

Type-B genetic correlations based on shared clones and shared full-sib families could only be calculated for sister trials (1-2, 3-4, 5-6, 7-8, and 9-10). Genetic correlations among sister trials yielded r_{GB} values around 0.7 with a standard error of approximate 0.08 for the first three pairs, indicating a relatively low degree of genotype × environment interactions ($G \times E$). Pairs 7-8 and 9-10 did not yield reliable estimates. Correlations of parental breeding values could be calculated for a larger number of trial pairs that shared parents through the partial factorial mating design (Fig. 4.3). Parent breeding values between sister trials in trials 1 through 6 were generally well correlated, which can be interpreted as low $G \times E$. The high elevation trial 8 showed negative correlation with any other trial, although the relationships are not statistically significant.

4.4.2 Genetic parameters for adaptive traits

The phenology traits bud break and leaf abscission had moderate broad- and narrow-sense heritabilities (Table 4.3). Also here, additive genetic variation was most important and dominance and epistatic variance components ranged from zero to 10% of the phenotypic variation. Moderate to strong genetic correlations were found between growth and phenology (r=-0.3 and 0.7) with tall trees being

associated with early budbreak and late leaf abscission (Table 4.4). Survival was not compromised by early bud break or late leaf abscission. In fact, the reverse appeared to be true with negative correlations between survival and bud break and positive correlations between survival and leaf abscission, just as for growth traits (Table 4.4).

4.5 Discussion

Positive associations between height and survival as well as increased growth and survival in trees that break bud early and abscise leaves late suggest that utilization of the growing season may be more important than avoidance of early fall frosts or late spring frosts across all test sites in this experiment. Strong additive genetic correlations between growth and phenology indicates that much of the genetic gain at the early stage of stand development will be due to expanding the growing season, which may increase the risk of frost damage in spring and fall.

We find that correlations between fall phenology and height and survival are generally stronger than those between spring phenology and height and survival, indicating that the adaptive value of fall phenology may be surprisingly high. Neither fall phenology or budbreak showed strong spatial patterns of breeding values across both breeding zones (Fig. 4.4). However, correlations between height and leaf abscission are visible in these maps: high breeding values for height (green dots) are often associated with late leaf abscission (pink and purple), with a Pearson's correlation coefficient of 0.65 (p<0.0001). Note that the

reliability of breeding value estimates indicated by the size of circles in Figs. 4.4 and 4.5 is driven by the representation of parents in northern and southern breeding regions (i.e. families of northern origin have been less thoroughly tested in the southern breeding region and vice versa).

In studies with other species, positive correlations among productivity and late senescence has previously been observed (Weih 2009), and the prolonged senescence in fall may increase the risks of frost (Howe et al. 2003). However, climate warming trends that have materialized over the last several decades in Alberta (Mbogga et al. 2009) may have decreased the risks of early fall frosts. This might potentially explain our positive association of an extended growing season in fall with high survival. Inadvertent selection for an extended growing season in fall (primarily controlled by day length and not influenced by warming trends) may therefore be an unplanned but effective climate change adaptation strategy.

In contrast, bud break is a highly plastic trait in response to interannual variation and long term trends in temperature. Populations can generally expected to respond appropriately to climate change trends as long as daily temperature variances do not change for given baseline values. In other words, the frost risk associated with a certain heatsum that triggers budbreak needs to remain the same even thought the day of year where this heatsum is reached has shifted. Late spring frosts are also considered a more severe threat than early fall frosts, as they may destroy buds and juvenile leaves and severely compromise early season growth (Wolken et al. 2009). In our study, genetic correlations between growth and bud break were negative (i.e. early bud break associated with better growth),

but they were not nearly as strong as genetic correlations with leaf senescence. Furthermore, survival was not compromised in genotypes that started the growing season relatively early. We therefore conclude that inadvertent selection for early budbreak poses only a small risk that managers may want to address by avoiding selection of the most extreme genotypes in this respect.

Heritabilities found in this study were generally much lower than those reported by Gylander (2012) in comparable trial series that investigated the parental clones that were used for generating the offspring in the trial series of this study. Gylander et al. (2012) reported broad-sense heritabilities of 0.51-0.58 from clonal trials. Kanaga et al. (2008) calculated broad-sense heritabilities for growth traits ranging from 0.30 to 0.50 in a short-term common garden study using 13 aspen clones. Our low heritability estimates for growth traits are likely due to harsh site conditions and the juvenile age at which trees were evaluated. Heritabilities were particularly low for trials 7 and 10, which were the only trials that could not be fenced and lacked vegetation control. We conclude that selection for growth traits at the current stage promises only small genetic gains, but higher heritabilities may emerge at a later date of trial evaluation.

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Table 4-1 Locations, experimental design, family structure, clonal structure and measurement averages for sapling height, day of the year of budbreak, and day of the year of leaf coloration for ten progeny trials of *Populus tremuloides* in Alberta. Note that the first two trials do not have clonal replications of genotypes.

Trial ID[1]	Latitude	Longitude	Elevation	Experimental. Design[2]	Half-sibs	Full-sibs	No of clones	No of trees	Height (m)	Budbreak (DoY)	Leaf col. (DoY)
Southern Breeding Region											
01-81-05	55°60'	-120°48'	662	6×10×9×3	33	51	-	1,620	4.77	-	-
02-35-05	53°18'	-116°30'	962	6×10×9×3	33	50	-	1,620	3.27	128	267
03-36-07	53°48'	-115°30'	968	9×24×24×1	37	83	560	5,184	1.36	135	268
04-83-07	55°12'	-120°48"	808	9×20×25×1	36	73	508	4,500	1.02	-	-
08-37-07	52°42'	-116°00'	1234	9×8×6×1	2	28	49	432	0.70	135	273
Northern Breeding Region											
05-11-07	56°24'	-118°48'	525	9×20×24×1	33	71	471	4,320	1.32	-	-
06-10-07	56°48'	-118°24'	570	9×21×21×1	32	77	455	3,969	2.00	-	-
07-13-07	56°48'	-119°36'	850	9×8×6×1	2	27	47	432	0.52	-	-
09-13-08	56°36'	-118°06'	650	9×21×20×1	31	61	491	3,780	2.01	-	-
10-11-08	56°24'	-118°48'	525	9×21×20×1	32	53	459	3,780	0.40	-	-

[1]) The Trial ID consists of a trial number, a site ID and the establishment year (e.g., the first entry is Progeny trial #1, planted at site #81, and established in 2005).

[2]) The experimental design consists of the number of replications × alpha blocks per replication × treatment plots per alpha block × trees per plot.

Table 4-2 Estimates of narrow-sense and broad-sense heritabilities at ten aspen progeny trials for tree height and diameter at breast height (DBH). Standard errors of the estimates are given in parentheses.

Trial Code[1]	Age of Measurement	Narrow-sense heritability ($\hat{h}^2$)		Broad-sense heritability ($\hat{H}^2$)	
		Height	DBH	Height	DBH
Southern Breeding Region					
01-81-05	8	0.55 (0.16)	0.54 (0.17)		
02-35-05	8	0.08 (0.10)	0.03 (0.09)		
03-36-07	5	0.21 (0.08)	0.19 (0.07)	0.33 (0.03)	0.25 (0.02)
04-83-07	5	0.11 (0.05)		0.14 (0.02)	
08-37-07	5	No estimate		0.03 (0.03)	
Northern Breeding Region					
05-11-07	5	0.10 (0.03)	0.06 (0.03)	0.14 (0.02)	0.09 (0.02)
06-10-07	5	0.13 (0.04)		0.20 (0.02)	
07-13-07	4	0.42 (0.45)		0.42 (0.22)	
09-13-08	5	0.11 (0.06)	0.14 (0.05)	0.19 (0.02)	0.17 (0.02)
10-11-08	3	0.07 (0.03)		0.080.02)	

[1]) The Trial ID consists of a trial number, a site ID and the establishment year (e.g., the first entry is Progeny trial #1, planted at site #81, and established in 2005).

Table 4-3 Estimates of narrow-sense and broad-sense heritabilities at three aspen progeny trials forbud break and leaf abscission. Broad sense heritabilities were not estimated for the seedling trial 02-35-05. Standard errors of the estimates are given in parentheses.

Trial Code	Age of Measurement	Narrow-sense heritability ($\hat{h}^2$)		Broad-sense heritability ($\hat{H}^2$)	
		Bud break	Leaf abscission	Bud break	Leaf abscission
Southern Breeding Region					
02-35-05		0.46 (0.15)	0.33 (0.14)		
03-36-07		0.37 (0.10)	0.42 (0.10)	0.46 (0.03)	0.46 (0.03)
08-37-07		0.36 (0.07)	no estimate	0.36 (0.07)	0.05 (0.05)

Table 4-4 Estimates of genetic and phenotypic correlations at three aspen progeny trials for bud break (BUD), leaf abscission (LAB), height (HT) and survival (SURV). Genetic and phenotypic correlations were estimated using an individual tree model for BUD, LAB and HT for the seedling trial 02-35-05, with half-sib families excluded. For the clonal trials 03-36-07, 08-37-07 we used an individual clone model. Phenotypic correlations of survival with all other traits were based on family means (trial 02-35-05) and clone means (trials 03-36-07, 08-37-07) with standard errors determined through bootstrapping.

	Trial 02-35-05		Trial 03-36-07		Trial 08-37-07	
Correlation	Genetic	Phenotypic	Genetic	Phenotypic	Genetic	Phenotypic
HT - BUD	-0.30 (0.21)	-0.23 (0.06)	-0.19 (0.05)	-0.25 (0.02)	no estimate	-0.42 (0.05)
HT - LAB	0.83 (0.09)	0.57 (0.04)	0.58 (0.04)	0.37 (0.02)	no estimate	0.28 (0.06)
BUD - LAB	-0.08 (0.22)	-0.05 (0.07)	0.15 (0.07)	0.00 (0.03)	no estimate	-0.20 (0.06)
SURV - BUD		-0.34 (0.09)		-0.07 (0.06)		-0.03 (0.20)
SURV - LAB		0.55 (0.11)		0.29 (0.04)		0.20 (0.12)
SURV - HT		0.58 (0.11)		0.42 (0.04)		0.00 (0.15)

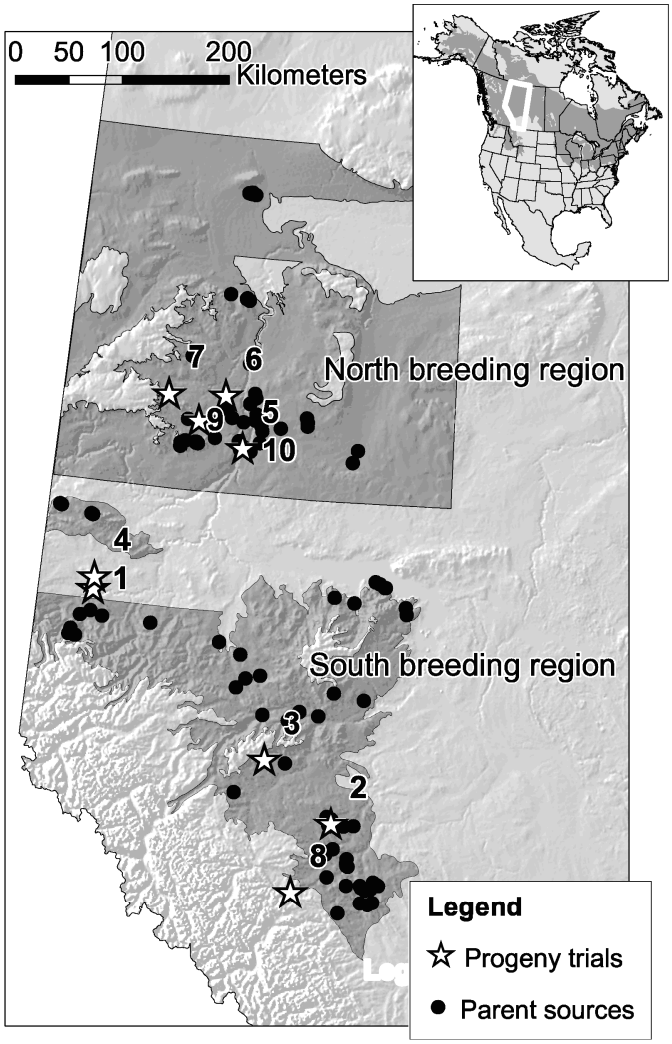

Figure 4-1 Study area showing forests and protected areas in 17 states and provinces that were evaluated in this case study. Circles are the parental sources. Stars and numbers represent the location of trials.

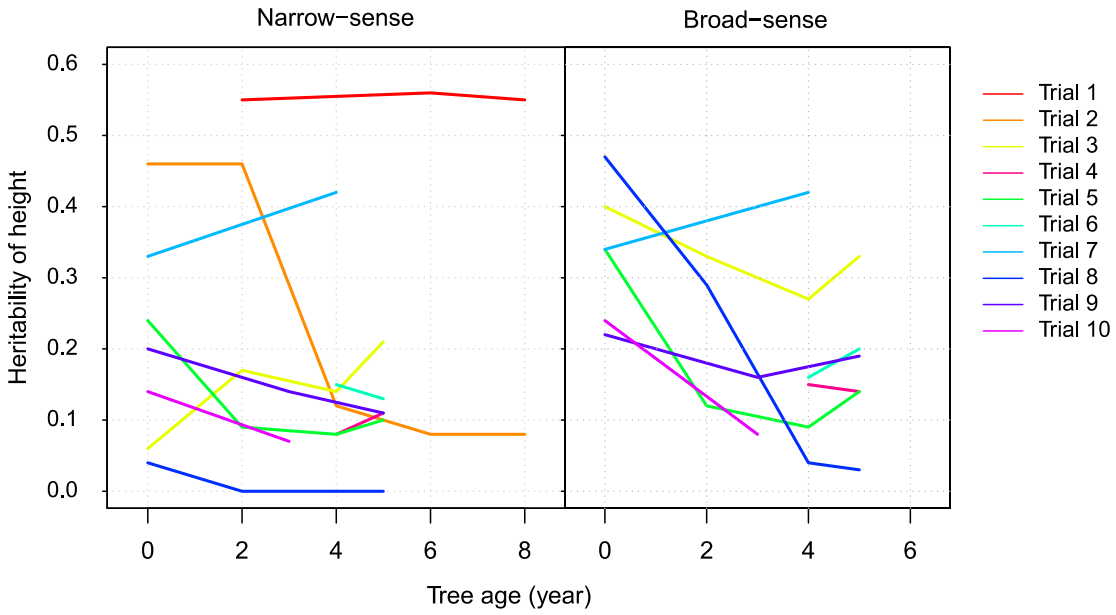

Figure 4-2 Changes to heritability in height during early stand development. Trials 1 and 2 are seedling trials and no broad-sense heritabilities were estimated.

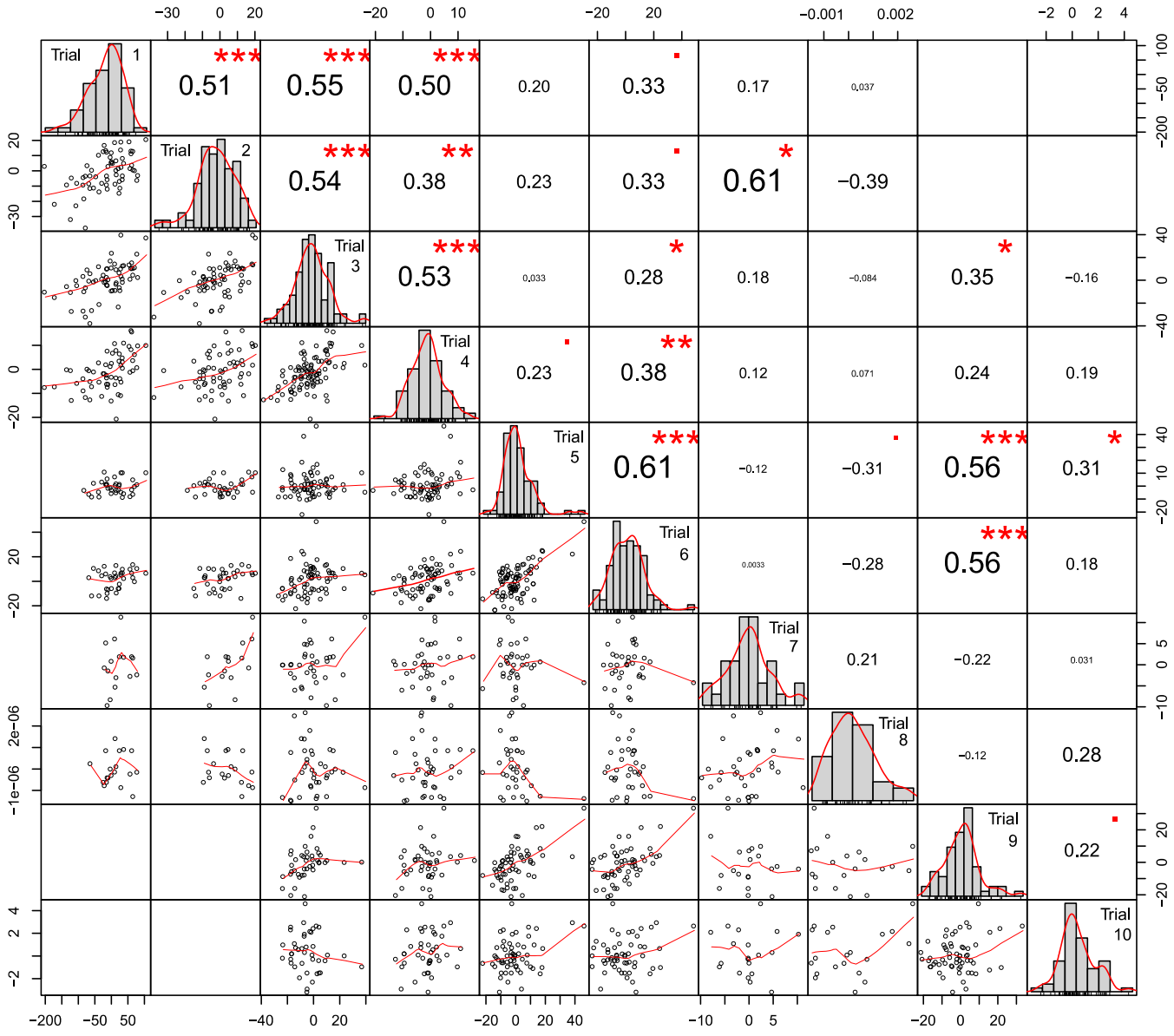

Figure 4-3 Correlation of breeding value estimates for parents among 10 progeny trials for height measured at the oldest age available.

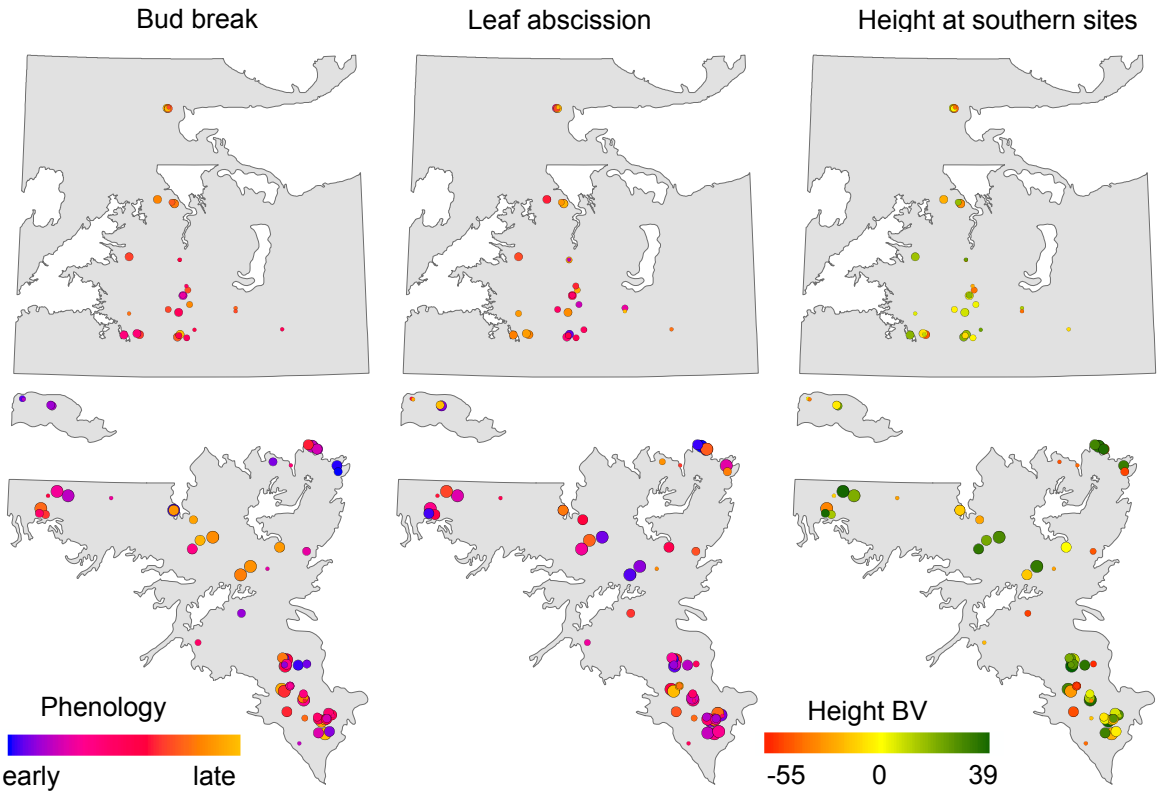

Figure 4-4 Breeding values of parents on southern breeding region test sites for phenology and growth. The size of the circle indicates the reliability of the estimate (reliability: 0.1 = small circles, 0.7 = large circles).

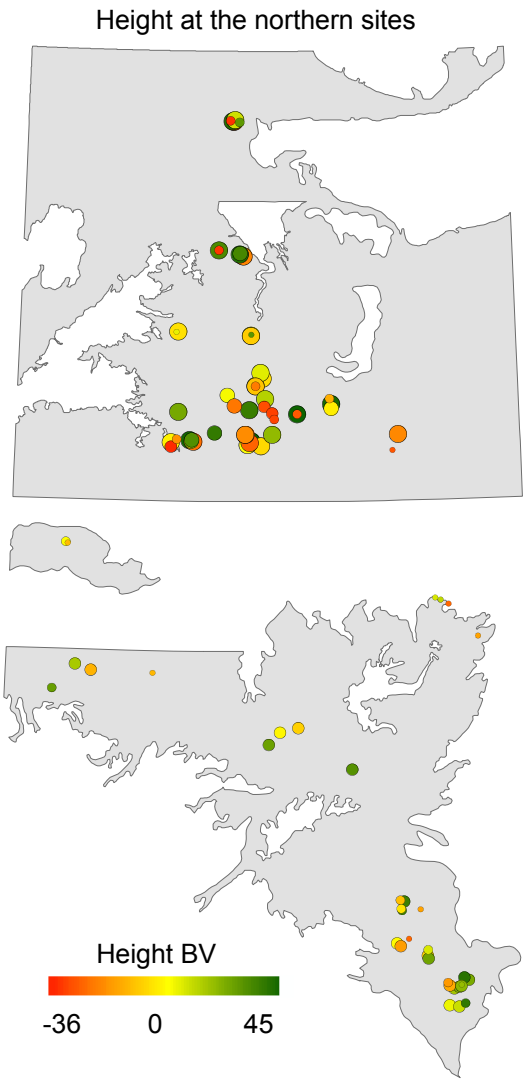

Figure 4-5 Breeding values of parents on northern breeding region test sites for height. The size of the circle indicates the reliability of the estimate (reliability: 0.1 = small circles, 0.7 = large circles).

Chapter 5 - Synthesis and Conclusions

In this thesis I explored questions related to trade-offs between growth and adaptive traits, and potential causes for sub-optimatlity of local populations. According to the range limit hypothesis, the distribution of many temperate species is restricted by a trade-off between their capacity to survive winter extremes in the north (or high elevation), and their ability to compete with better-adapted species in the south (or low elevation range limits). This trade-off has important implications in forestry, in the context of managed seed movement under climate change as well as in tree breeding programs that may inadvertently shift the balance in traits that favor growth versus traits that favor survival.

Previous studies have documented that movement of aspen seed sources in northwest direction leads to higher productivity relative to local seed sources. Better growth could be the result of transferred provenances utilizing a longer growing season, with bud break earlier and leaf senescence occurring later than in local provenances, thus increasing the vulnerability to late spring frosts and early fall frosts. I found that this is generally not the case. Experimental cold hardiness testing and phenology observations in a common garden experiment revealed that seed transfer to more northern locations results in delayed timing of leaf senescence, but the onset of dormancy and frost hardiness suggests that there should be no severe risks involved with northward transfers of planting material. Northward movement was also associated with a slightly delayed onset of growth of introduced genotypes relative to local provenances, and therefore pose no additional risks. I conclude that benefits in growth outweigh potential risks to survival associated with a northward movement of aspen populations in forestry operations. Even extreme long-distance northward movements had positive or neutral effects on growth and survival, while southward movement had clear negative consequences, highlighting the risk of inaction in the face of a warmer

climate in the future. I therefore recommend that seed transfer guidelines in western Canada allow a moderate movement of aspen planting stock to account for adaptational lag.

Results from estimating genetic parameters in a progeny trial series for Alberta sources corroborates this assessment. Moderate to strong genetic correlations were found between growth and phenology, with tall trees being associated with early budbreak (r_G=-0.3) and late leaf abscission (r_G=0.7). I conclude that selection for growth traits will likely result in an expanded growing season, which may increase the risk of frost damage somewhat in spring and more severly in fall. However, survival was not compromised by early bud break or late leaf abscission. In fact, the reverse appeared to be true: survival was favored by early bud break and late leaf abscission, indicating that utilization of the growing season was more important than avoidance of early fall frosts or late spring frosts across all test sites in both the provenance trial series and progeny trial series. Climate warming trends can potentially explain the lack of observed tradeoffs between growing season utilization and productivity. Growth does not necessarily equal evolutionary fitness, and it is possible that local sources could better survive very rare unseasonal frost events that never occurred at the 15 common garden experiments investigated in this study. However, a more likely explanation for both the provenance and progeny trial results is that local populations are suboptimally adapted. A potential explanation for suboptimality is the longevity of aspen clones, where populations could be adapted to climates present during post-glacial recolonization.

This hypothesis was explored with habitat reconstructions to the last glacial maximum and I hypothesized that low productivity of aspen populations found in northeast British Columbia and northern Alberta could be due to these populations being the result of recolonization from glacial refugia in Alaska. Paleoclimatic habitat reconstructions were, however, inconclusive. The model suggested a low probability of an Alaska refugium, but suggested aspen

recolonization exclusively from the east. This was further supported by a southeast to northwest gradient of decreasing genetic variance in quantitative traits from the provenance experiment, and such a gradient would be expected because of repeated founder effects during post-glacial migration northwards. Patterns of adaptational lag in quantitative traits also fits well with this migration history. Aspen provenances from northeastern British Columbia appear to be the most mal-adapted populations in terms of growth, survival, phenology and frost hardiness compared to populations from Alberta and Minnesota. Decreasing genetic diversity in combination with aspen's clonal life history slow the process of adaptation to new environmental conditions, with current populations apparently being adapted to fossil climates to some degree.

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Appendix A - Origin of parent clones used in the northern breeding region. Lat (ºN) , Long (ºW), Elev(m).

Parent	Sex	Lat	Long	Elev
1004	M	56.4097	117.9064	719
1005	F	57.6753	117.6036	420
1006	M	57.1317	118.0831	660
1007	M	57.1281	118.1028	689
1008	F	56.4097	117.9328	728
1010	M	57.6753	117.6036	420
1016	M	56.6336	117.0464	515
1017	M	56.7703	117.1503	492
1018	M	56.7697	117.1508	492
1019	F	56.7728	117.1478	492
1020	M	56.6861	117.0381	485
1022	F	56.5839	116.6539	598
1023	M	56.5839	116.6539	598
1026	F	56.3625	118.1497	866
1027	M	56.3933	118.1503	886
1028	M	56.4131	118.0836	749
1029	F	56.4011	117.9111	768
1030	M	56.3997	117.8872	810
1167	F	56.7099	117.4850	504
1231	F	56.4480	116.9532	569
1232	F	56.8590	117.0885	546
1244	F	56.4577	117.6372	615
1245	F	56.3788	117.2418	493
1249	F	56.5889	116.9495	534
1250	F	56.5497	116.9284	543
1251	F	56.8215	117.0653	526
1273	F	56.3718	117.0828	519
1274	F	56.3934	117.2156	517
1275	F	56.4472	117.2724	547
1352	F	56.5956	118.0713	710
1352	F	56.5956	118.0713	710
1423	F	56.4038	117.1958	502
1425	F	56.4486	117.2681	555
1482	M	-	-	-
1483	M	56.6116	117.2262	721
1484	M	56.6382	117.4044	721
1485	M	-	-	-
1489	M	56.4437	115.4578	485
1490	M	-	-	-
1491	M	56.3392	115.5259	511
1493	M	56.6863	116.2657	708
1495	M	56.6203	116.2467	605
1901	M	57.1061	117.2091	-
1902	M	56.6500	116.2500	695
1903	F	57.1073	117.2086	-
1905	F	57.1129	117.2033	-
1913	F	57.6476	117.3487	-
1922	F	57.6537	117.3428	-
1927	F	57.6348	117.3019	-
1929	F	57.6367	117.3047	-
2011	M	58.5264	111.4655	-
2012	F	-	-	-
2013	M	-	-	-
2014	M	58.5367	117.4710	367
2015	F	58.5328	117.4368	362
2016	F	58.5328	117.4220	355
2017	M	58.5354	117.4452	364
2018	F	58.5261	117.3569	349
2019	F	58.5441	117.4160	357

Appendix B - Origin of parent clones used in the southern breeding region. Lat (ºN) , Long (ºW) Elev(m)

Parent	Sex	Lat	Long	Elev
3001	F	53.7728	116.2089	907
3004	M	52.6408	114.8639	1066
3006	M	52.5364	115.3353	1054
3007	F	52.8372	115.5164	977
3009	M	53.4997	116.8996	1076
3010	M	52.7747	115.2400	-
3011	M	53.0792	115.4567	958
3012	M	53.0789	115.4578	948
3013	M	52.9383	115.2328	885
3014	M	53.0792	115.4578	952
3015	F	53.2681	115.5283	960
3017	M	53.2792	115.5389	987
3020	M	-	-	-
3021	F	-	-	-
3023	M	-	-	-
3025	F	53.2744	115.3256	923
3026	M	53.2828	115.1856	827
3221	M	52.8147	114.8683	966
3222	M	52.7967	114.8185	1041
3223	M	52.7872	114.7965	1048
3224	M	52.7656	114.8962	936
3225	M	52.7572	114.9020	923
3226	M	52.7526	114.9271	975
3227	F	52.9449	115.2509	946
3228	M	52.9666	115.2578	935
3229	M	53.0024	115.2538	868
3230	F	53.2850	115.5081	942
3232	F	53.3384	115.5434	935
3233	F	53.3474	115.5698	936
3234	F	53.3289	115.5028	973
3235	F	52.7437	114.9786	1016
3237	M	52.7626	115.0312	1037
3238	M	52.7811	115.0481	1049
3239	F	52.6213	114.9305	1048
3240	M	52.6308	115.0334	960
3241	F	53.0316	115.5535	966
3242	F	53.0517	115.5893	967
4002	F	54.1892	115.7822	769
4003	F	54.2153	116.0572	799
4008	F	54.4936	116.6597	844
4009	M	54.4622	116.8661	798
4010	F	54.3892	115.5786	1040
4011	F	54.1364	116.2158	931
4013	M	54.3808	116.9928	902
4014	M	54.6583	116.9708	791
4016	F	54.7481	117.2942	768
4017	F	54.7481	117.2942	768
4020	F	-	-	-
5001	F	55.3047	114.9175	-
5002	M	55.3047	114.9306	-
5005	F	55.3483	115.0714	-
5007	F	55.3339	115.0075	-
5008	M	55.1956	115.6611	-
5011	F	54.3447	115.1442	-
5017	M	55.0867	114.5919	-
5019	F	55.1592	115.3664	-
5021	F	55.1447	114.6097	-
8001	M	55.7668	119.8359	823
8004	M	55.7594	119.8103	823
8007	F	55.7086	119.3315	907
8008	M	55.7158	119.3574	869
8069	F	54.6831	119.3828	985
8078	F	54.9053	119.2036	738
8086	F	54.8547	118.3150	790
8087	M	54.8608	119.3472	820
8093	F	54.6908	119.4794	912
8094	F	54.7381	119.4614	952
8119	F	54.8683	119.0243	770

Appendix C - Partial factorial mating design for the northern breeding region (BR1). Male 4-digit clone numbers are shown in the horizontal row and female clone numbers are given in the first column. The 3-digit cross ID is followed by the successfully propagated clones in parentheses**.**

♀ \ ♂	1006	1030	1017	1023	1028	1027	1020	1004	1007	1491	1493	1018	1482	1490	2014	1489	2013	2017	1902	1483	2011	1484	1901	1010	1016	1485	1495	Poly
1005	102 (16)	101 (15)																										125 (15)
1008	121 (15)	172 (13)																										122 (15)
1019			104 (16)																									173 (15)
1274			112 (12)	113 (4)	111 (3)																							128 (14)
1273				109 (11)		110 (15)																						127 (15)
1245						108 (14)	107 (14)																					126 (15)
1022								115 (16)	116 (3)																			117 (15)
1026								118 (3)	119 (1)																			120 (1)
1029										174 (2)	175 (4)																	176 (13)
1167												105 (2)																129 (14)
1231													151 (16)															152 (13)
1232													157 (14)															158 (14)
1913													137 (1)	138 (12)														139 (13)
1425														193 (7)														194 (14)
1249															167 (4)													168 (3)
1251																153 (13)	154 (13)											155 (15)
1352																159 (7)	160 (14)											161 (14)
1423																		177 (15)	178 (15)									179 (15)
2015																		180 (16)	181 (16)									182 (9)
1903																			140 (10)									142 (12)
1927																				147 (14)	148 (11)							149 (11)
1929																					145 (10)	144 (15)						146 (15)
2016																						135 (15)	134 (13)					136 (15)
2012																								131 (2)	132 (4)			133 (15)
1905																										165 (1)		
2019																											190 (15)	191 (15)
1250																												171 (5)
1244																												130 (14)
1275																												123 (16)
1352																												161 (14)
1922																												143 (8)
2018																												188 (1)

Appendix D - Partial factorial mating design for the southern breeding region (BR2). Male 4-digit clone numbers are shown in the horizontal row and female clone numbers are given in the first column. The 3-digit progeny identification number is followed by the successful crosses (number of families) in parentheses.

♀ \ ♂	3006	4014	3026	5008	3004	5002	3009	3011	5017	3221	3222	8001	3223	3225	8087	3226	3228	3229	3238	3237	3240	3013	3224	8008	4009	4013	3020	3023	8004	3012	3014	3010	3017	Poly
3001	217 (2)	218 (2)																																235 (1)
5021		254 (8)																																255 (8)
3007			230 (2)	231 (1)																														232 (8)
4010			213 (3)	214 (3)																														238 (7)
3015					220 (8)	219 (8)																												239 (7)
5007					227 (7)	221 (8)																												249 (7)
3021							205 (2)	206 (4)																										237 (8)
3025									223 (2)																									240 (7)
5005									211 (3)																									241 (8)
3227										278 (8)	279 (8)																							280 (8)
3234										261 (6)	262 (7)																							263 (8)
3233												259 (1)	260 (1)																					293 (7)
3230												281 (1)																						283 (8)
3232														257 (6)	256 (1)																			258 (3)
8094														277 (4)																				228 (8)
3235																288 (7)	289 (7)																	290 (7)
3239																285 (7)	286 (8)																	287 (8)
3241																		294 (8)	295 (6)															296 (8)
3242																				298 (8)	297 (7)													299 (7)
8119																				301 (8)	300 (8)													302 (8)
4002																						265 (1)												
8078																						274 (1)	275 (5)	273 (7)										276 (6)
8086																							271 (1)											
4003																									204 (8)	203 (7)									234 (6)
4008																									202 (8)	201 (8)									236 (8)
4011																											243 (1)	242 (7)							244 (8)
5011																												250 (3)							252 (2)
4016																													284 (1)						247 (8)
4017																														207 (4)	208 (6)				245 (8)
5001																														209 (4)	210 (3)				246 (8)
4020																																216 (3)	215 (8)		
5019																																			268 (1)
8007																																			233 (7)
8069																																			248 (8)
8093																																			229 (8)
8094*																																			292 (6)

*) Female parent 8094 was assigned two different family names in separate polycrosses 292(6) and 228(8).

Appendix E - List where northern families were tested.

Northern	Northern test sites					Southern test sites				
families	5	6	7	9	10	1	2	3	4	8
101	•	•	•	•	•	-	-	•	•	•
102	•	•	•	•	•	-	-	•	•	•
104	•	•	•	•	•	-	-	•	•	•
105	•	•	-	•	-	-	-	-	-	-
107	•	•	•	•	•	-	-	•	•	•
108	•	•	•	•	•	-	-	•	•	•
109	•	•	-	•	•	-	-	•	-	-
110	•	•	•	•	•	-	-	•	•	•
111	•	•	-	•	•	-	-	-	-	-
112	•	•	•	•	•	-	-	•	•	•
113	•	•	-	•	•	-	-	-	-	-
115	•	•	-	•	•	-	-	-	-	-
117	•	•	-	•	•	-	-	-	-	-
118	•	•	-	•	•	-	-	-	-	-
119	•	•	-	-	•	-	-	-	-	-
120	•	•	-	•	•	-	-	-	-	-
121	•	•	-	•	•	-	-	•	•	-
122	•	•	-	•	•	-	-	•	-	-
123	•	•	-	•	•	-	-	-	-	-
124	•	•	-	•	•	-	-	-	-	-
125	•	•	-	•	•	-	-	•	-	-
126	•	•	-	•	•	-	-	-	-	-
127	•	•	-	•	•	-	-	-	-	-
128	•	•	-	•	•	-	-	-	-	-
129	•	•	-	•	•	-	-	-	-	-
130	•	•	-	•	•	-	-	-	-	-
131	•	-	-	•	•	-	-	-	-	-
132	•	•	-	•	•	-	-	-	-	-
133	•	•	-	•	•	-	-	-	-	-
134	•	•	-	-	•	-	-	•	•	-
135	•	•	•	-	•	-	-	•	•	•
136	•	•	•	-	•	-	-	-	-	-
138	•	•	-	-	•	-	-	•	•	-
139	•	•	-	-	•	-	-	•	•	-
140	•	•	-	-	•	-	-	-	-	-
142	•	•	-	-	•	-	-	•	-	-
143	•	•	-	-	•	-	-	-	-	-
144	•	•	•	-	•	-	-	•	•	•
145	•	•	•	-	•	-	-	•	•	•
146	•	•	-	-	•	-	-	-	-	-
147	•	•	•	-	•	-	-	•	•	•
148	•	•	•	-	•	-	-	•	•	•
149	•	•	-	-	•	-	-	-	-	-
151	•	•	-	-	•	-	-	•	•	-
152	•	•	-	-	•	-	-	-	-	-
153	•	•	-	-	•	-	-	•	•	-
154	•	•	-	-	•	-	-	•	•	-
155	•	•	•	-	•	-	-	-	-	-
157	•	•	-	-	•	-	-	•	•	-
158	•	•	-	-	•	-	-	-	-	-
159	•	•	-	-	•	-	-	-	-	-
160	•	•	-	-	•	-	-	•	•	-
161	•	•	-	-	•	-	-	-	-	-
165	•	•	-	-	-	-	-	-	-	-
167	•	•	-	-	•	-	-	-	-	-
168	•	-	-	-	•	-	-	-	-	-
171	•	•	-	-	•	-	-	-	-	-
172	•	•	-	-	•	-	-	•	•	-
173	•	•	-	-	•	-	-	-	-	-
174	•	-	-	-	•	-	-	-	-	-
175	•	-	-	-	•	-	-	-	-	-
176	•	•	-	-	•	-	-	-	-	-
177	•	•	•	-	•	-	-	•	•	•
178	•	•	-	-	•	-	-	•	•	-
179	•	•	-	-	•	-	-	-	-	-
180	•	•	•	-	•	-	-	•	•	•
181	•	•	-	-	•	-	-	-	-	-
182	•	•	-	-	•	-	-	-	-	-
188	•	•	-	-	•	-	-	-	-	-
190	•	•	-	-	•	-	-	•	•	-
191	•	•	-	-	•	-	-	-	-	-
193	•	•	-	-	•	-	-	-	-	-
194	•	•	-	-	•	-	-	-	-	-

Appendix F - List where southern families were tested.

Southern	Southern test sites					Northern test sites				
families	1	2	3	4	8	5	6	7	9	10
201	•	•	•	•	•	•	•	•	-	-
202	•	•	•	•	•	•	•	•	-	-
203	•	•	•	•	•	•	•	•	-	-
204	•	•	•	•	-	•	•	-	-	-
205	•	•	•	•	-	-	-	-	-	-
206	•	•	•	•	-	-	-	-	-	-
207	•	•	•	•	-	-	-	-	-	-
208	•	•	•	•	-	-	-	-	-	-
209	•	•	•	•	-	-	-	-	-	-
210	•	•	•	•	-	•	•	-	-	-
211	•	-	•	•	-	•	•	-	-	-
212	-	•	-	-	-	-	-	-	-	-
213	•	•	•	•	-	-	-	-	-	-
214	•	•	•	•	-	-	-	-	-	-
215	•	•	•	•	-	•	•	-	-	-
216	•	•	•	•	-	-	-	-	-	-
217	-	•	•	•	-	-	-	-	-	-
218	•	•	•	-	-	-	-	-	-	-
219	•	•	•	•	-	-	-	-	-	-
220	•	-	•	•	-	•	•	-	-	-
221	•	•	•	•	-	-	-	-	-	-
222	•	•	•	•	-	-	-	-	-	-
223	-	•	•	•	-	•	•	-	-	-
227	-	-	•	-	-	-	-	-	-	-
228	•	•	•	•	-	-	-	-	-	-
229	•	•	•	•	-	-	-	-	-	-
230	•	•	•	•	-	-	-	-	-	-
231	•	•	•	-	-	-	-	-	-	-
232	•	•	•	•	-	-	-	-	-	-
233	•	•	•	•	-	•	•	-	-	-
234	•	•	•	•	-	-	•	-	-	-
235	•	•	•	•	-	-	-	-	-	-
236	•	•	•	•	-	-	-	-	-	-
237	•	•	•	•	-	-	-	-	-	-
238	•	•	•	•	-	-	-	-	-	-
239	•	•	•	•	-	-	-	-	-	-
240	•	•	•	•	-	-	-	-	-	-
241	•	•	•	•	-	-	-	-	-	-
242	•	•	•	•	•	-	-	-	-	-
243	•	•	•	-	-	-	-	-	-	-
244	•	•	•	•	-	-	-	-	-	-
245	•	•	•	•	-	-	-	-	-	-
246	•	•	•	•	-	-	-	-	-	-
247	•	•	•	•	-	-	-	-	-	-
248	•	•	•	•	-	-	-	-	-	-
249	•	•	•	•	-	-	-	-	-	-
250	•	•	•	•	-	-	-	-	-	-
251	•	-	-	-	-	-	-	-	-	-
252	•	•	•	•	-	-	-	-	-	-
253	-	•	-	-	-	-	-	-	-	-
254	•	•	•	•	-	-	-	-	-	-
255	•	•	•	•	-	-	-	-	-	-
256	•	•	•	-	-	-	-	-	-	-
257	•	•	•	•	-	•	•	-	-	-
258	•	•	•	•	-	-	-	-	-	-
259	•	•	•	-	-	-	-	-	-	-
260	•	•	•	-	-	-	-	-	-	-
261	•	•	•	•	-	•	•	-	-	-
262	•	•	•	•	•	•	•	•	-	-
263	•	•	•	•	•	-	-	-	-	-
265	-	-	•	•	-	-	-	-	-	-
268	-	•	•	-	-	-	-	-	-	-
271	•	-	•	-	-	-	-	-	-	-
272	•	•	-	-	-	•	-	-	-	-
273	•	•	•	•	•	•	•	•	-	-
274	-	-	•	•	-	-	-	-	-	-
275	•	•	•	•	-	•	•	-	-	-
276	•	•	•	•	-	-	-	-	-	-
277	•	•	•	•	-	•	•	-	-	-
278	•	•	•	•	-	•	•	-	-	-
279	•	•	•	•	•	•	•	•	-	-
280	•	•	•	•	-	-	-	-	-	-
281	-	-	•	•	-	-	-	-	-	-
283	•	•	•	•	-	-	-	-	-	-
284	-	-	•	•	-	-	-	-	-	-
285	•	•	•	•	•	•	•	•	-	-
286	•	•	•	•	•	•	•	•	-	-
287	•	•	•	•	-	-	-	-	-	-
288	•	•	•	•	•	•	•	•	-	-
289	•	•	•	•	•	•	•	•	-	-
290	•	-	•	•	-	-	-	-	-	-
292	•	•	•	•	-	-	-	-	-	-
293	•	•	•	•	•	-	-	-	-	-
294	•	•	•	•	-	•	•	-	-	-
295	•	•	•	•	-	•	•	-	-	-
296	•	•	•	•	-	-	-	-	-	-
297	•	•	•	•	-	•	•	-	-	-
298	•	•	•	•	-	•	•	-	-	-
299	•	•	•	•	-	-	-	-	-	-
300	•	•	•	•	•	•	•	-	-	-
301	•	•	•	•	•	•	•	•	-	-
302	•	•	•	•	-	-	-	-	-	-

Appendix G - R code to estimate variance components and heritabilities for seedling trials using an individual tree model with random effects. The theory of the model is according to equations 5,6,8,9 in Costa e Silva, *et al.*(2004). The code implementation is according to the ASReml-R manual (Butler, 2009).

```
library(asreml)
pin <- dget("pin.R")

dat1 <- read.csv("Progeny01-81-05.csv")
dat2 <- dat1[dat1$FAMILY!="", ]  # drop surround and filler trees
dat2 <- dat2[dat2$MALE!="",]     # drop families with unknown males
dat2$GENOTYPE <- as.factor(dat2$ORDER)
dat2 <- droplevels(dat2)

### Making the pedigree file ###
ped1 <- dat2[,c("GENOTYPE","MALE","FEMALE")]
ped2 <- asreml.Ainverse(ped1)
ped3 <- ped2$ginv
table(ped2$inbreeding) # Returns the degree of inbreeding

### Random effect individual tree model ###
out1 <- asreml(fixed = HT2013 ~ 1,
               random = ~ REP/BLOCK/PLOT + FAMILY + ped(GENOTYPE, var=T),
               ginverse = list(GENOTYPE=ped3), rcov = ~idv(units),
               na.method.X = 'omit', na.method.Y = 'omit', data= dat2)
plot(out1)
summary(out1)
VA <- pin(out1, VA~V5)[1,1:2]; VA         # Additive variance & SE
VD <- pin(out1, VD~V4*4)[1,1:2]; VD       # Dominance variance & SE
VG <- pin(out1, VG~V5+4*V4)[1,1:2]; VG    # Additive variance & SE
VP <- pin(out1, VP~V5+V4+V7)[1,1:2]; VP   # Additive variance & SE

pin(out1, h2~V5/(V5+V4+V7))[1,1:2]        # Narrow sense h2 & SE
pin(out1, H2~(V5+4*V4)/(V5+V4+V7))[1,1:2] # Broad sense H2 & SE

### breeding value estimates for individual trees and parents ###
blup1 <- data.frame(summary(out1,all=T)$coef.random)
blup2 <- blup1[grep('ped*', dimnames(blup1)[[1]] ),]
blup2$reliability = 1-blup2$std.error^2/VA[1,1]

### pin function to estimate standard errors with the delta method ###
function (object, transform) {
   pframe <- as.list(object$gammas)
   names(pframe) <- paste("V", seq(1, length(pframe)), sep = "")
   tvalue <- eval(deriv(transform[[length(transform)]], names(pframe)),
                  pframe)
   X <- as.vector(attr(tvalue, "gradient"))
   tname <- if (length(transform) == 3) transform[[2]] else ""
   n <-length(pframe); i <-rep(1:n, 1:n); j <-sequence(1:n); k <-1 + (i > j)
   Vmat <- object$ai
   se <- sqrt(sum(Vmat * X[i] * X[j] * k))
   data.frame(row.names = tname, Estimate = tvalue, SE = se)
   }
```

Appendix H - R code to estimate variance components and heritabilities for progeny trials with clonal structure. The theory of the model is according to equations 5, 6, 8, and 9 in Costa e Silva, et al. (2004). The code implementation is produced according to the ASReml-R manual (Butler, 2009).

```
library(asreml)
library(Matrix)
library(MCMCglmm)
pin <- dget("pin.R")

dat1 <- read.csv("Progeny03-36-07.csv")
dat2 <- dat1[dat1$FAMILY!="",]          # drop the surround and filler trees
#dat2 <- dat2[dat2$MALE!="0",]          # keep only full-sibs
dat2 <- dat2[dat2$MALE!="",]            # drop families with unknown males
dat2$GENOTYPE <- paste0("G",dat2$CLONE) # create unique names for pedigree
dat2 <- droplevels(dat2)

### Making the pedigree file ###
ped1 <- aggregate(dat2, by=list(GENOTYPE=dat2$GENOTYPE, MALE=dat2$MALE,
                  FEMALE=dat2$FEMALE), mean)[,1:3]
ped2 <- asreml.Ainverse(ped1)
ped3 <- ped2$ginv

### Create the identity matrix for clones ###
Inv <- diag(nrow(ped1))
Iinv <- as.matrix(solve(inv))
Iinv <- as(iinv,"sparseMatrix")
colnames(iinv) <- ped1$GENOTYPE
rownames(iinv)<- ped1$GENOTYPE
in.inv <- sm2asreml(iinv)
dat2$GENOTYPE2 <- dat2$GENOTYPE

### Random effect individual clone model ###
out1 <- asreml(fixed = HT2012 ~ 1, random = ~ REP/BLOCK + FAMILY
               + ped(GENOTYPE, var=T) + ide(GENOTYPE2), ginverse =
               list(GENOTYPE=ped3, GENOTYPE2=in.inv), rcov = ~ idv(units),
               na.method.X='omit', na.method.Y='omit', data=dat2)
plot(out1)
summary(out1)

VA <- pin(out1, VA~V4)[1,1:2]; VA             # Additive variance & SE
VD <- pin(out1, VD~V3*4)[1,1:2]; VD           # Dominance variance & SE
VI <- pin(out1, VI~(V5-3*V3) ); VI            # Epistatic variance & SE
VG <- pin (out1, VG~V4+V3+V5)[1,1:2]; VG      # Genetic variance & SE
VP <- pin(out1, VP~V4+V3+V5+V7)[1,1:2]; VP    # Phenotypic variance & SE

pin(out1, h2~V4/(V4+V3+V5+V7))[1,1:2]         # Narrow sense h2 & SE
pin(out1, H2~(V4+V3+V5)/(V4+V3+V5+V7))[1,1:2] # Broad sense H2 & SE

### breeding value estimates for individual clones and parents ###
blup1 <- data.frame(summary(out1, all=T)$coef.random)
blup2 <- blup1[grep('ped*',dimnames(blup1)[[1]] ),]
blup2 <- blup2[order(row.names(blup2)),]
blup2$reliability <- 1 - blup2$std.error^2/VA[1,1]

### pin function as in Appendix G above ###
```

Appendix I - R code to estimate genetic correlations. The code implementation is based on the website *http://www.quantumforest.com/2012/05/bivariate-linear-mixed-models-using-asreml-r-with-multiple-cores*.

```
### Data preparation and pedigree generation as in Appendix G and H ###

### Selection of pairs of traits ###
dat2$DEN1 <- dat2[,"HT2013"]
dat2$DEN2 <- dat2[,"LAB7"]

### Model to estimate genetic correlations ###
out1 <- asreml(cbind(DEN1,DEN2) ~ trait, random = ~ REP
               + ped(GENOTYPE):us(trait),
               ginverse = list(GENOTYPE = ped3),
               rcov = ~ units:us(trait), data=dat2)

summary(out1)
pin(out1, rG~V3/sqrt(V4*V2))                  # Genetic correlation & SE
pin(out1, rP~(V3+V7)/sqrt((V4+V8)*(V2+V6)))   # Phenotypic correlation & SE

### pin function for delta method estimation of SE as in Appendix G ###
```

Appendix J - R code to estimate type-B genetic correlations for sister trials. The code implementation is based on the website *http://www.quantumforest.com/2012/05/bivariate-linear-mixed-models-using-asreml-r-with-multiple-cores*.

```
dat3 <- read.csv("Progeny03-36-07.csv")
dat4 <- read.csv("Progeny04-83-07.csv")
dat1=rbind(dat3,dat4)

dat2 <- dat1[dat1$FAMILY!="",]  # drop the surround and filler trees
dat2 <- dat2[dat2$MALE!="",]    # remove families with unknown males
dat2 <- dat2[dat2$CLONE!="",]   # remove clones with unknown clone ID
dat2$GENOTYPE <- paste0("G",dat2$CLONE)
dat2 <- droplevels(dat2)
nrow(dat2)
str(dat2)

dat2$HT <- dat2$HT/100          # variable needs decimal (bug fix)
dat2$DEN1 <- ifelse(dat2$SITE_ID == "PROG03-36-07", dat2$HT, NA)
dat2$DEN2 <- ifelse(dat2$SITE_ID == "PROG04-83-07", dat2$HT, NA)
str(dat2)

### Making the pedigree file ###
ped1 <- dat2[ ,c("GENOTYPE","FEMALE","MALE")]
ped2 <- aggregate(ped1, by=list(GENOTYPE=ped1$GENOTYPE, MALE=ped1$MALE,
FEMALE=ped1$FEMALE), mean)[,1:3]
ped3 <- asreml.Ainverse(ped2)
ped4 <- ped3$ginv
table(ped3$inbreeding)

### Bivariate random effect individual tree model ###
out1 <- asreaml(cbind(DEN1,DEN2) ~ random=~ped(GENOTYPE):us(trait),
         ginverse =list(GENOTYPE = ped4), rcov = ~ units:us(trait),
         data=dat2)
summary(out1)
pin(out1,rb~V2/sqrt(V1*V3))

### pin function for delta method estimation of SE as in Appendix G ###
```

Appendix K - Climate conditions at test sites within the northern and southern aspen breeding regions.

Trial code[1]	Climate Variables[2]									
	MAT	MWMT	DD>5	MCMT	DD<0	MAP	MSP	AHM	SHM	NFFD
Southern Breeding Region										
01-81-05	2.5	16.2	1326	-13.2	1370	445	268	29	64	158
02-35-05	2.8	15.9	1246	-10.9	1169	534	378	25	44	155
03-36-07	2.9	15.9	1252	-10.7	1122	475	307	28	55	162
04-83-07	2.1	15.7	1244	-13.3	1408	463	279	27	59	159
08-37-07	2.6	15.1	1093	-9.6	1056	550	399	23	40	141
Northern Breeding Region										
05-11-07	1.7	17.0	1437	-17.1	1752	385	228	31	77	155
06-10-07	0.8	16.4	1298	-17.6	1872	414	250	27	67	150
07-13-07	0.1	14.9	1081	-17.2	1884	469	295	22	52	149
09-13-08	0.9	16.3	1297	-17.3	1850	429	265	26	63	155
10-11-08	1.7	17.0	1437	-17.1	1752	385	228	31	77	155

[1]) The Trial ID consists of a trial number, a site ID and the establishment year (e.g., the first entry is progeny trial #1, planted at site #81, established in 2005).

[2]) MAT, Mean annual temperature (°C); MWMT, Mean warmest month temperature (°C); DD>5Degree-days above 5°C; MCMT, Mean coldest month temperature (°C); DD<0, Degree-days below 0°C; MAP, Mean annual precipitation (mm); MSP, Mean annual summer (May to September) precipitation (mm); AHM, Annual heat moisture index; SHM, Summer heat moisture index; NFFD, The number of frost-free days. All these variables were extracted From ClimateAB software (Mbogga et al. 2010).

Appendix L – Alternate SAS code to the R code of Appendices G and H to estimate the general combining ability (GCA), specific combining ability (SCA) and heritabilities for clonal trials. The theory of the model is according to equations 1 to 3 in (Xiang and Li 2001) and equations 1, 2, 4, 5 in (Xiang and Li 2003). The code implementation is produced according to Isik (2012).

```
PROC IMPORT OUT=dat1 DATAFILE="C:\path\Progeny09-13-08.csv" DBMS=CSV REPLACE;
   getnames=yes; guessingrows=3000;
   run;

data dat2; set dat1;
  if FAMILY = "" then delete;  /* drop the surround and filler trees*/
  if MALE = "" then delete;    /* remove families with unknown males */
  if CLONE = "" then delete;   /* remove clones with unknown clone ID */
 run;

/* Create a simple list of all male and female parents in one column */
proc sort data=dat2; by FEMALE MALE; run;
data ped1; set dat2; PARENT=MALE; keep PARENT; run;
data ped2; set dat2; PARENT=FEMALE; keep PARENT; run;
proc append base=ped1 data=ped2; run;
proc sort data=ped1 nodupkey; by PARENT; run;
data ped4 ;
  set ped1;
  pn+1;
  call symput('pn',compress(pn));
run;

/* Generate a matrix of individual trees (rows) and their parents (columns) */
proc iml;
  use dat2; read all var{FEMALE MALE} into d; close dat2;
  n = nrow(d);
  use ped4; read all var { parent} into P; close ped4;
  pcode = char(1:nrow(p),5,0)`;
  p = p||pcode;
  create pcode from pcode [colname={'p'}]; append from pcode; close pcode;
  a = shape(0,n,nrow(p));
  do i=1 to n;
    do k=1 to nrow(p);
      if d[i,1]=p[k,1]|d[i,2]=p[k,1] then a[i,k] = 1;
      end;
    end;
  create ped5 from a; append from a; close dummy;
quit;

/* Combine data and pedigree matrix */
data dat3;
  merge dat2 ped5;
run;
```

```
/* Mixed model execution */
title 'GCA, SCA estimates for clonal trials with proc mixed';
proc mixed data=dat3 covtest asycov update;
  class SITE_ID REP BLOCK FEMALE MALE FAMILY CLONE;
  model HT2013=REP BLOCK(REP) /residual solution;
  random col1-col&pn /type=toep(1) solution;    /*GCA effect*/
  random FAMILY /type=toep(1) solution;         /*SCA effect*/
  random FEMALE*REP MALE*REP CLONE(FAMILY) /solution;
  ODS output solutionR=BLUP;                    /* Breeding value estimates */
  ODS output solutionF=BLUE;
  ODS output covparms=_varcomp asycov=_cov;
run;

/* Error calculation and extraction of relevant results */
title 'Genetic parameters estimates with standard errors';
proc iml;
  use _varcomp;
  read all var {Estimate} into VC;
  read all var {probZ} into P;
  read all var {Zvalue} into Z;
  close _varcomp;

  use _cov; read all into C; COV = C(|1:nrow(C),2:ncol(C)|);
  close _cov;

  /* Numerator and denominator for narrow-sense heritability */
  AU = shape(0,nrow(VC),1); AU[1,1]=1*4;
  AV = shape(1,nrow(VC),1); AV[1,1]=1*2;
  PHEN = AV`*VC;                              /* Phenotypic variance */
  H2 = AU`*VC/PHEN;                           /* Narrow sense h2 */
  VAR_VC = vecdiag(COV);
  SE_VC = sqrt(Var_VC);                       /* SE of variance components*/
  VAR_U = AU`*COV*AU;                         /* Variance of numerator */
  VAR_V = AV`*COV*AV;                         /* Variance of denominator */
  COV_UV = AU`*COV*AV;                        /* Covariance between variances */
  SE_H2 = sqrt( (h2**2)*( (VAR_U/(AU`*VC)**2)+(VAR_V/(PHEN)**2)-(2*COV_UV/
          (AU`*VC)/(PHEN)) ) );               /* SE of  Narrow sense h2 */

  /* Numerator and denominator for broad-sense heritability */
  AU2 = shape(0,nrow(VC),1); AU2[1,1]=4; AU2[2,1]=1;AU2[5,1]=1;
  AV = shape(1,nrow(VC),1); AV[1,1]=2;
  H2B = AU2`*VC/PHEN;                         /* Broad-sense H2*/
  VAR_U = AU2`*COV*AU2;                       /* Variance of numerator */
  VAR_V = AV`*COV*AV;                         /* Variance of denominator*/
  COV_UV = AU2`*COV*AV;                       /* Covariance between variances */
  SE_H2B = sqrt( (H2B **2)*( (VAR_U/(AU2`*VC)**2)+(VAR_V/(PHEN)**2)-(2*COV_UV/
           (AU2`*VC)/(PHEN)) ) );             /* SE of broad-sense H2 */
```

```
  /* Create output */
  ITEM =  {"A","D","REP*F","REP*M","C(FAM)","RES"};
  create varcmp  var {ITEM VC SE_VC P}; append;
  create table1  var {H2 SE_H2 H2B  SE_H2B PHEN }; append;
  print
  ITEM VC [format=6.4] SE_VC [format=6.3] p[format=6.5] PHEN [format=6.3]
       H2 [format=6.3] SE_H2 [format=6.3] H2B [format=6.3] SE_H2B [format=6.3];
  run;
quit;
```