Partitioning of Multivariate Phenotypes using Regression Trees Reveals Complex Patterns of Adaptation to Climate across the Range of Black Cottonwood (*Populus Balsamifera L. Spp. Trichocarpa*)

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Partitioning of Multivariate Phenotypes using Regression Trees Reveals Complex Patterns of Adaptation to Climate across the Range of Black Cottonwood (*Populus Balsamifera L. Spp. Trichocarpa*)

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ABSTRACT

Local adaptation to climate in temperate forest trees involves the integration of multiple physiological, morphological, and phenological traits. Latitudinal clines for the relevant component traits are frequently observed for species that have a north-south distribution, but these relationships do not account for climatic variation within a given latitudinal band, which may be reflected in adaptive traits. We used black cottonwood (*Populus trichocarpa*) as a model to characterize the interplay between geography, climate, and adaptation to abiotic factors. Twelve traits (height, diameter, volume index, crown diameter, number of branches, number of sylleptic branches, relative number of branches, Relative canopy depth, Bud set, Bud flush, cold index of injury, carbon isotope ratio) were measured in a range-wide sample of 124 *P. trichocarpa* genotypes grown in a common garden. Heritability's were moderate to high (0.24 to 0.55) and significant population differentiation ($Q_{ST} > 0.3$) suggested adaptive divergence. When climate variables were taken as predictors and the 12 traits as response variables in a multivariate regression tree analysis, aridity (Eref) explained the most variation, with subsequent splits grouping individuals according to mean temperature of the warmest month, frost-free period (FFP), and mean annual precipitation (MAP). This grouping matches relatively well the splits using geographic variables as predictors: the northernmost groups (short FFP and low Eref) had the lowest growth performance, and the highest cold hardiness. The groups spanning the south of British Columbia (low Eref and intermediate temperatures) displayed an average growth and cold hardiness. The group from the coast of California and Oregon (high Eref and FFP) had the best growth performance and the lowest cold hardiness. The southernmost and high-elevated group (with High Eref and low FFP) performed poorly, had a low cold hardiness and a significantly lower WUE.

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List of Abbreviations

δ^{13}C: carbon Isotope ratio

Δ **^{13}C**: Carbon Isotope Discrimination

AH:M: Annual heat:moisture index (MAT+10)/ (MAP/1000))

bFFP: The Julian date on which FFP begins

CMD: Hargreaves climatic moisture deficit

DD<0 (DD_0): Degree-days below 0°C, chilling degree-days

DD<18 (DD_18): Degree-days below 18°C, heating degree-days

DD>18 (DD18): Degree-days above 18°C, cooling degree-days

DD>5 (DD5): Degree-days above 5°C, growing degree-days

eFFP: The Julian date on which FFP ends

EMT: extreme minimum temperature over 30 years. For an individual year, the EMT is estimated for a 30-year normal period (one of the nine normal periods included in the package) where the individual year is nearest to the center of the normal period.

Eref: Hargreaves reference evaporation

EXT: extreme minimum temperature over 30 years. For an individual year, the EMT is estimated for a 30-year normal period where the individual year is nearest to the center of the normal period.

FFP: Frost Free Period (day)

F_{ST}: population differentiation in molecular markers

MAP: Mean Annual Precipitation (mm)

MAT: Mean Annual temperature (°C)

MCMT: Mean Coldest Month Temperature (°C)

MRT: Multivariate Regression Trees

MSP: Mean Annual Summer P (May to Sept.) precipitation (mm),

MWMT: Mean Warmest Month Temperature (°C)

NFFD: The number of frost-free days

PAS: Precipitation as snow (mm). For an individual year, PAS is calculated for the period between August in previous year and July in current year.

Q_{ST}: population différentiation in quantitative traits

SH:M: Summer heat:moisture index ((MWMT)/(MSP/1000))

SPEI12: Mean annual Standard Precipitation Evapotranspiration Index

TD: temperature difference between MWMT and MCMT, or continentality (°C)

WUE : Water Use Efficiency

Introduction

Climate changes in conjunction with anthropogenic activities are threatening forest tree species growth and compromising the economic and ecological services they provide. Increasing global mean temperature and change in amount and pattern of precipitation, as predicted by most of the climate change models, are likely to have an impact on forest trees local adaptation (Solomon et al. 2007). About 10% (8765) of the approximately 100,000 tree species worldwide are on the endangered species list for various reasons (Newton and Oldfield 2008). The projected relatively rapid change in climate could increase this percentage and the risk of extinction of some tree species.

Tree species populations can respond to climate change by migrating to new areas with environmentally suitable condition, phenotypic plasticity, genetically adapting to the new conditions, or a combinations of any of these responses (Aitken et al. 2008). Migration rates are estimated to be less than 100 m per year (Savolainen et al. 2007), which might not be sufficient enough to cope with the predicted climate change rate. Therefore adaptation to climate change will also require phenotypic plasticity (the capacity of a single genotype to exhibit variable phenotypes in different environments), or local adaptation, which is the genetic differentiation in tree populations due to the varying selection force induced by geographically different climatic conditions. The efficiency of tree species response to climate change will depend upon the amount of variability in adaptive traits, the strength of selection, their fecundity, interspecific competition, and biotic interactions (Aitken et al. 2008). Genetic variability in life history traits is an adaptive asset as it increases the likelihood that appropriate variation will be available to facilitate adaptation to the new environment. Indeed numerous studies have demonstrated that adaptive genetic responses to environmental changes have occurred in a relatively short period (a few years to hundreds of years) suggesting that much of local adaptation comes from standing variation, rather than from new mutations (Savolainen et al. 2013).

Many approaches have used phenotypic, genetic and/or environmental data to study variability and local adaptation of tree species. Common gardens and transplant experiments traditionally used to evaluate phenotypic differences among populations and define seed transfer guidelines, have demonstrated their ability to detect local adaptation, identify traits under divergent selection, and quantify the genetic basis of the adaptive traits (Sork et al. 2013). Moreover knowledge of population phenotypic variation can be combined with genotypic and environmental information using approaches such as QTL mapping, association mapping or landscape genomics for a better understanding and prediction of tree responses to climate change.

Studies have found that local adaptation can occur with respect to many selective factors (climate, edaphic factors, parasites, etc.) and that different traits can respond to selection (Savolainen et al. 2007). Fitness related traits have revealed substantial differentiation and close association with geographic variables such as latitude and altitude, and with climate variables such as minimum annual temperature (Benowicz et al. 2000, Andersson and Fedorkov 2004, Gornall and Guy 2007, Hall et al. 2007, Vitasse et al. 2009a, Keller et al. 2011, McKown et al. 2013). For instance, the synchronization of phenotypic traits with their local photoperiod and seasonal temperature regimes constitute the strongest evidence of local adaptation in boreal and temperate forest trees (Aitken et al. 2008).

The natural distribution of *Populus trichocarpa* covers a wide latitudinal range encompassing environments differing largely in photoperiod, temperature and water availability. In addition to its large range the species possess numerous characteristics (easy to propagate, fast growing, whole genome sequenced, and ecologically and economically important) that make it a suitable model for adaptation studies. In this study, we investigated genetic variability in 12 traits encompassing *Populus trichocarpa* trees morphology, phenology and physiology, and used multivariate regression trees to partition these variabilities according to geography and climate.

Objectives

1. Measure variability of adaptive traits in *Populus trichocarpa* genotypes from across the native range of the species.
2. Infer the species local adaptation pattern by the correlation of these variations with climate and geography variables.
3. Explore the relationships among traits

Literature Review

Populus Trichocarpa Species

Species of the genus Populus (about 40 species) are deciduous or, rarely semi-evergreen, and occur primarily in the boreal, temperate, and subtropical zone of the northern hemisphere (Eckenwalder 1996, Dickmann DI 2001, Cronk 2005). Poplar trees are one of the superior hardwood trees grown in North America in temperate latitudes and are used for wood production, pulp production, plywood confection, and bioenergy (Zsuffa et al. 1996). *Populus trichocarpa Torr. & Gray* (Black cottonwood) is a deciduous broad leaf tree species native to western North America, and occupies riparian corridors with diverse topography and climate from northern California to southern Alaska (Gornall and Guy 2007). Its rapid growth, ease of propagation, wide intraspecific variability, and substantial hybrid vigor for many important commercial and adaptive traits have contributed to its use for commercial forestry (Zsuffa et al. 1996) and potential use for carbon sequestration (Marron et al. 2005). Considerable research on productivity and key adaptive traits in *Populus* species has been completed with substantial results used in breeding programs for selecting the most productive genotypes. For instance

Dillen et al. (2009) found that the number of sylleptic branches and individual LA of the largest leaf along the main stem were the best growth predictors for 2 hybrid poplar families.

Local Adaptation

The forces of natural selection often vary in space causing each local population to evolve traits that provide an advantage under its local environmental conditions, regardless of the consequences of these traits for fitness in other habitats (Kawecki and Ebert 2004). By definition, local adaptation is the pattern and the process leading to the highest fitness for the locally adapted populations when compared with other populations at their growing site (Kawecki and Ebert 2004).

The genetic basis of local adaptation can be determined by genetic tools, such as quantitative trait locus (QTL) mapping (Tanksley 1993) and association studies, but provenance trials must first be undertaken to detect the traits that are under spatially diversifying selection. Provenance trials allows (1) the study of genotype by environment interaction (GxE) when two or more common garden are established, which is a necessary condition of local adaptation (Morgenstern 1996) (2) the comparison of population differentiation in quantitative traits (Q_{ST}) and neutral molecular markers (F_{ST}) which can indicate the role of divergent selection pressure in shaping the adaptive genetic differences (Slavov and Zhelev 2010a) and (3) the detection of a gradual phenotypic changes along a geographical or environmental gradient (Clinal variation).

Q_{ST} was developed by Spitze (1993) to test whether genetic differentiation among populations arises by demographic processes (genetic drift and migration) or diversifying selection. Theoretical analyses and simulations have indicated that under a strictly neutral model, F_{ST} should be equal to Q_{ST} (Miller et al. 2008). If Q_{ST} is significantly higher than F_{ST}, the adaptive population divergence in the trait is not only explained by demography but also by divergent or disruptive selection (i.e., selection for different traits optima in the different populations). If $Q_{ST} < F_{ST}$ the trait is under stabilizing selection (i.e., selection for the same optimum in all population) that constrains population divergence (O'Hara and Merilä 2005).

Clinal variations for adaptive traits along geographic and climatic gradients also indicate the effect of diversifying natural selection. Geographic position affects many environmental selective agents such as temperature, precipitation, light, wind, soil nutrient availability, growing season length, photoperiod and biotic agents, which can bring about a geographical pattern in adaptive trait variation (Morgenstern 1996, McKown et al. 2013).

Phenology Traits, Branching Pattern and Carbon Isotope Ratio

Trees adaptive traits such as growth, phenology, physiology and fecundity have been used individually or collectively as a surrogate to fitness in common garden experiments and clinal variation studies, and their variations have been considered to be robust measures of local adaptation (Morgenstern 1996, Benowicz et al. 2000, Savolainen et al. 2007, Savolainen et al. 2007). Numerous studies on trees species have revealed high variability that follow geographic and / or climatic patterns. For instance, cold adaptation traits such as bud-set day, commonly display the strongest clines with environmental variables and the highest differentiation in quantitative traits (Q_{ST}) in temperate and boreal tree species suggesting that these traits are under strong natural selection (Howe et al. 2003). Variation in cold adaptation traits reflect the synchronization between the annual growth and the dormancy cycle (Aitken et al. 2008), which allows species to avoid cold injury in the winter and take advantage of the favorable conditions in summer. Also, latitudinal clines for growth and photosynthesis have been observed in tree species such as black cottonwood (Gornall and Guy 2007), paper birch, and Sitka alder

(Benowicz et al. 2000), which suggests a general adaptive response among woody angiosperms to be due to selective pressures encountered at higher latitudes. Similarly, Soolanayakanahally et al. (2009) monitored spring and autumn phenology in balsam poplar planted in a common garden and found strong clines with latitude of origin. Also, altitudinal clines were noticed in growth rate (height and stem diameter) among populations of 6 woody species by Vitasse et al. (2009a). In addition, they found that leaf phenology (flushing and senescence) differences for two of the species where linked to the annual temperature at the provenance native locality. Some studies on poplar trees have shown significant correlation between δ13C and latitude, elevation (Guy and Holowachuk 2001, Vitasse et al. 2009a) and longitude or distance from the coast (Gornall and Guy 2007).

One main adaptive feature of trees in temperate and boreal climates is the seasonal timing of the beginning and cessation of growth, which reflects an evolutionary compromise between avoiding frost damage and maximizing growth (Rehfeldt et al. 1999, Howe et al. 2003, Friedman et al. 2011). The annual growth cycle consists of growth cessation and bud-set, initiation of cold acclimation, development of endodormancy, development of maximum cold hardiness, endodormancy release via chilling, loss of cold hardiness, and initiation of primary growth and vegetative bud-flush (Howe et al. 2003). Studies have revealed that dormancy break in spring is controlled largely by temperature (chilling and warming requirements), and endodormancy induction in the fall by photoperiod and temperature (Pauley and Perry 1954, Rohde et al. 2011). Other environmental factors such as soil moisture and nutrient availability can interact with these cues to regulate the annual growth cycles (Howe et al. 2003).

Water use efficiency (WUE), the amount of carbon gained per unit of water loss, is a physiological trait that plays an important role in plant species adaptation, productivity and probability of survival under drought (Pereira and Osório 1995, Lauteri et al. 1997). It can therefore be used in breeding programs as a criterion for selecting plants with higher productivity under water limiting conditions. WUE is traditionally measured as the ratio between net CO_2 assimilation and transpiration rate (instantaneous WUE), or the dry weight accumulated and the amount of water transpired during a defined time (intrinsic WUE).

Farquhar and Richards (1984) and Hubick et al. (1986) have observed a linear negative correlation between $\Delta^{13}C$ in leaves and WUE and productivity in crop plants, and suggested that $\Delta^{13}C$ can be used as a selection criterion for water use efficient plants. The same negative correlation was reported in tree species such as *Quercus robur L.*, *Quercus petraea (Matt.)* (Ponton et al. 2002), and *Poplar hybrids* (Ripullone et al. 2004, Monclus et al. 2006). This led to the wide use of the $\Delta^{13}C$ as surrogate measurement of WUE, in various tree species including Populus species (Rae et al. 2004, Marron et al. 2005, Monclus et al. 2005, Dillen et al. 2008). Substantial intraspecific variation has been reported for $\Delta^{13}C$ in poplar hybrids under both optimal and moderate water-deficit conditions (Monclus et al. 2009). Study on 5 provenances of *Populus trichocarpa* genotypes occupying a wide latitudinal range (Gornall and Guy 2007) reported that instantaneous WUE varied among and not within provenances, and $\Delta^{13}C$ varied strongly within and not among.

Discrimination against ^{13}C during the incorporation of CO_2 into plant biomass is indicated by the lower ratio of ^{13}C over ^{12}C ($\delta^{13}C$) in plant tissue than in ambient air. This discrimination occurs during both diffusion through the stomata and the carboxylation process (Farquhar et al. 1989). Indeed, the rate of diffusion of $^{13}CO_2$ across the stomatal pore is 4.4‰ lower than that of $^{12}CO_2$ and ribulose bisphosphate carboxylase (Rubisco) prefers $^{12}CO_2$ over $^{13}CO_2$ by a factor of ~27‰. The extent of the discrimination depends mainly on the ratio of intercellular to atmospheric CO_2

partial pressures (p_i/p_a) (Farquhar and Sharkey 1982) which is controlled in opposite directions by both stomatal aperture and photosynthetic activity (Lauteri et al. 2004) creating the negative linear relationship between $\Delta^{13}C$ and WUE.

Divergent correlations were found between $\Delta^{13}C$ and productivity of different poplar species in the literature. While a positive relationship has been reported for *Populus davidiana (Dode)* Schneider (Zhang et al. 2004), no relationship was found for *Populus deltoides* ×*Populus nigra* hybrids (Marron et al. 2005, Monclus et al. 2005), and *Populus deltoides Bartr. ex Marsh* × *Populus trichocarpa L.* (Rae et al. 2004, Monclus et al. 2009). This led the authors to conclude that high productivity is not always linked with low WUE and that for many poplar species there might be a possibility to select for genotypes having both high productivity and high WUE.

Tree productivity traits such as biomass production, height and growth increment have been largely and successfully used to determine trees fitness in specific environments. For instance, growth traits confer important competitive advantages in some environments, but constitute a disadvantage in others due to their support and maintenance costs, as well as increased exposure to storm winds (King 1990). Numerous studies have focused on determinants of crop and tree productivity to select superior genotypes and improve their yield. Branching pattern is an important component of crown architecture and is intimately linked to a tree productivity as it determines the amount of leaf display, light interception and subsequent carbon assimilation in trees (Ceulemans et al. 1990). The relation between tree branch characteristics and biomass production has been investigated and large genotypic variability has been reported for a number of traits within the genus Populus (Ceulemans et al. 1990, Dunlap and Stettler 1996, Gielen et al. 2001, Zeleznik 2007). Strong genetic control and heritability for the sylleptic branches (i.e. branches developing from auxiliary buds without undergoing dormant period) have also been observed for some poplar species (Wu and Stettler 1996, Rae et al. 2004, Marron et al. 2006, Dillen et al. 2009). Sylleptic branches produced by hardwood trees including poplar species were found to contribute additional leaf area in the first few years of plantation establishment when crown canopy is not yet closed (Ceulemans et al. 1990). Also, sylleptic branches allocate more carbon to the stem than prolepsis branches hence contributing more to tree growth (Scaracia-Mugnozza et al. 1997). Ceulemans et al. (1990) reported that tree height and branch dimensions were more decisive criteria for maximum leaf area index than the total number of branches. Broeckx et al. (2012) found that tree height and branch dimension (diameter and length) were the most important determinants of wood production and maximum leaf area index followed by the number of sylleptic branches. Some studies reported correlation between branch characteristics of *Populus trichocarpa* genotypes and geographic variables as latitude and altitude (Weber et al. 1985, Rogers et al. 1989, Ceulemans et al. 1990). Indeed, they found that northern genotypes had smaller number of branches and wider branch angles than southern genotypes. In general, these studies focused on a small sample range or used few geographical and climate variables and might have missed some important interaction between predictor variables.

Materials and Methods

Plant Materials and Common Garden

In 2010, branch cuttings of *P. trichocarpa* genotypes were collected all over the native range of the species, from Alaska to California (ranged between 37.54 to 61.01 latitude and 118.70 to 153.58 longitude), and used to produce plantlets. The Plantlets were grown for 6 month in a greenhouse at Blacksburg, VA, USA before being transplanted in May 2011 in a common garden (located South- East of the southern periphery of the species range) at the Reynolds Homestead Forest Resource Research Center located in Patrick County Virginia (36° 37’ N and 80° 09’ W at

359 m). 789 different genotypes were planted following a Randomized block design with 4 blocks. The annual climate parameters (means of 1981-2009 years) for the closest weather station (Patrick County (VA)) showed a mean annual precipitation (MAP) of 1275 mm, a mean annual temperature (MAT) of 14.1 °C, a mean warmest month temperature (MWMT) of 24.4 °C, and a mean coldest month temperature (MCMT) of 4.2 °C (obtained from the weatherbase.com). The field was drip irrigated and weeds were controlled both mechanically and chemically. No fertilizers or other soil supplements were used at any time in the common garden. For this study, we selected 124 genotypes from the 789 available genotypes in such a way that we cover a wide latitudinal gradient (37 N to 58 N) (Figure 1).

Figure 1. Distribution of the *Populus trichocarpa* accessions source localities (red dots). The green area represents the species native range and the red star the common garden location.

Carbon Isotope Discrimination

A branch-section from the current growth year was collected in March 2013 for each tree on a branch oriented towards east and located at the 3/4 of the tree height. The branch section was oven-dried at 65°C until a constant weight is reached and ground to 0.5 mm size. 1.8 to 2 mg of each sample was combusted in an IsoPrime100 isotope ratio mass spectrometer (Isoprime Ltd, Cheadle Hulme, UK) and the isotope ratio $R_{sample} = {}^{13}CO / {}^{12}CO$ was determined. The analyses were conducted by the Virginia Tech Forest Soils and Biogeochemistry Lab and the carbon isotope ratio (δp) was calculated as the $^{13}C/^{12}C$ ratio relative to the international Pee Dee Belemnite standard (Farquhar et al., 1989):

$\delta_p = \frac{R_{sa} - R_{sd}}{R_{sd}} * 1000\ [‰]$, where R_{sa} and R_{sd} are the ^{13}C:^{12}C ratios of the sample and the standard, respectively (Craig, 1957).

The following relationship was used to estimate the carbon isotope discrimination ($\Delta^{13}C$):

$$\Delta^{13}C = (\delta_a - \delta_p)/(1 + \delta_p)$$

Where δ_p is the isotopic composition of the plant material and δ_a is isotopic composition of the air (assumed to be 8‰) (Craig, 1957).

Tree Dimensions and Branching Pattern

In March 2013, when the trees were in a dormant state, dimensions and branch characteristics traits (Figure 2) were measured on the 124 genotypes; Tree height (H), Insertion height of the highest branch (I_h), and insertion height of the lowest branch (I_l) (Broeckx et al. 2012), were measured using a tape and a telescopic pole. Tree stem diameter (D) at 22cm above ground level was measured using a digital caliper. The Volume Index (Diameter squared × height) was calculated and used to estimate the stem volume according to Causton (1985). The total number of branches (NB) and the number of sylleptic branches produced during the 2012 growing season (Nsyll2012) on the main stem were counted for each tree. The relative number of branches (RNB), density of branches per unit of stem height, was calculated by correcting the number of branches of each tree for H. The crown diameter was obtained by computing the mean of the crown diameters taken from 2 perpendicular directions (east to west and north to south). The relative canopy depth (RCD), percentage of the stem carrying branches, was computed as (I_h – I_l)/H.

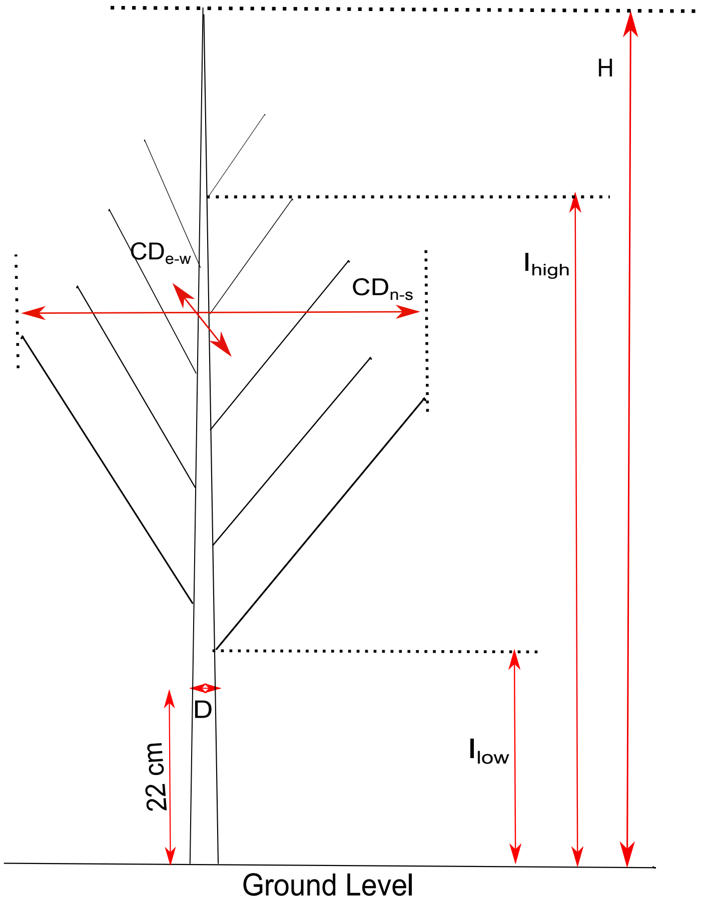


Figure 2. Trees dimension and branch characteristics measured. H: Tree height, D: Tree stem diameter at 22cm above ground level, CD: Crown Diameter (measured North-South and East-West), I_{high}: Insertion height of the highest branch and insertion, I_{low}: height of the lowest branch.

Bud Phenology and Cold Hardiness

Bud stage was scored on a weekly basis over 1 month. The main apical bud states were scored in April 2012 (bud-flush) and September 2012 (Bud-set), and the week at which the bud event (flushing or setting) occurred was converted to the Julian day (the number of days from the 1st January). The bud was considered flushed when a leaf had emerged 1 cm from the bud, and set when fully developed and covered by dark brown scales. The bud set/flush that occurred before the first week or after the last week of scoring were recorded as happened 1 week before, or later respectively.

Fall cold Injury were assess by measuring electrolytic leakage on branches sections collected in October 2012 on the same subsample and exposed to different freezing temperatures (Hannerz et al. 1999). For each genotype, 2 samples containing 3 segment discs (1 - 2mm) were placed in solutions of 500 ul of distillated water and a trace of Silver iodide (Agl) for ice nucleation. While control samples for all genotypes were kept at 4°C, the temperature of a second sample was gradually lowered (4°C/hour) until -20°C, and then held there for 2 hrs. The second samples were then heat-killed at 95°C for 4 hours. The conductivity was determined after the temperature treatments, and after the heat-kill process with a digital conductivity meter.

The freezing damage was express on an index of injury scale (0 for non-frozen tissue to 100 for Heat killed tissues) according to the method used by Flint et al. (1967):

$$I_t = 100(R_t - R_0)/(1 - R_0);\ R_t = \ L_t/L_k\ ;\ R_0 = \ L_0/L_d$$

Where I_t is the index of injury (or percent injury), R_t and R_0 are the relative conductivities for -20° and 4°C respectively, L_t is the specific conductivity of leachate from sample frozen at -20°C, L_k is the specific conductivity of leachate from sample frozen at -20°C and then heat-killed, L_0 is the specific conductivity of leachate from non-frozen control samples (-4°), and L_d is the specific conductivity of leachate from non-frozen (-4°C) heat-killed samples (Flint et al., 1967).

Climatic Datasets

21 annual climate parameters (means of 1981 – 2009 years) were extracted from the ClimateWNA version 4.72 climate variable simulator (www.genetics.forestry.ubc.ca/cfgc/climate-models.html#v2) developed by Hamann and Wang (2005), and a drought index, the Standard Precipitation Evapotranspiration Index (SPEI), from the Global SPEI database (SPEIbase v.2.2). These variables were used as predictor variables to explain the observed plant trait variability among genotypes and groups (Appendix).

Statistical Analysis

All statistical analyses were done using R version 2.15.1. The Data was tested for normality and homoscedasticity and a log transformation was performed on the number of sylleptic branches before the analysis. Pearson's correlation coefficients (r) with a significance test were calculated on a genotypic mean basis using the 'corstarsl' function of the 'Hmisc' Package in R.

We built Multivariate Regression trees (MRT) with the geographic and climate variables as candidate predictors using the R package MVpart v1.2–6. MRT is an extension of Classification and regression Tree (CART) that can be used to explore, describe, and predict relationships between multivariate response data and multiple predictor variables. A set of clusters are grown by repeated binary splits of the dataset to produce nodes as homogeneous as possible with respect to the response variables. This homogeneity is evaluated as sums of squares of traits around the multivariate mean of observations in a cluster (De'ath 2002). Before the MRT building, Principal Component analysis was run on the 8 growth and branch characteristic variables in R using the "prcomp" function of the stats Package, and the retained principal components were used with the phenology and δ^{13}C variables as response variables. The response variables were then standardized to give them an equal weight.

The MRT split the dataset into groups that are homogeneous in the measured adaptive traits using geographic variables as predictors. These groups were considered as populations for computation of the Population differentiation in quantitative traits (Q_{ST}) and the broad-sense heritability (H^2).

Population differentiation (Q_{ST}) and broad-sense heritability (H^2) for the measured quantitative traits were calculated following the procedure in Luquez et al. 2008. The variance components of genotypes within a population and of populations were obtained using a REML model with block as a fixed factor and genotype and population as random factors and used to compute H^2 and Q_{ST}. To estimate the heritability of the traits we used the linear model:

$$Y_{jkl} = \mu + b_j + g_k + e_{jkl},$$

Where Y_{jkl} is the measured variable phenotype of the l_{th} individual in the kth block from the jth genotype, μ denotes the grand mean and e_{jkl} is the residual error term. The Broad-sense heritability was calculated from estimates of total genetic variation (σ^2_G) and total phenotypic variation (σ^2_P) using the following formula: $H^2 = \frac{\sigma^2_G}{\sigma^2_P}$

Where σ^2_G is the genotype variance component and σ^2_p the total variance component

For Q_{ST} estimates, we used the model:

$$Y_{ijkl} = \mu + a_i + b_j + g_k + e_{ijkl},$$

Where the population (a_i) and genotype (b_{ij}) effects provide estimates of genetic variation between populations (σ_B^2) and among genotypes within populations (σ_W^2) respectively. Q_{ST} were estimated for each treat using the formula $Q_{ST} = \frac{\sigma_B^2}{(\sigma_B^2+2\sigma_W^2)}$

This calculation method is based on clonal replication of trees and has been shown by Lynch and Walsh (1998) to give Q_{ST} estimates confounded by non-additive genetic variances and maternal effects, which result in a slight deflation of Q_{ST}. According to Hall et al. (2007) the Q_{ST} estimates found with this method are likely to represent lower bounds and are therefore conservative with finding evidence for adaptive population differentiation.

Results

Relationships between Geography and Climate

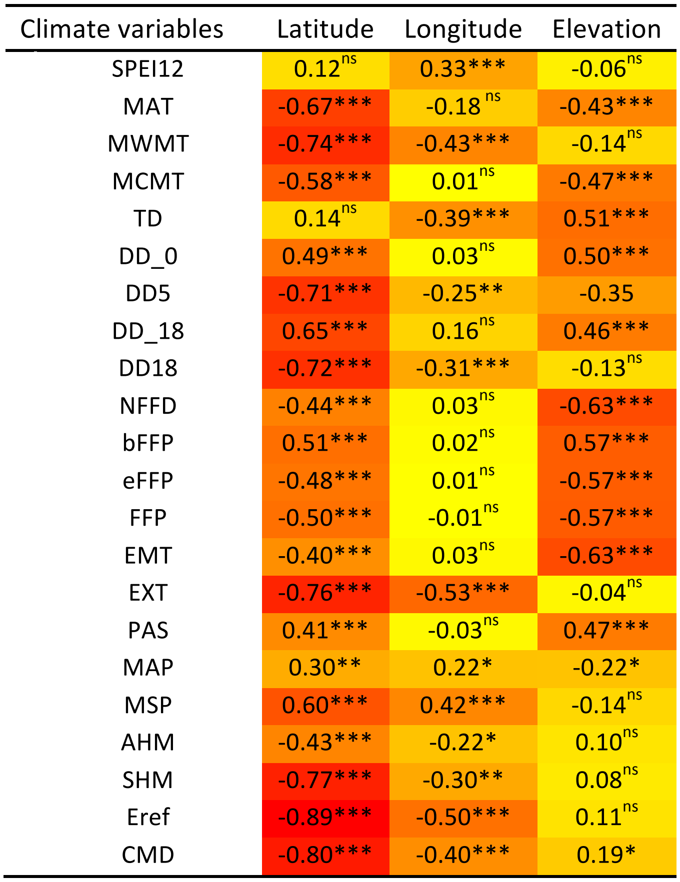
Latitude was the geographical variable with the highest number of significant correlations (20/22) and high correlations (10/22 variables had $|r| > 0.6$) with the climate variables (Table 1). The general trend is that with increasing latitude, precipitation increases whereas temperature, evaporation and climate moisture deficit decrease.

Longitude of origin had a moderate correlation with the temperature and the precipitation variables (Table 1). It is also significantly correlated with latitude ($r = 0.64$) due to the southeast – northwest orientation of the species range, and altitude ($r = -0.49$) because of the southern location of our highest elevation collection. The confounding effect of latitude and elevation makes it difficult to estimate the relationship between longitude and the climate variables. Nevertheless, the central part of our sample range (between latitude 48 and 54), which encompasses a large longitudinal range, meant there was an increase in temperature differential

(TD) and water deficit (Hargreaves reference evaporation, Hargreaves climatic moisture deficit) from the coast to the interior within that latitudinal band.

Elevation was moderately correlated with the temperature variables (MAT, MCMT, MWMT, DD's, FFP, and EMT).

Table 1. Pearson correlation coefficient and level of significance between climate and geographic variables.



Climate variables	Latitude	Longitude	Elevation
SPEI12	0.12^{ns}	0.33***	-0.06^{ns}
MAT	-0.67***	-0.18^{ns}	-0.43***
MWMT	-0.74***	-0.43***	-0.14^{ns}
MCMT	-0.58***	0.01^{ns}	-0.47***
TD	0.14^{ns}	-0.39***	0.51***
DD_0	0.49***	0.03^{ns}	0.50***
DD5	-0.71***	-0.25**	-0.35
DD_18	0.65***	0.16^{ns}	0.46***
DD18	-0.72***	-0.31***	-0.13^{ns}
NFFD	-0.44***	0.03^{ns}	-0.63***
bFFP	0.51***	0.02^{ns}	0.57***
eFFP	-0.48***	0.01^{ns}	-0.57***
FFP	-0.50***	-0.01^{ns}	-0.57***
EMT	-0.40***	0.03^{ns}	-0.63***
EXT	-0.76***	-0.53***	-0.04^{ns}
PAS	0.41***	-0.03^{ns}	0.47***
MAP	0.30**	0.22*	-0.22*
MSP	0.60***	0.42***	-0.14^{ns}
AHM	-0.43***	-0.22*	0.10^{ns}
SHM	-0.77***	-0.30**	0.08^{ns}
Eref	-0.89***	-0.50***	0.11^{ns}
CMD	-0.80***	-0.40***	0.19*

Significant correlations are indicated as: *** = P < 0.001; ** = P < 0.01; * = P < 0.05; ns = non-significant. Strong correlation coefficient is indicated by dark red and weak correlation by yellow.

Relation between Traits and Explanatory Variables

Pearson correlation coefficients and their level of significance were computed for each pair of variables (Table 2 and appendix) to evaluate the relationship between the studied traits and the geography and climate. All traits had a statistically significant ($p<0.05$) relationship with latitude of origin except relative number of branches (Table 2). The phenology traits showed the highest correlations and the best-defined latitudinal clines, with BS having the highest coefficient ($r = -0.79$). However the relationship between latitude and some of the morphology and physiology traits (H, D, CD, NB and $\delta^{13}C$) were not linear but rather an inverse U-shaped relationship (Figure 3), with the southernmost and northernmost genotypes having the lowest values for these traits. The southern high elevated genotypes had significantly lower $\delta^{13}C$ than the other genotypes which had approximately the same values (Table 3). This suggests that the latitudinal cline for $\delta^{13}C$ was created by the confounding effect of elevation.

$\delta^{13}C$ had correlations coefficients of 0.20 and -0.46 with latitude and elevation respectively, suggesting that the trees from highest latitude or lowest elevation had the highest $\delta^{13}C$. The Tukey Pairwise difference test (table 3) showed that pattern of this relationship was profoundly influenced by the genotypes from the Population 1, which had a significantly higher mean $\boldsymbol{\delta}^{13}C$ (-24.77) compare to the other populations that had similar means (between -23.02 and -23.32).

Dimension traits (H, D, Vi) and the $\delta^{13}C$ had a statistically significant negative correlation and a weak clinal variation with elevation. Longitude had weak or no correlations with most of the variables except for bud-set and bud-flush, which were moderate (0.39 and 0.47 respectively). As noted above, this correlation may have been induced by the high correlation existing between latitude and longitude of origin for our sample range.

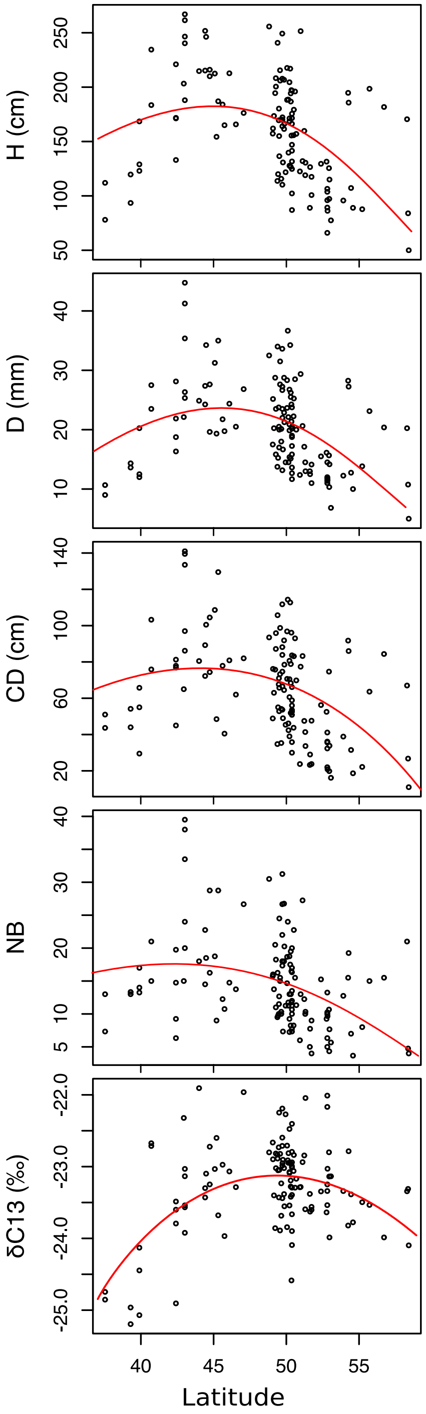


Figure 3. Inverse U-shaped relationship between latitude and some traits: tree height, stem diameter, number of branch, and carbon isotope ratio. The red line represents a 2nd degree polynomial curve.

Table 2. Pearson's correlation coefficients (r) and level of significance calculated from genotypic means between the studied traits and geographic variables

Traits	Latitude	Longitude	Elevation
H	-0.33***	-0.20*	-0.23**
D	-0.26**	-0.16^{ns}	-0.25**
CD	-0.35***	-0.19*	-0.20*
NB	-0.32***	-0.21*	-0.18*
NSyll2012	-0.33***	-0.15^{ns}	-0.13^{ns}
RNB	-0.17^{ns}	-0.14^{ns}	0.03^{ns}
RCD	-0.18*	-0.19*	-0.08^{ns}
Vi	-0.28**	-0.12^{ns}	-0.24**
***δ*13C (‰)**	0.20*	-0.04^{ns}	-0.46***
BF	0.45***	0.47***	-0.09^{ns}
BS	-0.79***	-0.39***	-0.03^{ns}
I_{-20}	-0.59***	-0.20*	-0.11^{ns}

Significant correlations are indicated as: *** = P < 0.001; ** = P < 0.01; * = P < 0.05; n.s. = non-significant.

Table 3. Least squared means of δ^{13}C for the 7 *Populus trichocarpa* populations

Population	N	Mean δ^{13}C (‰)*	SE
1	7	-24.77^{a}	0,19
2	32	-23.17^{b}	0,09
3	24	-23.02^{b}	0,10
4	29	-23.03^{b}	0,09
5	31	-23.28^{b}	0,09

Means followed by the same superscript are not significantly different at the 0.05 level (experimentwise), using the Tukey – Kramer HSD

Relationships between Traits

Among the traits, the highest correlations with climate were with temperature related variables (MAT, MWMT, MCMT, DDs, FFPs, EMT, EXT). BS showed the highest correlations and was statistically correlated with all the variables except with the drought index (SPEI12). MAP displayed statistically significant correlation with only BS whereas the MSP did show some statistically significant correlation with the branch characteristics traits, the BF, BS, and the cold index of injury at -20°C (I_{-20}). δ^{13}C had a weak negative but statistically significant correlation with mostly precipitation variables integrating temperature (Degree-days above 18°C: $r = -0.18$, Hargreaves climatic moisture deficit: $r = -0.32$, Annual heat:moisture index: $r = -0.22$, Summer heat:moisture index: $r = -0.27$, Hargreaves reference evaporation: $r = -0.28$).

The Pearson correlation coefficients between the measured traits are presented in Table 4. Relatively strong positive correlations ($r > 0.50$) were found between the dimension traits (H, D, Vi) and branch characteristics (NB, NSyll2012 and CD). The trees with large dimension (H, D, Vi) generally displayed the higher number of branches (including Sylleptic branches). δ^{13}C had a weak positive and not statistically significant correlation with the volume index ($r = 0.14$) suggesting a weak link between WUE and productivity. I_{-20} had a strong positive correlated with BS ($r = 0.78$) and a weak but statistically significant negative correlation with BF ($r = -0.23$). Dimension and branching characteristics traits had a statistically significant negative relationship with BF and a positive relationship with BS and I_{-20}.

Table 4. Pearson's correlation coefficients (r) between studied traits with level of significance calculated from black cottonwood genotypic means

	H	D	VI	CD	NB	NSYLL12	RNB	RCD	$\boldsymbol{\delta}^{13}$C	BF	BS
D	0.89***										
VI	0.84***	0.95***									
CD	0.85***	0.90***	0.84***								
NB	0.67***	0.74***	0.72***	0.78***							
NSYLL12	0.51***	0.60***	0.62***	0.61***	0.85***						
RNB	0.07^{ns}	0.20*	0.48***	0.31***	0.72***	0.59***					
RCD	0.43***	0.50***	0.20*	0.52***	0.73***	0.60***	0.69***				
$\boldsymbol{\delta}^{13}$C	0.18^{ns}	0.15^{ns}	0.14^{ns}	0.13^{ns}	0.10^{ns}	0.00^{ns}	-0.08^{ns}	-0.02^{ns}			
BF	-0.41***	-0.40***	-0.24**	-0.33***	-0.45***	-0.37***	-0.32***	-0.20 *	0.13 ^{ns}		
BS	0.55***	0.49***	0.49***	0.52***	0.48***	0.48***	0.17^{ns}	0.31***	-0.08^{ns}	-0.35***	
I-20	0.55***	0.49***	0.32***	0.48***	0.54***	0.42***	0.37***	0.14 ^{ns}	-0.05^{ns}	-0.23*	0.78***

Significant correlations are indicated as: *** = P < 0.001; ** = P < 0.01; * = P < 0.05; n.s. = non-significant.

Multivariate Regression Trees

The explanatory variables (geography and climate) were used to partition the data into groups according to their similarity in the traits using multivariate regression trees (MRT). The dimension and branching traits were subjected to a principal component analysis to remove their colinearity. The first 2 principal components were retained as they explained most of the variation in the trees and branches traits (88 %). The loadings of the traits (Table 5) indicate that the first principal component (PC1) is positively influenced by all the 8 variables (0.56 to 0.95) whereas the PC2 was related negatively to the trees dimension variables (H, D, Vi and CD) and positively to the branch characteristics (NB, NSyll2012, RNB and RCD).

Table 5. Trait loadings of the first (PC1) and second (PC2) principal components of analysis on trees dimension and branch traits, with PC1 explaining 69.9% and PC2 explaining 17.8% of the cumulative variation in the data set.

Trait	PC1	PC2
H	0.83	-0.46
D	0.92	-0.34
Vi	0.90	-0.31
CD	0.91	-0.25
NB	0.95	0.22
NSyll2012	0.82	0.27
RNB	0.56	0.78
RCD	0.74	0.46

The multivariate regression tree using the geography as predictors split the 124 genotypes in 5 groups, which explains 42 % of the variation in the tree dimension, branch characteristics, δ^{13}C and the tree phenology traits (Figure 4 A & C). Trees originating from below 49.28° latitude were split into the groups 1 and 2 according to their elevation of origin. This split could also have been made based on latitude (40.30°) or longitude (120.9°) of origin with the same amount of variance explained. The 7 genotypes from the group 1 (the southernmost population) had lower than average dimension and branch characteristics, flushed early, set buds late, had a high cold injury index, and the lowest (more negative) δ^{13}C. The 32 genotypes from the group 5, located between 49.34° and 40.30° latitude (Coastal), performed significantly above average for all the tree dimension and branch characteristic, had the earliest BF, the latest BS and the highest susceptibility to cold injury. Trees originating between latitude 51.05° and 49.28° (Southern BC) were further differentiated into group 3 and 4 according to their longitude of origin. Group 2 originated below 123° longitude (interior group) and consisted of 29 genotypes best characterized by dimensions (H, D, CD) slightly below the sample mean and a low cold Injury index whereas the 24 genotypes from the group 3, located above 123° longitude (Coastal group), had dimensions and cold Injury index slightly above the sample mean. Group 5 consisted of 31 genotypes originating above 51.05° of latitude (northernmost). This group had the lowest dimension and branch characteristic traits, the latest BF, the earliest BS and the lowest cold injury index.

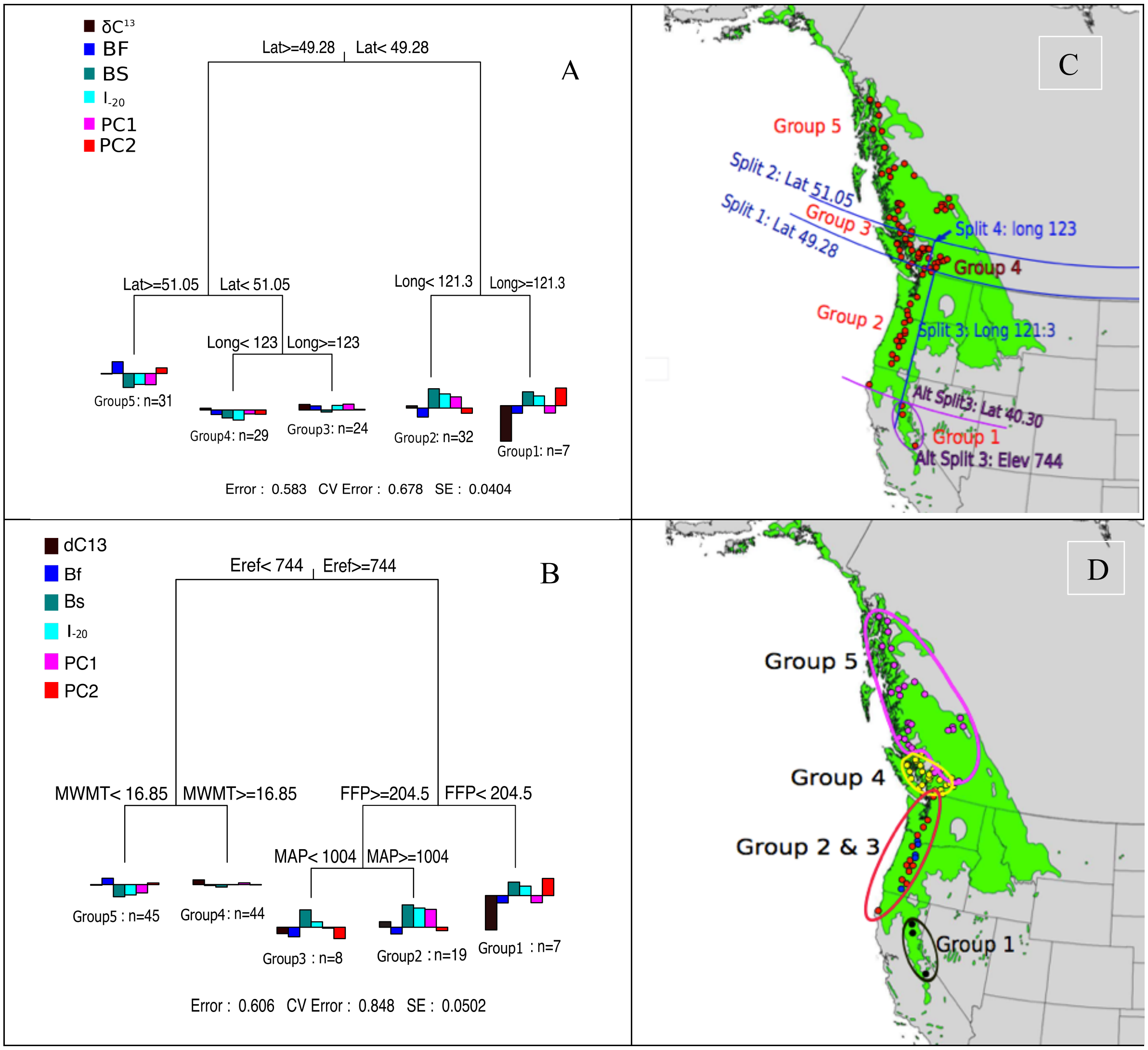


Figure 4. Multivariate regression tree (MRT) analyses of the 124 black cottonwood genotypes. A - with latitude, longitude and elevation as predictor variables. B - with climate as predictor variables. The bar charts at the leaves represent the average population means for the studied traits. C & D - Geographic representation of the splits.

Multivariate regression tree that uses climate variables as predictors also split the 124 genotypes in 5 groups, which explain 39 % of the genetic variation in the studied traits (figure 4 B & D). Groups 1 contained 7 genotypes that match with the high-elevated genotypes (group 1) in the MRT with the geographic variables. These genotypes originate from areas with Eref greater than 744 and FFP lower than 204.5 day. These trees had low dimensions and branch characteristics, early BF, late BS and the lowest δ^{13}C (lowest WUE). Groups 2 and 3 originating from areas with

Eref greater than 744 and FFP higher than 204.5 days correspond to the group 2 in the MRT with the geographic variables. They were further split according to the MAP, which show a spatial heterogeneity in precipitation regime for this locality. The 19 genotypes from the group 2 (from MAP higher than 1004 mm) flushed their buds early, had the latest BS, the highest susceptibility to cold injury, the highest performance in growth, branch characteristics and the highest δ^{13}C (highest WUE). The 8 genotypes of the group 3 (from MAP lower than 1004 mm) had some growth and branch characteristics close to the dataset average, the earliest BF, late BS, and low cold hardiness. Group 4 had 44 genotypes corresponding mainly to the genotypes of the group 3 obtained in the MRT with the geographic variables. Its locality of origin is characterized with Eref and MWMT respectively lower than 744 and 16.85°C. Trees from this population were characterized by some trait values close to the dataset average. Finally, Group 5 had 45 genotypes and comprises mostly genotypes from the group 5 and 4 obtained in the MRT with the geographic variables. These genotypes originate from localities with the Hargreaves reference evaporation (Eref) lower than 744 and MWMT higher than 16.85°C. Trees from this group had the lowest dimension and branch characteristics, set their buds earlier, flush their buds later and had the lowest cold injury index.

Genetic Variability and Heritability

The variability between genotypes within populations (refers to the groups obtained using the MRT1: Figure 5 & Table 6) and among populations was assess using ANOVA and by computing the quantitative trait differentiation (Q_{ST}).

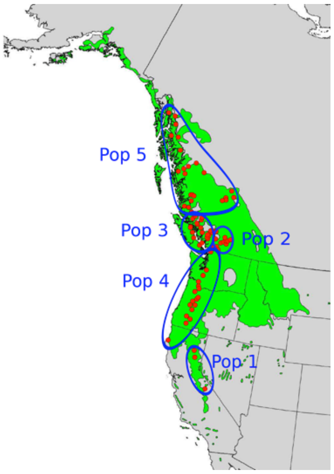


Figure 5. Sorting of the sampled genotypes into 5 geographically differentiated populations (blue circles)

Table 6. Source geographical ranges of the *P. trichocarpa* groups and the common garden location.

		Number of genotypes	Latitude (DD)	Longitude (DD)	Elevation (m)
Common garden			36.6667	80.15	359
Populations	1	7	37.54-39.90	118.7-120.78	1227-2363
	2	32	40.72-49.28	121.73-124.22	2-365.8
	3	24	49.28-50.93	123-125.83	5-538
	4	29	49.39-50.98	121-126.12	61-1143
	5	31	51.12-58.42	121.17-134.6	23-823

The proportion of the phenotype explained by the genetic variances was quantified using the broad sense heritability (H^2). Significant variability ($P < 0.05$) was found among genotypes in a population and among populations for all the studied traits (Table 7 & Figure 6). The variation among populations was higher than the variation within populations for the trees dimension traits (height and diameter) bud-set (BS) and the cold index of injury (I_{-20}). Relative branch number and relative canopy depth showed low variation among populations but high within population variance. Bud-set day (BS) had the highest variation among populations, which explained 45 % of the total variation for the trait (Table 7). Although we monitored bud-flush and set for only one month, we found significant variability within and among populations. Indeed, BS showed net differentiation among populations whereas the variability of the bud-flush day (BF) was mostly within population.

Table 7. Traits means range and percent of total variance explained for black cottonwood genotypes and groups

Trait	*Genotypes*		*Populations*	
	Range	**Variance (%)**	**Range**	**Variance (%)**
H (cm)	50 – 267	20.06	117.55 – 202.64	25.02
D (mm)	5 – 44.75	18.27	13.2 – 25.88	18.72
Vi (cm^3)	10.5 – 5237.65	15. 05	227.31 – 1833.6	13.30
CD (cm)	11 – 141	21.39	42.34 – 84.94	16.72
NB	3.67 – 39.5	27.31	10.3 – 19.28	15.49
NSyll2012	0 – 20.5	23.83	1.15 – 6.07	13.03
RNB	3.43 – 19.325	24.62	7.90 – 11.50	2.49
RCD	0.137 – 0.792	20.22	0.38 – 0.49	4.27
$\delta^{13}C$ (‰)	-25.19 – - 21.91	18.97	-24.54 – -23.01	18.67
BF (Julian day)	101^a – 130^b	26.12	108.52^a – 117.71^b	15.15
BS (Julian day)	218^a – 247^b	12.77	225.32^a – 238.13^b	45
I_{-20} (%)	25.21 - 76.33	15.38	36.49 - 58.49	26.19

a = 4.9 % and 11.0% of the trees had respectively already flushed and set their buds before this date.
b = 11.8% and 10.4% of the trees had respectively already flushed and set their buds after his date.

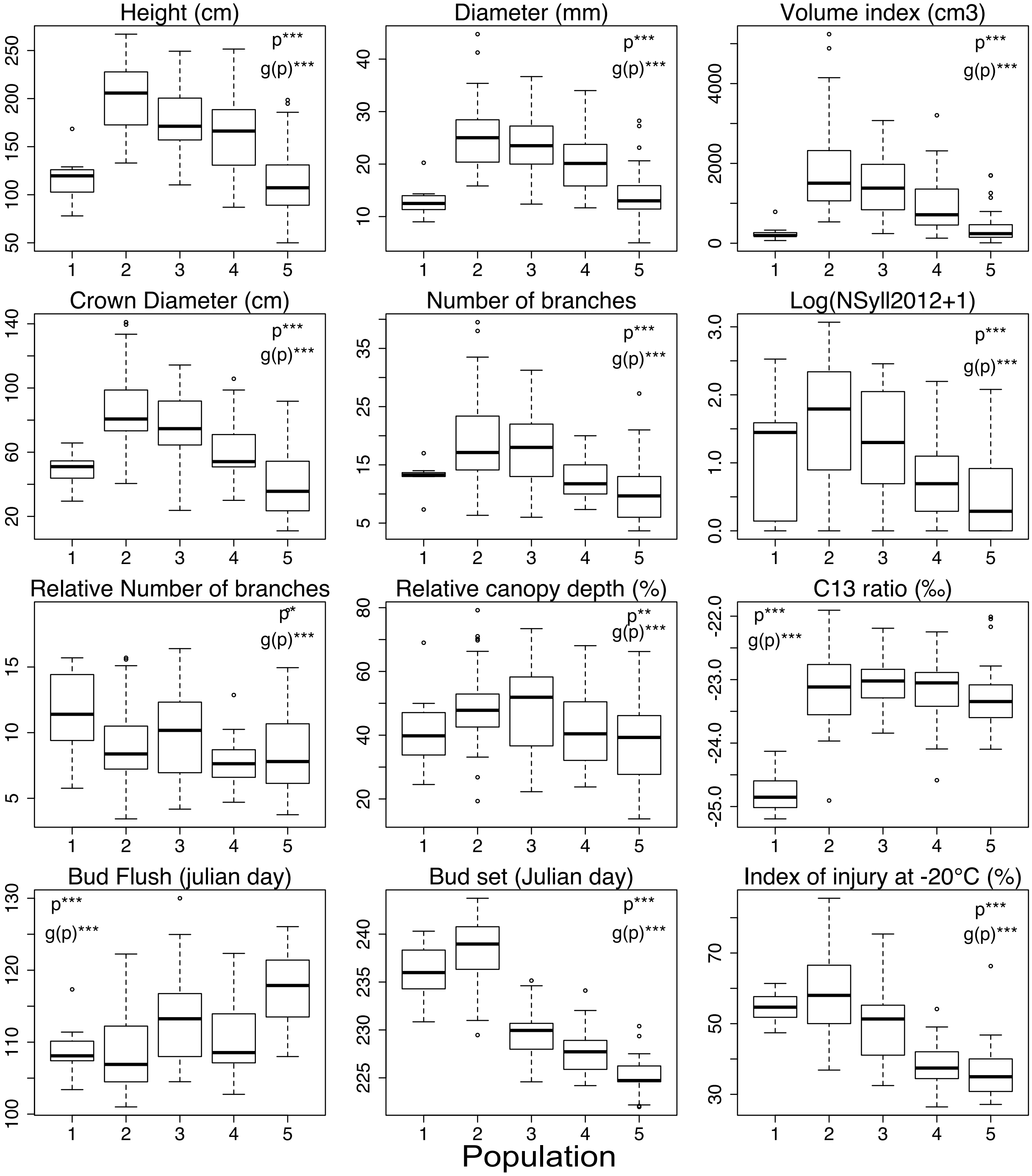


Figure 6. Box plots of black cottonwood genotype means for the 12 traits with some showing a latitudinal cline. Results of the ANOVA are indicated, where “p” is the population effect and “g(p)” is the genotype nested in the population effect. Levels of significance are *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ns = non-significant

Population differentiation in the quantitative traits (Q_{ST}) was low to moderate. Dimension traits (H, D and Vi) and cold adaptation traits (BS and I_{-20}) had the highest among population differentiation ($Q_{ST} > 0.25$) whereas the branch characteristics (NB, NSyll2012, RNB and RCD) had lower among population differentiation ($Q_{ST} < 0.25$). H^2 were moderate to high (0.24 to 0.56: table 8) with the phenology traits (BS and the index of injury) having the highest values ($H^2 \geq 0.50$), the tree dimension and branch number had moderate values (H_2 between 0.25 to 0.42), and the heritabilities for computed indexes (Vi, RNB, RCD) and $\delta^{13}C$ were lower than 0.30.

Table 8. Black cottonwood population differentiation in the quantitative traits (Q_{ST}) and broad sense Heritability (H^2) for the studied 12 traits.

Trait	**Q_{ST}**	**H^2**
H (cm)	0.38	0.42
D (mm)	0.33	0.34
Vi (cm^3)	0.31	0.26
CD (cm)	0.28	0.37
NB	0.22	0.42
NSyll2012	0.21	0.36
RNB	0.05	0.27
RCD	0.10	0.24
$\delta^{13}C$	0.34	0.27
BF	0.22	0.40
BS	0.64	0.56
I_{-20}	0.46	0.40

Discussion

Species occupying a wide geographical range generally display variability in important traits due to the effect of diversifying natural selection pressure. Studying the distribution of trait variability along environmental gradients can help predicting adaptive potential in a changing climate, and allow for selection of the best genotypes for planting in specific environments. *P trichocarpa,* a tree model for plant biology research, is suitable for adaptation studies as its range covers a relatively wide diversity of environmental conditions.

In this study, we investigated genetic variability in 12 traits (trees dimensions, branch characteristics, bud phenology and WUE through δ^{13}C) for 124 genotypes collected along the latitudinal gradient of the species native range (21 latitudinal degrees), and grown in the same common garden. We partitioned variability in the 12 traits according to the environment (geography and climate) and found patterns of adaptation to cold and water limitation for this species. Also, we found that selection is the main evolutionary process that has shaped the variability in most of the traits.

Traits Variability and Heritability

The high variability found within and between populations for all the studied traits was expected as the species spans a relatively wide geographical range encompassing habitats differing in photoperiod, temperature, and water availability. Studies have revealed that forest trees generally display high levels of adaptive genetic variation both within and among populations due to their large population size and the high gene flow among populations (Farmer Jr 1996, Morgenstern 1996, Howe et al. 2003, Savolainen et al. 2007, Aitken et al. 2008). All studied traits except BS had a within-population variation higher or similar to the among population variation (Table 6).

High within-population variation is thought to be a characteristic inherent to pioneering riparian species, which allows them to cope with the highly fluctuating nature of the riparian environment (Weber et al. 1985). The considerably lower within than among population's variability for BS is consistent with the strong selective pressure often found on this trait in tree species occupying wide latitudinal ranges (Keller et al. 2011, Soolanayakanahally et al. 2013).

Although the method we used to compute Q_{ST} is conservative and likely to provide underestimated values (Goudet and Büchi 2006), neutral markers are very heterogeneous in their F_{ST} (Whitlock 2008), and genetic differentiation in quantitative traits (Q_{ST}) may have low precision (O'Hara and Merilä 2005). Caution should hence be taken when comparing Qst to F_{ST} values.

In general, long-lived and widely distributed woody species harbor low levels of population differentiation in molecular markers ($F_{ST} = 0.084$; Hamrick et al. (1992)), with a median value of 0.047 for the *Populus* genus (Slavov and Zhelev 2010b), and values between 0.063 to 0.112 for *Populus trichocarpa* (Weber et al. 1985, Slavov et al. 2009, Ismail 2010). Compared with F_{ST} levels in poplar species, we found considerably higher Q_{ST} (more than 2 fold) for all the traits except the relative number of branches (RNB) and the crown canopy depth (RCD). This suggests that the genetic differentiation in H, D, CD, Vi, BS, BF, I_{-20} and $\delta^{13}C$ was mainly shaped by divergent selection instead of random genetic drift and gene flow.

Highest value of Q_{ST} for BS found in this study is in agreement with values observed in range-wide studies on woody species including poplar species, and confirm the relative importance of traits related to cold adaptation for trees in temperate and boreal regions (Morgenstern 1996, Hurme et al. 1997, Hall et al. 2007, Mimura and Aitken 2007, Keller et al. 2011). Indeed, phenology traits are important targets for natural selection in temperate tree species because they need to synchronize their annual growth cycle with the annual climatic cycle of their locality to avoid cold damage and take advantage of favorable conditions (Howe et al. 2003). While Morgenstern (1969) and Keller et al. (2011) found high Q_{ST} values for BF ($Q_{ST} = 0.52$ in *Picea mariana* and 0.66 in *Populus balsamifera l.* respectively), most of the other studies done in woody species are in agreement with our results of low Q_{ST} for BF ($Q_{ST} < 0.3$). This suggests that there is less genetic control of natural selection and a higher environmental control on this trait. Howe et al. (2003) explained that BF is determined by heat sum accumulation whereas BS is determined mainly by the photoperiod. The branch characteristics (NB, NSyll, RNB and RCD) had Q_{ST} slightly higher than F_{ST} values for *Populus trichocarpa,* but due to the low precision of Q_{ST}, these differences may not be enough to suggest that divergent selection are acting on these traits.

The moderate to high H^2 for the 12 traits ($0.2 < H^2 < 0.6$) indicates that relatively important proportions of the observed variation are due to genetic differences among individuals. This also suggests that all the traits can be directionally selected in breeding programs. Similar to our results, in studies of poplar hybrids, phenological traits were under stronger genetic control (high H^2) than ecophysiological traits (Bradshaw and Stettler 1995, Rae et al. 2004, Rohde et al. 2011). Similar results were found for *Populus tremula* (Luquez et al. 2008), *Populus nigra* (Chamaillard et al. 2011), *Populus balsamifera* (Keller et al. 2011), and a range wide sample of *Populus trichocarpa* (McKown et al. 2013). However, heritability varies with the genetic architecture of the trait and the population sample analyzed (Lynch and Walsh, 1998) and cautious should be taken when extrapolating to other populations.

Patterns of Local Adaptation

Latitude is the geographical variable with the strongest effect on tree dimension, branch characteristics, phenology variables and δ^{13}C. We observed a significant latitudinal gradient in trees dimension, BS, BF and cold hardiness (Figure 4). These clines were driven by a progressive change in climate factors from the south to the north of the species range (Figure 5), specifically a general decrease in temperature and water deficit with increasing latitude. BS had the strongest correlation with latitude (-0.79) followed by the cold index of injury (-0.59). Strong latitudinal clines for BS have been reported for numerous temperate and boreal tree species (Howe et al. 2003, Savolainen et al. 2007, Luquez et al. 2008, Soolanayakanahally et al. 2009, Keller et al. 2011, McKown et al. 2013).

Genotypes from the northern periphery of our range had the lowest performance, which reflects their adaptation to the short growing season (lower FFP) in their native environment. This is revealed by their late BF, early BS, and higher cold hardiness compare to the other genotypes (Figure 4). Experiments in common gardens have revealed for many tree species that high latitude populations achieve less height even when they display higher assimilation rates than low latitude populations (Burtt 1956, Kaurin et al. 1985, Junttila and Kaurin 1990, Soolanayakanahally et al. 2009, Savage and Cavender-Bares 2013). The small height and canopy area of the southernmost genotypes (originating from between 1227 and 2363 m above sea level) in our common garden could be explained by their local adaptation to the high elevation climate conditions in the Sierra Nevada of California. This locality has low minimum temperatures and a short growing season similar to the northern area of our sample (median EMT = -22.5 and FFP=169.3: Table 9) and is cooler than the common garden. Climate variables relating to water availability also reveal drier summers for this location compared to the rest of the collection range (MSP = 159.2, the highest median Eref and CMD). Decreasing tree height with altitude of origin has been described for a number of conifers and broadleaved tree species (Rehfeldt 1994, Oleksyn et al. 1998, Sáenz-Romero et al. 2006, Premoli et al. 2007, Rweyongeza et al. 2007, Vitasse et al. 2009a). Vitasse et al. (2009a) explained this pattern by the differential selection favoring high allocation to growth and competitive ability in low altitude populations, and low growth rate and robustness including cold-tolerance in high latitude or high altitude trees. Other possible explanations of the pattern of low growth rate for high-altitude populations could be their high respiration rates, their high allocation to roots, and/or their reduced shoot-growth period (Oleksyn et al. 1998).

The high-elevation genotypes from California (population 1) have displayed longer growing season (early BF and late BS) in the common garden although they were adapted to the fewer FFP (169 days) in their locality of origin. These two environments differ in temperature regime but not significantly in photoperiod (only 1° latitude), which means that the lengthening in growth season that occurred in the common garden was likely related to temperature differences. Indeed, the higher temperatures in the common garden compare to the high elevation where population 1 originates have led to a later BS or more probably an earlier BF as this trait is controlled mainly by temperature whereas the former is primarily controlled by photoperiod (Pauley and Perry 1954, Luquez et al. 2008, Keller et al. 2011). According to Friedman et al. (2011), the control of BF by temperature allow a more plastic response to climatic variation. Although these genotypes had a long growing season similar to the southern coastal genotypes, this did not result in a high growth rate but they rather displayed short sizes similar to the northern genotypes. This suggest that these genotypes are not only adapted to the cold temperatures in high elevation but also to other climatic factors which characterize these environments (higher water deficit, wind velocity, radiation, lower air pressure etc.).

The climate in the south of BC is characterized by low temperatures, high precipitation, and low evaporative demand compared with the other localities used in this study. The genotypes selected from this locality were differentiated into 2 groups according to continentality: The coastal group had a milder climate with MWMT > 16.85 and displayed better dimensions (slightly above the data overall mean for the measured dimensions), had a longer growing season and was more susceptible to fall cold damage than the more interior one (Figure 4). The interior population in southern BC was merged to the northern groups in the MRT using climate predictors (Figure 4 B & D) as the temperature parameter of these two population locations are similar.

Genotypes spanning the coast of Oregon and Washington were the tallest and had the largest number of branches in the common garden. This area is below 400 m altitude and has a mild climate (highest MAT, MWMT, EXT and low TD: Table 9). Moreover, this area has the closest temperature parameter to the common garden conditions, which may have impacted growth of these genotypes. The results of MRT using climate variables showed that these genotypes could be further differentiated according to Mean Annual Precipitation. This led to a heterogeneous distribution of the variability with the best growing genotypes originating from sites with MAP greater than 1004 mm (Figure 4). This spatial heterogeneity in climate could represent an important buffer in response to climate change. Indeed, spatial heterogeneity contributes to increased variability and facilitates migration by reducing dispersal distance required to find an appropriate climatic niche (Ackerly et al. 2010).

Table 9. Median values of source geographic and climatic variables for the different black cottonwood groups

	Groups					
Variables	1	2	3	4	5	Mean
Lat (degree)	7	32	25	29	31	-
Long (degree)	39.29	44.61	50.05	50.30	52.83	47.42
Elev (m)	120.77	123.08	123.37	122.35	126.95	123.30
SPEI12	1771	136	58	850	122	587.4
MAT (°C)	-0.22	-0.48	-0.36	-0.78	-0.29	-0.43
MWMT (°C)	10.7	12.3	9.8	6.4	7.4	9.32
MCMT (°C)	20.8	20.5	18.2	16.2	16.1	18.36
TD (°C)	3.1	4.85	2.5	-3.7	-0.1	1.33
MAP (mm)	18.6	15.9	16.1	19.5	16	17.22
MSP (mm)	1205	1055.5	2236	1401	1656	1510.7
AHM	138	173.5	417	315	407	290.1
SHM	18	21.35	9.1	12.1	10.8	14.27
DD_0 (°C)	144.7	120.45	44.6	49.7	42.2	80.33
DD5 (°C)	99	48	93	401	248	177.8
DD_18 (°C)	2409	2813	2062	1488	1605	2075.4
DD18 (°C)	2907	2285	3066	4254	3870	3276.4
NFFD (days)	259	266.5	107	39	42	142.7
bFFP (J-days)*	264	321	305	202	248	268
eFFP (J-days)	110	69	93	139	123	106.8
FFP (days)	295	319	310	272	285	296.2
PAS	185	249	220	132	164	190
EMT (°C)	89	13.5	74	354	158	137.7
EXT (°C)	-21.2	-9.3	-10.3	-24.2	-19.7	-16.94
Eref	38.2	39.85	35.8	34.9	34.2	36.59
CMD	918	865	650	610	601	728.8
CMD	571	410.5	138	147	100	273.3

*J-day: Julian day

Temperature variables where more correlated to the studied plants traits than precipitation variables (Appendix), suggesting that adaptation to water limitation is of less importance than adaptation to temperature for our sampled accessions. This is consistent with the role of temperature as an important signal determining tree phenology, which in turn influences tree physiology and morphology. Similar to our results, weak to moderate genetic clines to moisture gradients, particularly for summer precipitation, have been found in other species (e.g., Douglas-fir (St Clair et al. 2005); lodgepole pine (Rehfeldt et al. 1999)). However, our results showed that precipitation variables integrating temperature (AH:M, SH:M, Eref; CMD) had similar correlations to temperature variables with the traits. Andalo et al. (2005) found when studying the impact of climate change on growth of white spruce populations that the most efficient models among 90 were the ones that included both temperature and precipitation.

The relationship between longitude of origin and some of the traits were more likely created or increased by the fact that longitude is positively correlated with latitude for our sample range ($r = 0.64$). Instead of longitude, the distance from the coast (DC) seem to be a better variable as the environment is known to change substantially between coastal and interior regions. Nevertheless, the correlation of traits with the climate variable TD (continentality), which is highly correlated with DC, was lower and generally non-significant (Appendix).

Plants adapted to arid environments (high latitude and altitude) generally have higher δ^{13}C (higher WUE) than plants adapted to wet environments (Cohen 1970; Fischer & Turner 1978; Passioura 1982). An increase in δ^{13}C (increase in WUE) with altitude of origin was observed in situ for a hundred plant species from all over the world (Körner et al. 1988), shrub and tree species from deserts and mountains (DeLucia and Schlesinger 1991), and 3 riparian tree species (*Populus fremontii, P. angustifolia*, and *Salix exigua*) along altitudinal transects (Sparks and Ehleringer 1997). Similar results were also reported in common gardens and growth chambers for *Castanea sativa Mill* (Lauteri et al. 2004) and *P. nigra*, (Chamaillard et al. 2011). This trend contrasts with our observation of lowest δ^{13}C (lowest WUE) for genotypes from high altitude, which are likely to face extreme low temperatures and water limitation, compared to those from low altitude. Similar counterintuitive trends were observed in *Pseudotsuga menziesii, Larix occidentalis, Pinus ponderos* and *Pinus contorta Dougl. ex Loud* (Zhang et al. 1993, Zhang and Marshall 1994, Aitken et al. 1995, Zhang and Marshall 1995, Guy and Holowachuk 2001). According to Read and Farquhar (1991) and Aitken et al. (1995), possible explanations to lower (WUE) for higher elevation plants are (1) the difference in phenology leading in different environmental conditions (air isotopic composition and vapor pressure) during the growth period, (2) intraspecific differences in assimilation rate or stomatal conductance, (3) the difference in environmental conditions between common garden and population origins. The difference in phenology between populations does not hold in explaining the observed difference in $\boldsymbol{\delta}^{13}$C as there was no statistical correlation between $\boldsymbol{\delta}^{13}$C and phenology traits. Also, population 1 had its growing season overlapping with those of the other populations (Figure 6), suggesting that genotypes in population 1 accumulated their carbon within the same time period. It is more likely that the differences in $\boldsymbol{\delta}^{13}$C are attributable to the combination of differential adaptation to water limitation and dissimilarities between the origin site and the common garden environment. The lowest WUE for the population from the highest elevation may suggest that drought avoidance mechanisms play a more important role than WUE for adaptation to water limitation in this population. Indeed, plants cope with the fluctuation in environmental dryness by adjusting their leaf area, root depth and density, hydraulic properties, photosynthetic capacity and stomatal conductance, and the adaptive strategy they use determines the strength and the sign of the correlation between WUE ($\boldsymbol{\delta}^{13}$C) and the climate (Aitken et al. 1995, Lauteri et al. 2004). Genotypes in population 1 could have invested in a more efficient root system and maintained a high photosynthetic rate and stomatal conductance, whereas the others genotypes, which are generally not under water limitation, adapted by adjusting their stomata conductance. Also, the population's origins differed with respect to the combination of temperature and water regime: The native range of population 1 is characterized by a dry, hot summer compared with the native range of the southern coastal population (median MSP = 159 mm, EXT =39.1 °C, AHM = 19.0, SHM =135.8, Eref =991, CMD = 554.4), a short growing period (median FFP = 169 days), some low minimum temperature (median EMT = -22.5 °C) and large temperature fluctuations (median TD = 18.5 °C) similar to the native range of the northernmost populations (Table 9). Although population 1 locality had a similar seasonal distribution of precipitation compared with the southern coastal population and similar MAP in the common garden, differences in soil characteristics (soil depth, texture, and organic matter content), and air characteristics (Vapor deficit pressure, CO2 and O2 partial pressure) could have greatly affected water availability leading to a higher water stress. When grown in the common garden, accessions of population 1 could have encountered a moister climate and thus displayed a lower WUE (lower δ^{13}C), whereas accessions from the other populations experienced mild water stress and responded by reducing their water loss, which led to higher WUE (Higher δ^{13}C).

Relationships between Productivity and the Other Traits

The strong positive correlation between tree dimension (H, D, Vi) and branching variables ($r < 0.5$) suggest that branch characteristics play an important role in productivity. Branch characteristics are important features of crown architecture, which is known to be closely related to tree productivity as it defines the quantity of light interception and CO_2 assimilation (HallÈ et al. 1978). We found a higher number of sylleptic branches on high productive genotypes, which is consistent with a number of studies on poplar hybrids (Scarascia-Mugnozza et al. 1999, Rae et al. 2004, Marron et al. 2006, Dillen et al. 2009). Ceulemans et al. (1990) and Scarascia-Mugnozza et al. (1999) showed that sylleptic branches supply a larger proportion of carbon to the stem than proleptic branches during the early growing years of poplar trees. The lack of correlation between $\delta^{13}C$ and trees and branch characteristics combined with moderate H^2 found for these traits suggests that for *P. trichocarpa*, there is a possibility of improving WUE without compromising productivity. Similar results were reported for some interspecific poplar hybrids (Rae et al. 2004, Marron et al. 2005, Monclus et al. 2005, Monclus et al. 2006, Fichot et al. 2010), for *P. nigra* (Chamaillard et al. 2011) and *P. trichocarpa* (McKown et al. 2013). Contrary to that trend, negative (Cregg and Zhang 2001, Voltas et al. 2008, Aletà et al. 2009) and positive (Guehl et al. 1994, Lauteri et al. 2004, Zhang et al. 2004, Gornall and Guy 2007, Monclus et al. 2009) correlations were found between $\delta^{13}C$ and productivity traits in woody species. According to Farquhar et al. (1989) a lack of relationship between $\delta^{13}C$ and growth means that stomatal conductance drive WUE variations, and a positive correlation between the two traits generally supports that differences in photosynthetic capacity is the main driver for WUE. A previous study on a range–wide sample (Gornall and Guy 2007) has showed that the differences in *P. trichocarpa* genotypes WUE were more related to the variability in stomatal conductance than assimilation rate. Carbon allocation pattern, known to influence the relative growth potential, displayed a large variability in *P. trichocarpa* (Pallardy and Kozlowski 1979b, Scaracia-Mugnozza et al. 1997) and could also explain the lack of relation between WUE and growth.

The populations having the shortest growing season (originating from colder areas) were generally the ones with the smallest height indicating the trade-off usually observed between growth rate and adaptation to cold noted above. The northern genotypes are adapted to the longer summer day length encountered in high latitudes and when grown in a southern habitat, they set their buds early in the summer, which shortens their growth season length and their growth potential (Savage and Cavender-Bares 2013). Koehler et al. (2012) explained this trade-off by more resource allocation to freezing tolerance, which reduces the allocation to growth, as well as a shorter growing season length at northern latitudes, which limit growth. Studies in growth chambers have revealed that this tradeoff in poplar species results from variation in species' photoperiod cues for growth rather than in their inherent growth capacity (Soolanayakanahally et al. 2009, Savage and Cavender-Bares 2013).

Conclusion

We aimed to determine the geographic and climatic pattern of variability in 12 adaptive traits for a range wide collection of 124 *Populus trichocarpa* genotypes using multivariate regression trees (MRT). As commonly observed for tree species spanning a large range, we found high variability within and between populations in all the studied traits, and a moderate to high broad sense heritability. This suggests a significant genetic control of the studied traits and a possibility to select genotypes adapted to specific environmental conditions. Our data suggest that *P. trichocarpa* has considerable standing variation, which represent a substantial adaptive and selective potential. The significant population differences in tree dimension, phenology and carbon isotope composition combined with latitudinal clines suggests that neutral genetic drift and gene flow are not the only evolutionary processes that have driven these differences, but also adaptation to the local climate conditions. The multivariate regression trees showed that the population differentiation and variation according to the geography was mainly driven by adaptation to environments differing in photoperiod, temperature or water availability. Also, the MRT showed that heterogeneity in mean annual precipitation for accessions from the coastal California and Oregon resulted in a heterogenic distribution of the variability for the studied traits.

The genotypes from the coastal California and Oregon area were the best adapted to the climatic conditions of our Virginia common garden. This suggests that they can be used for conservation and breeding purpose in areas with similar environmental conditions. Indeed, with the growing interest in Poplar trees species for bioenergy, knowledge of the suitable genotypes for specific environments can constitute valuable informations for successful plantations. Future studies to enhance poplar productivity should couple phenotypic data with genomic data using methods such as association mapping and landscape genomic to uncover the underlying genetic architecture of adaptive traits and their variation along ecological gradients. Additional common garden sites will also help to tease apart the relationship between genetics and environment. To this end, the same accessions studied here have been planted in a common garden in coastal British Columbia, and provide the opportunity to compare the patterns revealed by this study with those in an area of very different environmental conditions. This will not only allow inferring local adaptation but also give insight to the extent of an evolutionary alternative to local adaptation; phenotypic plasticity.

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Appendix.

Heat map with correlation coefficient between traits and climate variables

Climate variables		Morphology								Physiology	Phenology		
		Dimensions			Branch pattern								
		H	D	Vi	CD	NB	NSyll12	RNB	RCD	δ^{13}C	BF	BS	I$_{-20}$
Temperature	MAT	0.48***	0.44***	0.46***	0.48***	0.45***	0.45***	0.16^{ns}	0.25**	-0.02^{ns}	-0.33***	0,75***	0.62***
	MWMT	0.42***	0.38***	0.39***	0.42***	0.40***	0.43***	0.17^{ns}	0.23**	-0.17^{ns}	-0.41***	0,73***	0.48***
	MCMT	0.41***	0.36***	0.40***	0.43***	0.41***	0.41***	0.16^{ns}	0.21*	0.04^{ns}	-0.22*	0,67***	0.63***
	TD	-0.19 ^{ns}	-0.16 ^{ns}	-0.20*	-0.21*	-0.20*	-0.17 ^{ns}	-0.06^{ns}	-0.08^{ns}	-0.21*	-0.07 ^{ns}	-0,26**	-0.43***
	EMT	0.47***	0.44***	0.45***	0.46***	0.43***	0.38***	0.12^{ns}	0.23*	0.21*	-0.19*	0,54***	0.55***
	EXT	0.39***	0.34***	0.35***	0.34***	0.33***	0.38***	0.09^{ns}	0.17 ^{ns}	-0.15^{ns}	-0.39***	0,74***	0.47***
	DD_0	-0.34***	-0.32***	-0.34***	-0.35***	-0.31***	-0.31***	-0.09^{ns}	-0.16^{ns}	-0.01^{ns}	0.18*	-0,56***	-0.54***
	DD5	0.50***	0.45***	0.48	0.50***	0.46***	0.48***	0.1 ^{ns}	0.26**	-0.05^{ns}	-0.38***	0,79***	0.60***
	DD_18	-0.48***	-0.44***	-0.46***	-0.48***	-0.44***	-0.44***	-0.16^{ns}	-0.25**	-0.01^{ns}	0.31***	-0,74***	-0.63***
	DD18	0.40***	0.36***	0.39***	0.40***	0.38***	0.44***	0.15^{ns}	0.21*	-0.18*	-0.38***	0,74***	0.48***
	NFFD	0.48***	0.44***	0.46***	0.47***	0.43***	0.41***	0.13^{ns}	0.24**	0.15^{s}	-0.20*	0,61***	0.58***
	bFFP	-0.52***	-0.47***	-0.50***	-0.52***	-0.46***	-0.43***	-0.14^{ns}	-0.25**	-0.15^{ns}	0.27**	-0,68***	-0.60***
	eFFP	0.50***	0.46***	0.47***	0.51***	0.46***	0.41***	0.17^{ns}	0.27**	0.12^{ns}	-0.21*	0,64***	0.61***
	FFP	0.51***	0.47***	0.49***	0.52***	0.47***	0.43***	0.15^{ns}	0.26**	0.14^{ns}	-0.25**	0,67***	0.61***
Temperature + precipitation	AHM	0.07 ^{ns}	0.01 ^{ns}	0.07 ^{ns}	0.07 ^{ns}	0.07 ^{ns}	0.13 ^{ns}	0.00^{ns}	-0.03^{ns}	-0.22*	-0.16	0.44***	0.21*
	SHM	0.25**	0.17	0.20*	0.24**	0.21*	0.27**	0.06^{ns}	0.06^{ns}	-0.27*	-0.33***	0.71***	0.50***
	Eref	0.33***	0.27**	0.30***	0.32***	0.31***	0.38***	0.14^{ns}	0.16^{ns}	-0.28*	-0.40***	0,78***	0.54***
	CMD	0.22*	0.15	0.19*	0.22*	0.23***	0.30***	0.13^{ns}	0.09 ^{ns}	-032**	-0.33***	0,72***	0.46***
	SPEI12	-0.19*	-0.18*	-0.16 ^{ns}	-0.12 ^{ns}	-0.04 ^{ns}	-0.02 ^{ns}	0.09^{ns}	-0.05^{ns}	-0.17^{ns}	-0.13 ^{ns}	-0,17 ^{ns}	-0.06 ^{ns}
Precipitation	MAP	0.00 ^{ns}	0.06 ^{ns}	0.01 ^{ns}	-0.01 ^{ns}	-0.04 ^{ns}	-0.06 ^{ns}	-0.05^{ns}	0.02^{ns}	0.16 ^{ns}	0.13 ^{ns}	-0,31***	-0.07 ^{ns}
	MSP	-0.19*	-0.12 ^{ns}	-0.14 ^{ns}	-0.20*	-0.25**	-0.21*	-0.17^{ns}	-0.12^{ns}	0.13^{ns}	0.28**	-0,55***	-0.31***
	PAS	-0.24*	-0.19*	-0.26**	-0.26**	-0.30***	-0.32***	-0.17^{ns}	-0.16^{ns}	0.02^{ns}	0.10	-0.56***	-0.50***

Strong correlation coefficient is indicated by dark red and weak correlation by Yellow. Significant correlations are indicated as: *** = P < 0.001; ** = P < 0.01; * = P < 0.05; n.s. = non-significant Traits are listed in order of category as follows: Morphology traits; Tree height, Stem diameter, Crown Diameter, Number of branch, Number of Sylleptic branches, Relative number of branch, Relative Canopy Depth. Ecophysiology traits; Carbon Isotope Ratio. Phenology traits; bud break, bud set, Cold Injury index (I$_{-20}$).