Which phenotypic traits are under selection under warm, dry climates in black spruce?

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ABSTRACT

• **Background and Aims** Trees are increasingly at risk of maladaptation to their environment as climates change rapidly world-wide. Although adaptive evolution by natural selection is a key mechanism by which populations and species can avoid extinction in changing environments, we have limited information regarding the phenotypic traits under selection under warm and dry environments. We answer the following research questions: (1) What ecophysiological traits are under selection in warm and dry environments? (2) Will intrapopulation trait integration affect the response to selection in the warmer, drier site? (3) Is the plastic response of traits under selection adaptive?

• **Methods** We studied 425 trees from seven provenances across three 50-year-old *Picea mariana* (black spruce) provenance trials located along a spatial climate gradient across eastern Canada. We measured height growth rate as a performance metric, and 10 traits that reflect water use, thermoregulation, structural support, and photosynthetic rate.

• **Results** All traits were under selection in at least one site, mostly in combination with other traits. For two trait combinations, the strength of selection gradients significantly increased from the colder, wetter site to the warmer, drier site: water use efficiency (WUE) with Huber value (HV), and carbon-to-nitrogen ratio (CN) with HV. In the warmer and drier site, trait-trait correlations among these three traits were largely absent, except for CN:HV in two provenances. The plastic response to the spatial climate gradient was adaptive for WUE but maladaptive for HV and CN.

• **Conclusions** Results suggest that adaptive evolution in response to climate change in *P. mariana* may favor phenotypes with fewer needles that are conservative for water and resource use. Intrapopulation trait integration should minimally impede adaptive evolution. However, the species' plastic responses to warmer and drier conditions may constrain the expression of optimally adapted phenotypes.

Keywords: *Picea mariana* (Mill.) B.S.P.; climate change; common gardens; functional traits; performance landscape; phenotypic integration; provenance trials; selection gradient analysis, Huber value; water use efficiency; leaf nitrogen to carbon ratio

INTRODUCTION

As climates shift under global change, the growth, productivity and long-term persistence of tree species are at risk (Allen *et al.* 2010; Choat *et al.* 2012). This threat is particularly urgent for boreal tree species, which are experiencing climate change more rapidly than those at lower latitudes (Peng *et al.* 2011; Aubin *et al.* 2018; Chagnon *et al.* 2022). Drought-induced tree mortality has increased under climate change (Allen *et al.* 2010; Choat *et al.* 2012), suggesting that plasticity has not been sufficient to keep pace with climate change (Malcolm *et al.* 2002; Iverson *et al.* 2004; Brecka *et al.* 2018) and projected rates of migration are expected to be insufficient (Boisvert-Marsh *et al.* 2022). Mismatches between tree species' distribution and climatic niche are becoming increasingly important (Gray and Hamann 2013; Lapenis *et al.* 2022).

In the face of climate change, tree species can avoid extinction via migration, adaptive plasticity, or adaptive evolution (Aitken *et al.* 2008). Mechanisms of adaptation increase the frequency of phenotypic traits that enhance fitness in a given environment. Adaptation can occur either through adaptive plasticity (where environmentally driven changes in phenotype of a given genotype improve fitness), or through adaptive evolution (where heritable traits that confer a fitness advantage increase in frequency across generations as a result of natural selection). Although both mechanisms can contribute to population persistence, research on phenotypic adaptation to climate change has predominantly focused on adaptive plasticity, and thus more studies on adaptive evolution are needed to gain a more complete understanding of phenotypic adaptation to climate change in trees (Lindner *et al.* 2010; Royer-Tardif *et al.* 2021). Indeed, while much research has examined adaptive plasticity in populations locally adapted to warm and dry environments (e.g. Blasini *et al.* 2021; Challis *et al.* 2022; Andrés-Hernández *et al.* 2023), we lack basic knowledge on what phenotypic traits are selected for (i.e. lead to high fitness, fitness component, or performance) under warming climates in trees (Alberto *et al.* 2013). While research on natural selection on plant phenotypes (i.e. phenotypic selection) in short-lived herbaceous species abounds (e.g. Etterson and Shaw 2001; Etterson 2004; Ludwig *et al.* 2004; Donovan *et al.* 2007, 2009), comparatively little research has quantified phenotypic selection on traits of forest tree species (e.g. Castro 2006; De La Mata *et al.* 2017; Warwell and Shaw 2018). Moreover, research on phenotypic selection in trees under warm or dry climates has largely focused on seed traits and early growth stages (e.g. Ramírez-Valiente *et al.* 2021; Costa E Silva *et al.* 2022, 2024).

To begin addressing this gap, we examine selection on ecophysiological traits from warming climates in mature trees of a dominant boreal species, black spruce (*Picea mariana* (Mill.) B.S.P.). Climate change is documented to affect black spruce growth (Sniderhan *et al.* 2021; Chagnon *et al.* 2022). This generalist species, which is present within the entire range of the North American boreal forest, occurs in a variety of habitats and soil types. It is common in habitats with wet soils such as peat bogs and swamps but also grows well on clays, loams, well-drained mineral soils and boulder pavements (Viereck and Johnston 1990). Black spruce generally grows in cold climate with humid to dry subhumid moisture regime. It has low drought and water-logging tolerance and is shallow rooted (Viereck and Johnston 1990; Niinemets and Valladares 2006). The warmer and drier conditions brought upon by climate change in much of its range are expected to lead to drought stress and growth declines in parts of its range (Girardin *et al.* 2016; Aubin *et al.* 2018; D'Orangeville *et al.* 2018). Climate models forecast increases in temperature in Canada (Swart *et al.* 2019; IPCC, 2018), leading to decreases in water availability for equivalent precipitation due to increased evapotranspiration (Dai *et al.* 2004; Barnett *et al.* 2005; Cholet *et al.* 2022). Indeed, climate moisture index (CMI), the difference between annual precipitation and potential evapotranspiration, is predicted to decrease across most of the boreal forest except for eastern-most regions (Boucher *et al.* 2020). Further, warmer air temperatures result in increases in atmospheric vapor pressure deficit (Ficklin and Novick 2017; Dai *et al.* 2018), which increases transpiration rates and can lead to soil water limitation and decreased growth (Yuan *et al.* 2019; López *et al.* 2021). Warming from climate change has affected black spruce growth unevenly across its range, promoting growth in the northern boreal forest, where growth is temperature limited, while reducing growth in southern regions, where water becomes limiting (Gamache and Payette 2004; Beck *et al.* 2011; Sniderhan *et al.* 2021; Chagnon *et al.* 2022). While black spruce's growth response to climate change is documented, the phenotypic traits that sustain growth under warmer and drier conditions remain unknown.

To address this gap, we test which traits are under selection for high growth rate in natural black spruce populations planted in provenance trials spanning a climate gradient. We expect that warmer and drier climates may select for traits related to water use, carbon economics, structural support and temperature regulation (Aubin *et al.* 2016; Boisvert-Marsh *et al.* 2020; Sniderhan *et al.* 2021). In water-limited environments, traits promoting conservative water use and durable leaf construction via higher investment in structural tissues are likely to increase performance and be the targets of selection. Although the relationship between wood density and hydraulic transport safety is complex (Chave *et al.* 2009; Hoffmann *et al.* 2011; Pratt and Jacobsen 2017), higher wood density can be associated with higher xylem cavitation resistance (Hacke *et al.* 2001; Hoffmann *et al.* 2011), which can result in higher performance in water limited environments (Cochard *et al.* 2007). Temperature controls metabolic rates and can impede photosynthetic activity above 35 °C, with the threshold varying among species (Münchinger *et al.* 2023). Boreal spruce species have shown photosynthetic inhibition above ca. 42 °C (Bigras 2000; Münchinger *et al.* 2023). Some research has shown that leaves can buffer variation in air temperature to maintain leaf temperatures near their metabolic optimum (Michaletz *et al.* 2015). As such, we expect enhanced capacity for leaf cooling (i.e., thermoregulation) to confer higher performance and thus be a target of selection in warmer climates.

Although assessing the adaptive nature of individual traits is important, phenotypic integration (i.e. trait correlations) may also affect adaptive evolution. The response of a highly integrated trait to selection is affected by selection on the correlated traits. This integration can constrain adaptive evolution when the direction of maximum trait covariance conflicts with the direction of selection, or enhance it when they are aligned (Björklund 1996; Schluter 1996). In contrast, a lack of trait covariance allows trait to evolve independently (Via and Lande 1985), provided that sufficient trait variation is present in the populations and that traits are under genetic control. This can be beneficial for populations as their response to climate change can proceed unconstrained by trait covariation. If different black spruce populations express different patterns of phenotypic integration, then certain populations might express phenotypic correlations better aligned with the direction of selection than others, and as a result have a higher potential for adaptive evolution across generations. Similarly, within generations, phenotypic plasticity can either help or hinder adaption: it can be adaptive if trait expression changes in the direction of selection and improves performance, or maladaptive if the phenotype moves away from the direction of selection and decreases performance (Bradshaw 1965; Pigliucci 2001; Whitman and Agrawal 2009).

Here, we use three black spruce provenance trials to address three research questions: (1) What ecophysiological traits are under selection in warm and dry environments? (2) Will intrapopulation trait integration affect the response to selection in the warmer, drier site? (3) Is the plastic response of traits under selection adaptive?

METHODS

STUDY SITES

We studied 425 trees from three sites belonging to a set of *P. mariana* (Mill.) B.S.P. range-wide provenance trials established in 1974 by the Canadian Forest service (Morgenstern and Kokocinski 1976; Keable 1978). These provenance trials were established for forestry purposes to determine which of various provenances (populations of different origin from the across the species' range) grew the best at various geographic location. We selected three provenance trials that shared seven provenances and covered a climate gradient, both in terms of temperature and water availability (Supplementary Table S1, Figure 1). The warmest and driest site was located in Petawawa Research Forest located near Chalk River, ON. A site with an intermediate climate was located near Chapleau, and the coldest and wettest site was located near Chibougamau QC. During establishment, each site was divided into three to six replicate blocks. Within each block, 16 trees per provenance were planted in grids of 4x4 trees, forming small plots (Morgenstern and Kokocinski 1976). Since the range-wide cooperative black spruce provenance program was designed to identify the optimal genetic stock for each site, tree spacing was adjusted among sites to maximize productivity according to local site conditions. The sites also differ in factors other than climate, such as soil texture, depth, and water holding capacity, and planting density (Table S1).

The trees originated from seven provenances (Table S2). The provenances were selected because they were present on most sites, there were sufficient individuals for sampling at each site, and their geographic origins covered a large portion of the species' range. Further, black spruce descends from three distinct glacial refugia with distinct genetics: the Western, Central and Eastern lineages(Jaramillo-Correa *et al.* 2004; Gérardi *et al.* 2010), and the study populations are different admixtures of these lineages (Table S2). At each site, an average of 23 trees per provenance were sampled (ranging from 14 to 41). Five provenances were common to all three sites: 6804 Roddickton, NL, 6855 Matagami, QC, 6859 Parc Mistassini, QC, 6901 Bancroft, ON and 6907 Timmins, ON. Two provenances were common to Petawawa and Chibougamau only: 6856 Manicouagan, QC and 6979 Rocky Mtn. House II, AB.

<< Figure 1. here >>

PERFORMANCE METRICS

We used relative height growth rate of individual trees as a performance metric. Growth rate has been shown to affect survival and reproduction, two fitness components (Farris and Lechowicz 1990; Arntz *et al.* 1998) and is broadly used as a performance metric in selection gradient analyses (Geber and Griffen 2003; Caruso *et al.* 2020) and in forestry (Younginger *et al.* 2017). Vegetative growth rate is also the only accessible indicator of performance in mature trees in natural setting. Here, by using the phenotypic selection analyses with growth rate as a performance metric, we identify the ecophysiological traits conferring a high aboveground growth rate in mature black spruce. The height of each individual tree was measured in 2022 in Petawawa, 2023 in Chapleau, and 2016 in Chibougamau and relative height growth rate since planting was calculated by dividing tree height by the age at the time of measurement (RGR; m/yr). In 2023, survival of all trees of the study provenances was measured at each site.

SAMPLE COLLECTION

In July and August 2022 and 2023, one branch per tree was sampled from at least three blocks at each site. To standardize light exposure, in the morning of each sampling day, we collected a healthy, sun-exposed, lateral branch from the top third of the tree crown using a 13.5m HV-245 telescopic pole pruner. It was then trimmed to contain the 0 to 5-year-old needles and an additional length of branch which was placed in florist tubes filled with water to rehydrate them prior to trait measurements. Branches were then placed in plastic bags with a damp paper towel and stored in the dark in a cooler on ice until measurements in the afternoon (Garnier *et al.* 2001). On average, 27 trees per provenance per site (range of 15-41) were sampled, resulting in a total of 143 trees sampled at Petawawa, 156 at Chapleau, and 132 at Chibougamau.

TRAIT MEASUREMENTS

We measured 10 traits associated with structural support, photosynthesis, water use, and thermoregulation and known to be associated with plant response to temperature or soil moisture deficit (Table 1). To characterize investment in structural support at the needle and branch levels, leaf dry matter content (LDMC; g/g) and twig specific density (TSD; g/cm^3) were measured.

LDMC, the ratio of dry to water-saturated mass, reflects needle resource investment into structural support versus metabolic processes (Shipley *et al.* 2006) and has been shown to increase with drought (Anderegg *et al.* 2021). TSD affects mechanical support, storage and hydraulics (Pratt and Jacobsen 2017) and closely related traits such as wood density can increase with water limitation (Anderegg *et al.* 2021). Leaf mass per area (LMA, g/cm^2) reflects the carbon cost of light interception and in global interspecific comparisons is an important indicator of plant carbon-use strategies (Westoby *et al.* 2002; Wright *et al.* 2004), with high values indicating high structural support and needle persistence, and low values indicating high photosynthetic rate. Among species, high LMA values has also been shown to correlate with tolerance to heat stress (Münchinger *et al.* 2023) and drought stress (Poorter *et al.* 2009; Scoffoni *et al.* 2014; Rosas *et al.* 2019). Carbon to Nitrogen ratio (CN; g g^{-1}) reflects the nutrient concentration in the leaves. High nitrogen concentration (low CN) is largely driven by high concentration of RUBISCO and is therefore correlated with high photosynthetic rate (Reich *et al.* 1999). Water use efficiency (WUE; ‰) was measured to characterize the grams of water lost per unit of carbon fixed. We used carbon isotope ratio ($\delta^{13}C$) to estimate intrinsic water use efficiency (Ma *et al.* 2023). During photosynthesis, $^{12}CO_2$ is preferentially fixed over $^{13}CO_2$ primarily because the enzyme RuBisCO discriminates against $^{13}CO_2$ and only binds it when $^{12}CO_2$ is limited, which occurs when stomata are closed (Farquhar *et al.* 1982; Lambers *et al.* 2008). The ratio of ^{13}C to ^{12}C present in plant tissue, therefore reflects the amount of time leaves spent with their stomata closed. $\delta^{13}C$ gives the ^{13}C isotope composition relative to the primary reference scale of Vienna Pee Dee Belemnite. Within species, $\delta^{13}C$ often increases with water limitation (Limousin *et al.* 2015; Hajek *et al.* 2016; Rosas *et al.* 2019; Ahrens *et al.* 2020; Csilléry *et al.* 2020; Anderegg *et al.* 2021; Rabarijaona *et al.* 2022; Lochin *et al.* 2024). Stomatal density (SD; number/cm) affects leaf gas exchange and is thus a strong correlate of both transpiration rate and photosynthetic rate (Lambers *et al.* 2008) and has been shown to vary within species with drought (Aragón *et al.* 2023). Huber value (HV; mm/g), the ratio of sapwood area to needle weight, characterizes water supply relative to water demand at the branch level (Carter and White 2009). High Huber values typically indicate increased hydraulic efficiency and thus increased drought avoidance (Mencuccini and Grace 1995; Mencuccini *et al.* 2019). It has been shown to increase within species with increasing water limitation (Rosas *et al.* 2019; Anderegg *et al.* 2021). To assess thermoregulation, needle cooling (NC; ºC) was measured as the difference between needle temperature and ambient temperature. Leaf cooling allows a leaf to partly control its temperature and stay within the optimal temperature range for photosynthesis (Michaletz *et al.* 2015). Chlorophyll concentration (CHL; mg/m^2) was measured because it is strongly correlated with photosynthetic capacity (Lambers *et al.* 2008). Needle length (NL; mm) affects total light capture and water loss of the plant, as well as needle thermoregulation by modifying the thickness of boundary layer and thus passive heat loss (Lambers *et al.* 2008).

<< Table 1 >>

On each branch, we measured traits on one- to four-year-old needles. This age range was selected because many needles that formed during the year of collection (age 0) were not yet mature and needles older than 4 years were frequently absent. Three traits were measured at the branch level (HV, WUE and CN, Table 1). The other seven traits were first measured from each needle age class, then averaged at the branch level by abundance-weighting by the relative needle biomass of each age class. The relative dry needle biomass was used for needle traits and relative dry branch biomass for TSD.

Two time-sensitive traits (CHL and NC) were measured indoors in the afternoon immediately following sample collection and while the samples were still in the water-filled tubes. Branches were brought indoors and taken outside the cooler for 30 minutes to acclimate to ambient air temperature. Total chlorophyll content (CHL) was measured on the adaxial side of needle bundles using a CCM-300 chlorophyll content meter (Opti-Sciences; Gitelson *et al.* 1999). Two measurements per needle age class were taken, except when they differed by more than 10%, in which case a third measurement was taken. NC was determined from needle and ambient temperature measured using a TCAM-300 thermal camera (Infrared Camera INC, 2021), a high accuracy temperature sensor (TMP117 High-Accuracy Digital Temperature Sensor) and FlashImagePro software. NC values are positive when the needle is warmer than the ambient temperature and negative when it is below, with more negative NC values indicating stronger cooling.

The remaining eight traits were measured in the laboratory while branches were still in the florist tubes, within 7 days of sampling. Two perpendicular diameter measurements (mm) were taken at the base of the fourth year of growth using digital calipers (Series 500 ABSOLUTE Digimatic Caliper). HV was calculated as the average branch diameter divided by the dry mass (g) of needles supported by the branch. Branches were then cut and separated into twigs based on growth year identified from terminal bud scars. For each growth year, 12 to 15 intact, healthy, mature needles were removed for trait measurements. Needle fresh weight was measured using analytical balances (AG104 Metler Toledo and XSR205 Metler Toledo). The needle length (mm) and fresh needle area (cm^2) were then determined from scans of 600 dpi (STD4800 scanner and WinSeedle V.2020). Needle dry weights were taken after drying at 60 ºC to constant weight. LDMC was calculated as the ratio of dry to fresh weight of each set of needles. LMA for each set was calculated by dividing the dry weight by total fresh area. The total dry needle weight of each growth year was used to calculate abundance-weighted needle trait values at the branch level. To determine TSD, three twigs of each growth year were haphazardly sampled (excluding the largest and smallest sizes to obtain median values), their fresh volume was determined by water displacement and then dried at 60 ºC to constant weight. TSD of each growth year was then calculated as twig dry mass (g) divided by twig fresh volume (cm^3). Finally, branch-level TSD were determined by abundance-weighing each year's TSD by their relative dry weight.

To measure WUE, 12 needles for each age class used for previous trait determinations were ground into a fine powder using a ball mill. Stable carbon isotope analysis was performed at the Environmental Isotope Laboratory at the University of Waterloo using a 4010 Elemental Analyzer (Costech Instruments, Italy) coupled to a Delta Plus XL continuous flow isotope ratio mass spectrometer (Thermo-Finnigan, Germany). To determine the variation within individual ground samples, duplicate measurements were performed for 34 samples, spaced at regular intervals throughout measurement. Images of the abaxial surface of three needles were taken using Leica EZ4 W stereo microscope at 35X magnification and LAS X for Life Sciences microscope imaging software (Leica Microsystems, 2021). The number of stomata were counted along the maximum visible needle length using WinSeedle Software (Regent Instruments, 2020), and SD was calculated as the number of stomata per unit length.

STATISTICAL ANALYSES

All statistical analyses were conducted in R version 4.4.0 (R Core Team 2025). Two percent of all data was missing due to lost samples or measurement errors (3% in Petawawa, <1% in Chapleau, <1% 3% in Chibougamau). Missing values were imputed for each site from other traits from linear mixed effect models, using the *lme()* function from the *nlme* package (Pinheiro *et al.* 2025). The model with the highest predictive power for missing values were identified using the *dredge()* function from the *MuMIn* package (Bartoń 2025). Data were only imputed when the R^2 of the predictive model was > 0.6. Otherwise, the missing values were left as NA. After imputation < 0.01% of data was missing from each site, resulting in n = 142 trees in Petawawa, n = 151 trees in Chapleau, and n = 132 trees in Chibougamau. Differences in survival among sites and among provenances within sites were tested with one-way ANOVAs using *aov()* from the *stats* package. Correlations among traits were described using *rcorr()* from *Hmisc* package (Harrell 2025) and principal component analyses (PCA) using the *PCA()* of the *FactoMineR* package (Lê *et al.* 2008). PCA were conducted on trait data across all sites as well as within each site. The statistical significance of the principal component axes was tested using *PCAsignificance()* from the *BiodiversityR* package (Kindt and Coe 2005), which compares the amount of variance explained by each axis to the variance explained in broken-stick null models.

To assess selection at each site, we used the Lande-Arnold regression approach to measure directional (β) and correlational (γ) selection gradients at each site (Lande and Arnold 1983). Here, the partial regression coefficients in multiple linear regressions of height RGR against traits represent the strength and direction of selection. We standardized trait values within each site to a mean of zero and a variance of 1 (z-transformation) and relativized RGR values within each site by dividing individual RGR values by the mean RGR for that site (Conner and Hartl 2004). Within each site we assessed βs from multiple regressions of RGRs on all 10 traits with provenance as a random effect, and we assessed γs from multiple regressions of RGRs on all 10 traits, their 2-way interactions and provenance as a random effect (Lande and Arnold 1983). We used the *lme()* function from the *nlme* package (Pinheiro *et al.* 2025). We confirmed the absence of multicollinearity by calculating the variance inflation factor (VIF) of the fixed term in the mixed model, using *vif()* for the car package. All VIFs were below 2.3. Following Zurr & Ieno (2016), we verified the data met model assumptions by examining diagnostic plots.

To identify which of the selection gradients in Petawawa might be caused by climate, we tested for correlations between the selection gradients and the climate among site (Wade and Kalisz 1990). We used two criteria: (1) the selection gradient showed clinal variation (i.e. continuous gradual change with the ecological gradient) with climate among sites, and (2) the selection gradients significantly differed between Petawawa and Chibougamau, the two sites at opposite ends of the climate gradient (hereafter called ‘climate-consistent selection gradients’ for brevity). We used the *tsum.test()* function from the *BSDA* package to perform a two-sample t-tests from the coefficient estimates and their standard errors (Arnholt and Evans 2023). While specific traits may be under selection from many factors at a given site, we interpret the clinal changes among sites as primarily driven by differences in climate. Indeed, other than differences in tree spacing, which we do not expect to impact the results (see discussion), we are not aware of any clinal differences in the environment among those sites.

To evaluate whether trait integration might affect the response of the provenances to selection, we examined whether trait integration was aligned with the direction of selection. This approach makes the common simplifying assumption that the phenotypic variance-covariance matrix is a proxy for the genetic variance-covariance matrix (Merilä and Björklund 2004). Since we are interested in selection under warm, dry climates, we used selection gradients at Petawawa, our warmest, driest site, to create performance surfaces using the *geom_contour()* function in the *ggplot2* package (Wickham 2016). Smooth contour lines were generated using the *interp()* function from the *akima* package (Akima and Gebhardt 2022). For each provenance, the residuals of the traits from the regression models were extracted using the *residuals()* function from the *stats* package, and correlations between the residuals were tested with the *cor.test()* function from the *stats* package.

To test whether traits changed plastically across sites, we first performed a two-way ANOVA, with site and provenance as fixed factors using *aov()* from the *stats* package. To test for significant differences among the sites' mean trait values, we performed a post-hoc LSD test on site alone using *LSD.test()* from the *agricolae* package (de Mendiburu 2023).

RESULTS

TRAITS UNDER SELECTION AT EACH SITE

All 10 traits measured were under directional or correlational selection in at least one site, for a total of 21 significant or marginally significant selection gradients across the three sites (eight in Petawawa, three in Chapleau and 10 in Chibougamau; Figure 2, Table S3). Of the 21 selection gradients, 7 were for individual trait values and (directional selection, β) and 14 were for trait combinations (correlational selection, γ). The traits and trait combinations under selection largely differed among sites. Specifically, in Petawawa three traits under directional selection - increased needle cooling, increased needle length and increased water use efficiency – and five trait combinations were under correlational selection - Chlorophyll concentration : Leaf dry matter content, Needle length : Carbon to Nitrogen ratio, Leaf dry matter content : Water use efficiency, Water use efficiency : Huber value and Carbon to Nitrogen ratio : Huber value.

<< Figure 2. here >>

DIFFERENCES IN SELECTION ASSOCIATED WITH CLIMATE

The shifts among sites were consistent with a role for climate in two of the eight selection gradients in Petawawa: CN:HV and WUE:HV. Selection for CN:HV trait combination showed a marginally significant clinal increase from non-significant in Chibougamau to + 0.021 in Petawawa ($p = 0.092$). Selection for WUE:HV trait combination showed a significant clinal increase from -0.078 in Chibougamau to +0.025 in Petawawa ($p = 0.025$) (Figure 3, Table S3). Changes in the other selection gradients in Petawawa were either not clinal or were not significantly different from Chibougamau (Figure S3).

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PHENOTYPIC INTEGRATION WITHIN PROVENANCES

Intraspecific integration in black spruce was weak, with the strongest correlations found among LDMC and LMA ($r= 0.42$, $p < 0.0001$, Table S4). In both the experiment-wide and within-site PCAs, principal component axes were not significant, and the first two axes accounted for a small fraction of the total variation (all data: 34%, Petawawa: 33%; Chapleau 38%; Chibougamau: 42%, Supplementary Figure S2). The site-specific PCAs for Chibougamau were similar to the experiment-wide PCA, but the Petawawa and Chapleau PCAs differed from each other and from the experiment-wide PCA (Figures S2B).

Since results indicated climate-consistent selection gradients for CN:HV and WUE:HV combinations, we explored the intraspecific trait correlations among the residuals of WUE, HV and CN. The trait residuals were not correlated within provenances except significant negative correlations between HV and CN in provenances 6855 ($p = 0.012$) and 6979 ($p = 0.042$). The negative correlation between HV and CN in both provenances is largely perpendicular to the direction of selection, which favours combinations of high values of both traits (Figure 4).

TRAIT PLASTICITY

All traits showed a significant plastic response across sites, with most provenances showing similar patterns of trait variation among sites (Figures 5 and S4). Since results indicated climate-consistent selection for positive interaction among CN:HV and WUE:HV, plastic increases in CN, HV and WUE would indicate adaptive plasticity. Results show that water use efficiency increased from the coldest to the warmest site, following the direction of selection ($p \leq 0.0001$). In contrast, CN ratio decreased from the coldest to the warmest site ($p \leq 0.0001$). Huber value showed a non-linear plastic response across the climate gradient, with highest values at Chapleau (the site with intermediate climate, $p \leq 0.0001$, Figure 5). The plastic response of CN ratio and Huber value to the spatial gradient thus do not track the direction of selection at the warmer, drier site.

<< Figure 5. Here >>

DISCUSSION

PHENOTYPIC TRAITS UNDER SELECTION DIFFERED AMONG SITES

We found that all ten ecophysiological traits studied here were under selection in one of the three sites, whether individually or in combination with another trait, and that the traits under selection differed among sites. This finding has several implications for trait-based plant ecology. First, although functional traits are defined as individual-level traits that affect performance and fitness components (Violle *et al.* 2007), the adaptive nature of many commonly measured ‘functional’ traits often remains an untested assumption, with recurrent calls for its validation (Ackerly *et al.* 2000; Ackerly and Monson 2003; Shipley *et al.* 2016; Salguero-Gómez *et al.* 2018; Swenson *et al.* 2020). Our findings that our ten study traits were associated with growth performance of black spruce in at least one site thus builds on emerging evidence that commonly measured functional traits indeed affect performance and fitness components (Geber and Griffen 2003; Caruso *et al.* 2020). Second, most selection gradients were for trait combinations, which indicates that a given trait value is only adaptive when it occurs in combination with another trait value. A corollary is that measuring many traits was necessary to detect selection. These findings thus suggest that assessing the individual effects of few traits could be one reason why many studies fail to find relationships between traits and individual performance or demographic rates. For examples, studies examined the effects of three to five traits on individual growth rate (Adler *et al.* 2014; Paine *et al.* 2015) and population demographics (García Criado *et al.* 2023) found no or weak relationships. Third, our finding that traits under selection differed across sites indicates that natural selection differs among environments. This supports previous conclusions that the environment needs to be considered at a sufficiently detailed spatial scale to properly detect relationships between traits and performance (Shipley *et al.* 2016; Swenson *et al.* 2020). Lastly, the fact that only some traits associated with a given function were under selection at a site suggests that selection may act a specific aspect of that physiological function. This implies that detecting selection on a specific function in a species may require measuring multiple traits that represent different aspects of that function. For example, in Petawawa we detected selection for high water use efficiency, but not for other traits directly affecting water use (such as stomatal density) or drought tolerance (such as LMA). This is unfortunate as structural traits such as LMA are much faster and inexpensive to measure than intrinsic WUE estimated by stable carbon isotope composition. Characterizing the ecological strategies of plants by measuring a few common traits is a goal of trait-based ecology (Westoby *et al.* 2002; Pérez-Harguindeguy *et al.* 2013). However, the need for a detailed phenotypic assessment in this intraspecific-scale study suggests that meeting this goal may be limited to studies with broad phylogenetic and spatial scopes, as traits may only become correlated into trait axes when they span a large range of values (Funk and Cornwell 2013).

TRAITS SELECTED FOR AT THE WARMER AND DRIER SITE

The strength of selection for two trait combinations CN:HV and CN:WUE increased in warmest and driest sites (Petawawa, Figure 3). The trees growing best in Petawawa thus had a combination of high WUE, HV, and CN. To the degree that these traits are heritable, and that total height growth is correlated with fitness, our results suggest that black spruce populations may evolve these trait combinations in response to selection pressures exerted by the warming and drying change. High intrinsic water use efficiency indicates a conservative water-use, with many tree species showing higher WUE in drought-adapted populations (Hajek *et al.* 2016; Rosas *et al.* 2019; Ahrens *et al.* 2020; Csilléry *et al.* 2020; Anderegg *et al.* 2021; Rabarijaona *et al.* 2022; Lochin *et al.* 2024), or plastic increases in WUE in response to drought conditions (Craven *et al.* 2013; Limousin *et al.* 2015; Forner *et al.* 2018; Schimpl *et al.* 2019). A high HV indicates a superior hydraulic supply capacity per leaf area. In our study, high HV resulted mainly from the presence of fewer needles because variation in HV in Petawawa was more strongly correlated with total needle biomass ($r = -0.79$, $p \leq 0.0001$) than branch diameter ($r = -0.49$, $p \leq 0.0001$) and total needle biomass was more variable than branch diameter (CV = 71% and 29% respectively). Lower needle biomass could result either from lower production or from loss of needle during drought events. High Huber values have been found to be associated with drought conditions in many other species (Mencuccini and Grace 1995; Li *et al.* 2019; Rosas *et al.* 2019; Mencuccini *et al.* 2019; Anderegg *et al.* 2021). Fewer needles may also be beneficial under water limitation by decreasing the need for transpirational cooling as a means of thermoregulation. High CN reflects relatively higher investment in structural support than metabolic processes. Collectively, these findings indicate that fewer water- and resource-use conservative needles is a beneficial strategy for black spruce in warm, dry environments. In this experiment, Petawawa was the warmest and driest site, such that we cannot separate adaptation to heat from adaptation to drought. Nonetheless, the three traits under climate-consistent selection are more consistent with adaptation to drought, which is consistent with literature showing that black spruce are water-limited in the southern margin of its range (Sniderhan *et al.* 2021; Chagnon *et al.* 2022).

A caveat inherent to the experimental design is that the initial tree spacing was higher in Chibougamau than the other two sites. This factor thus covaries with the climate gradient. The wider spacing likely decreased resource competition among individuals and decreased mortality. Indeed, when the provenance trials were established, tree spacing was adjusted at each site to maximize tree growth (Morgenstern and Kokocinski 1976). Differences in initial spacing are likely to have limited effect on the traits measured here because we sampled light-exposed needles in all sites. Further, the traits experiencing significant clinal shift in selection across sites defined trees with few needles with conservative water and resource use. This is consistent with selection from water stress and heat stress, but not from limited light availability. Nonetheless, since we cannot rule out this possible confounding factor, we refer to this set of traits as traits under climate-consistent selection.

WEAK PHENOTYPIC INTEGRATION WILL HAVE LITTLE EFFECT ON RESPONSE TO SELECTION

Our results suggest that response to selection for the *P. mariana* provenances under study is largely unconstrained by phenotypic integration. Not only is the overall strength of trait integration weak, as shown by the trait correlation matrix (Table S4) and the PCAs within sites (Fig. S2), but only two of the seven provenances showed significant intra-provenance trait correlations for one of the two trait combinations under climate-consistent selection (Figure 4). The two trait correlations were largely perpendicular to the direction of selection, indicating that phenotypic integration would hinder evolutionary response to selection. However, given the rarity of these instances overall (only 2 out of 21), phenotypic integration is unlikely to meaningfully affect the response to selection of *P. mariana* growing in warmer climates. Over evolutionary time, given unknown future selective forces, low integration can be beneficial as is provides more flexibility for a species' phenotype to respond to selection. A corollary to the weak trait integration in all provenances is that their integration did not differ. Thus, except for the Matagami (6855) and Rocky Mtn. House II (6979) provenances which are expected to evolve more slowly than the five others in response to selection for high CHL:HV, none of the studied provenances showed a particularly high potential for adaptive evolution in the face of warming climates. This is consistent with Robert *et al.* (2024) who report that the productivity of all populations will likely decrease by the end of the century. Provenance 6979 contains the largest proportion of Western genetic lineage, which has been found to contribute to reduced population productivity in some common gardens (Girardin *et al.* 2021). In contrast, the other population exhibiting a pattern of trait integration (provenance 6855), is one of five with a high proportion of the Central genetic lineage. Thus, there is no clear signal of climate refugia lineage in the intrapopulation integration of CHL and HV. As we only studied seven provenances, it remains possible that unstudied populations would have a higher adaptive potential. However, given the absence of correlations for most traits under selection in most of the populations, our findings suggest that trait integration is not a main factor of concerns when identifying black spruce provenances best adapted to future climate.

INSUFFICIENT PLASTIC RESPONSE TO MAINTAIN PERFORMANCE AT THE SITE WITH THE WARMEST AND DRIEST CLIMATE

Adaptive plasticity can help a population maintain high performance in changing environments (Bradshaw 1965; Pigliucci 2001; Whitman and Agrawal 2009). Similar to other studies, we found mixed results, with both adaptive and maladaptive plastic responses to the spatial climate gradient (Caruso *et al.* 2006; Ramírez-Valiente *et al.* 2021). The plastic increase in WUE across the spatial climate gradient was adaptive, consistent with a study reporting a temporal increase in WUE in the southern, water-limited part of the black spruce range (Sniderhan *et al.* 2021), and with studies in other species reporting plastic shifts in WUE with water limitation (Craven *et al.* 2013; Limousin *et al.* 2015; Forner *et al.* 2018; Schimpl *et al.* 2019). In contrast, the plastic decrease of CN in warmer sites is in the opposite direction to selection, which could indicate a maladaptive plastic response of CN to warming. However, since CN is also affected by the amount of available nitrogen (Lambers *et al.* 2008), trait differences among sites might also be driven by soil differences. Lastly, Huber value did not show a plastic increase along the climate gradient but instead showed a humped response for reasons that elude us, as no disturbance has been documented at Chapleau (the site with intermediate climate) in the last 5 years. Leaf shedding in response to drought has been documented in conifers (Kouki and Hokkanen 1992; Żytkowiak *et al.* 2005; Nadal-Sala *et al.* 2021), but the severity of the water limitation necessary to trigger this mechanism is unclear. Alternatively, trees growing under warmer and drier climates may simply produce branches with less foliage. Since the plastic response of traits under selection show a combination of adaptive and maladaptive patterns and that selection for both trait combinations are of similar strength, it is unclear from this specific set of traits whether the net effect of plasticity on height growth rate is positive or negative. If findings from this spatial climate gradient are informative of temporal trends, the abundance of black spruce will likely decline at its southern edge as a result of further climate warming. This is consistent with expected declines in growth and survival in the more water-limited portion of the black spruce range (D'Orangeville *et al.* 2018; Sniderhan *et al.* 2021; Chagnon *et al.* 2022).

CONCLUSIONS

In this study we combined classical selection gradient analyses and used black spruce provenance trials established along a spatial climate gradient to assess which phenotypic traits increased height growth performance in warmer and drier climates. Provenance trials provide more accurate assessment of phenotypic selection from climate in natural settings than short-term studies performed under greenhouse conditions (Poorter *et al.* 2016). Selection gradient analysis has rarely been used to assess phenotypic traits under selection from climate change in trees (Warwell and Shaw 2018, 2019; Ramírez-Valiente *et al.* 2021), and to our knowledge this is the first study to do so for ecophysiological traits in mature trees in natural settings along a climate gradient. This work thus demonstrates the value of research at the intersection of functional ecology and evolutionary ecology to fill pressing knowledge gaps on tree response to climate change (Lindner *et al.* 2010; Alberto *et al.* 2013; Laughlin and Messier 2015; Royer-Tardif *et al.* 2021). Indeed, species can use three mechanisms to avoid extinction – migration, adaptive plasticity and adaptive evolution – and to our knowledge most research has focused on migration and plasticity, leaving an important gap regarding the evolutionary response of tree phenotypes to climate change (Lindner *et al.* 2010; Royer-Tardif *et al.* 2021). In general, this approach can help assess whether some populations are better adapted to warmer climates by screening for provenances with (1) phenotypes better adapted to the environment (2) phenotypic integration facilitating adaptive evolution and (3) adaptive plasticity improving in situ performance. Specifically, this study found that in *P. mariana,* adaptive evolution to climate change may favor phenotypes with fewer needles that are conservative for water and resource use, that the limited intrapopulation trait integration should minimally impede adaptive evolution, and that the plastic response of black spruce to warmer and drier conditions may constrain the expression of an optimally adapted phenotype. Existing long-term field experiments offer considerable potential for future collaborative research on other ecologically and commercially valuable species. Important directions for future research are 1. to explore selection on root traits, because there is evidence they mediate response to warmer and drier climates (Laughlin *et al.* 2021; Messier *et al.* 2024), 2. selection in mixed forest communities, because many natural and managed forests are mixed (Alberto *et al.* 2013), and 3. separating selection due to high temperature from water deficit, because some populations within a species range experience water limitation whereas others do not (D'Orangeville *et al.* 2018; Sniderhan *et al.* 2021; Chagnon *et al.* 2022).

SUPPLEMENTARY INFORMATION

Supplementary information is available online at https://academic.oup.com/aob and consists of the following. Tables S1: Site characteristics. Table S2: Characteristics of the seven study provenances. Table S3: Traits and trait combinations under selection across the three study sites. Table S4: Pearson correlation coefficients among standardized study traits calculated across all sites. Figure S1: Geographical location of provenance trials and seven study provenances. Figures S2: PCA of trait values across sites and within each site. Figure S3: Selection gradients across sites for the trait and trait combinations under significant selection at Petawawa. Figure S4: Comparative reaction norms of provenances across sites for all the study traits. Supplementary Methods on needle cooling measurements.

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DATA AVAILABILITY

The trait and height growth rate data used in this study are openly available at https://doi.org/10.5281/zenodo.15571506.

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TABLES

Table 1. Traits measured, abbreviations, units, and associated physiological function(s). 'B' denotes traits measured at the branch level.

Trait name and acronym	**Units**	**Physiological Function**
Leaf dry matter content (LDMC)	g/g	Structural support
Twig specific density (TSD)	g/cm^3	Structural support
Leaf mass per area (LMA)	g/cm^2	Structural support, Photosynthesis
Carbon to Nitrogen ratio (CN) [B]	g/g	Structural support, Photosynthesis
Chlorophyll concentration (CHL)	mg/m^2	Photosynthesis
Water use efficiency (WUE) [B]	‰	Water use
Stomatal density (SD)	count/cm	Water use
Huber value (HV) [B]	mm^2/g	Water use
Needle cooling (NC)	ºC	Thermoregulation
Needle length (NL)	mm	Thermoregulation, Photosynthesis, Water use

FIGURE CAPTIONS

Figure 1. Mean annual temperature and total annual precipitation of the three provenance trials and seven provenances, averaged from 2011-2040. The grey cloud of points represents the climate envelope for the black spruce range in Canada. Data from McKenney *et al.* (2011) and Beaudoin *et al.* (2014).

Figure 2. Significant and marginally significant selection gradients (β and γ) for each of the three sites. Black horizontal lines represent the standard error. Asterisks indicate significance at $p \leq 0.05$ level. All other traits are marginally significant ($0.05 < p \leq 0.10$).

Figure 3. Selection gradients for CN:HV and WUE:HV were clinal across sites and statistically or marginally different between Petawawa and Chibougamau. Bars represent the 95% confidence intervals of each site. Significance level provided.

Figure 4. Significant intra-provenance trait correlations for CN:HV in 6855 (Magatami, QC) and 6979 (Rocky Mtn. House II, AB).

Figure 5. Comparative reaction norms of provenances across sites for the 3 traits under climate-consistent selection. Black crosses show site means. For a given trait, sites with different letters indicate significantly different means ($p < 0.05$). See table S1 for provenance information.

FIGURES

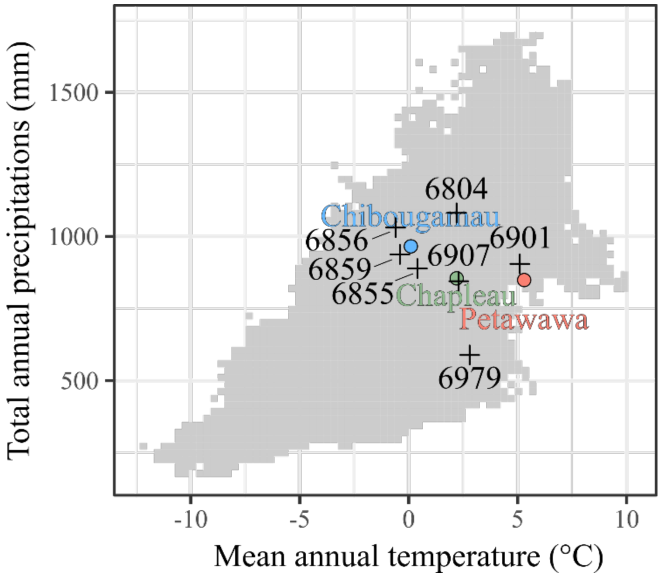


Figure 1.

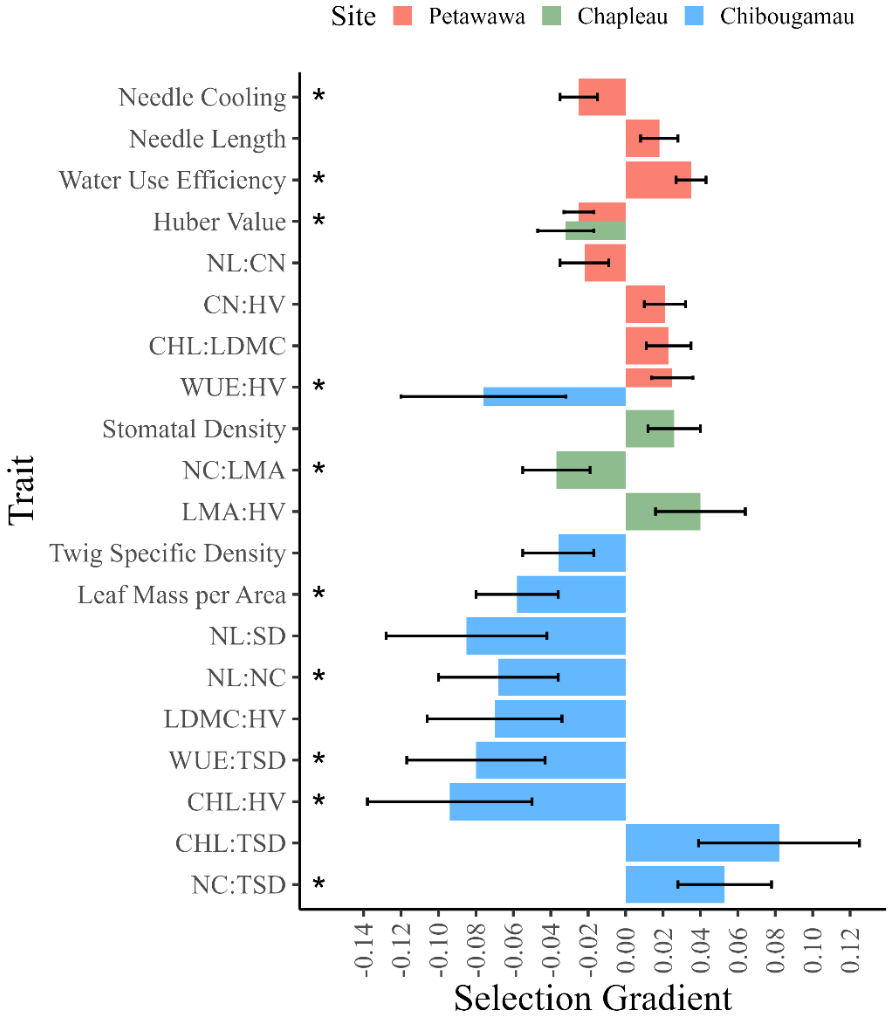

Site
Petawawa
Chapleau
Chibougamau
Trait
Needle Cooling *
Needle Length
Water Use Efficiency *
Huber Value *
NL:CN
CN:HV
CHL:LDMC
WUE:HV *
Stomatal Density
NC:LMA *
LMA:HV
Twig Specific Density
Leaf Mass per Area *
NL:SD
NL:NC *
LDMC:HV
WUE:TSD *
CHL:HV *
CHL:TSD
NC:TSD *
-0.14
-0.12
-0.10
-0.08
-0.06
-0.04
-0.02
0.00
0.02
0.04
0.06
0.08
0.10
0.12
Selection Gradient

Figure 2.

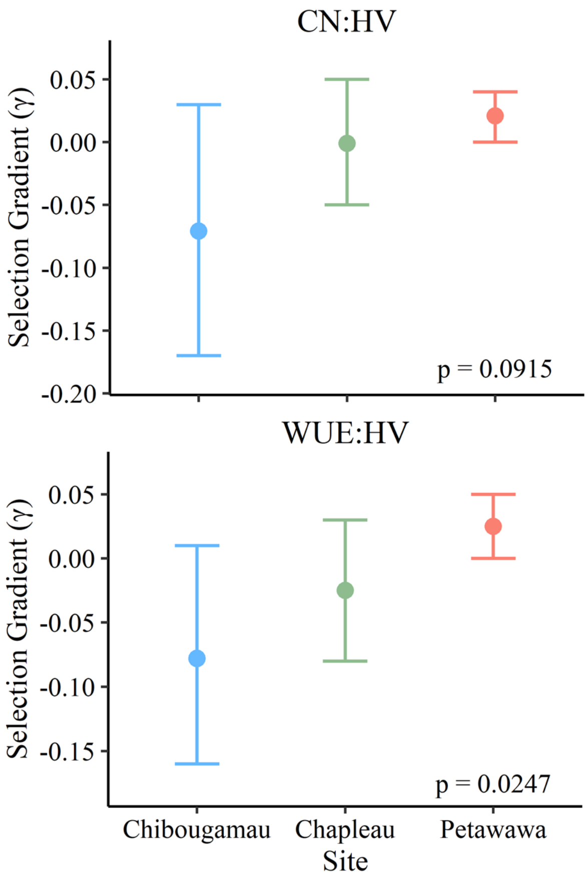

CN:HV
Selection Gradient (γ)
0.05
0.00
-0.05
-0.10
-0.15
-0.20
p = 0.0915
WUE:HV
Selection Gradient (γ)
0.05
0.00
-0.05
-0.10
-0.15
p = 0.0247
Chibougamau
Chapleau
Petawawa
Site

Figure 3.

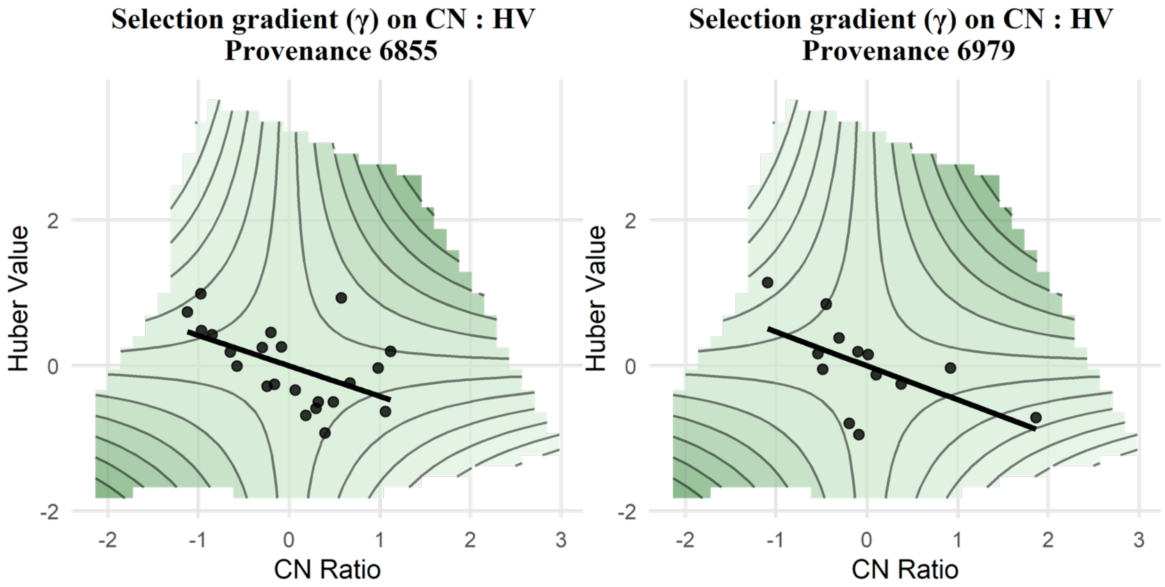
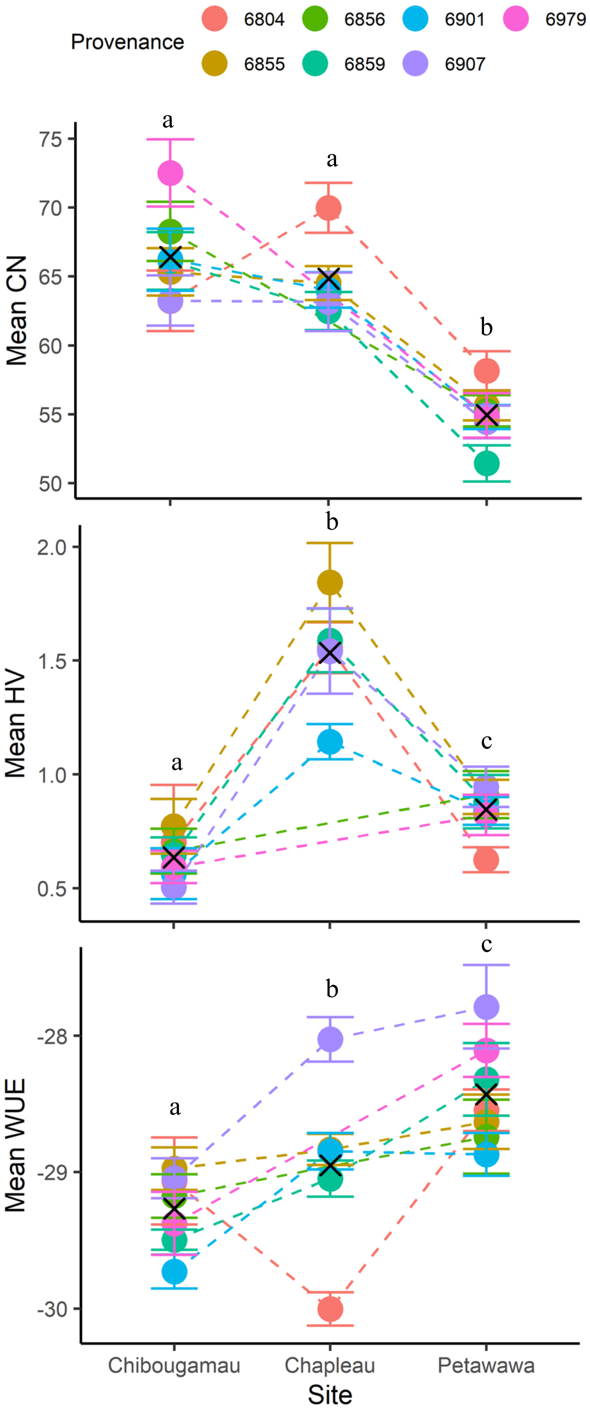

Selection gradient (γ) on CN : HV
Provenance 6855
Huber Value
CN Ratio
2
0
-2
-2
-1
0
1
2
3
Selection gradient (γ) on CN : HV
Provenance 6979
Huber Value
CN Ratio
2
0
-2
-2
-1
0
1
2
3

Figure 4.



944

945 Figure 5.

SUPPLEMENTAL INFORMATION

SUPPLEMENTARY TABLES AND FIGURES

Table S1. Site characteristics for Petawawa, Chapleau and Chibougamau. The climate moisture index (CMI) was retrieved from BioSIM (Fortin *et al.* 2022) and averaged from 1981-2010. The growing season spans the months of May to September, inclusively. MAT and MAP data retrieved from McKenney *et al.* (2011), and averaged from 1981-2010. Soil descriptions from Morgenstern and Kokocinski (1976) and Keable (1978).

Characteristic	**Petawawa**	**Chapleau**	**Chibougamau**
Mean annual temperature (ºC)	5.3	2.2	0.1
Total annual precipitation (mm)	849	855	966
Climate moisture index, annual and for the growing season	2.5; -0.8	3.1; 0.1	4.9; 3.1
Water holding capacity (%)	77.7	78.1	62.4
Spacing between trees (m)	1.8 x 1.8	1.8 x 1.8	2.4 x 3
Experiment number	353-H-5	353-H-4	353-B-3
Soil description	dry to moist, medium to fine sand	dry to fresh, shallow to moderately deep, medium to fine and silty acidic sand	shallow granite till and sand

Table S2. Characteristics of the seven study provenances. Latitude and Longitude are reported in decimal degrees. Elevation are reported in m above sea level. Mean Annual temperature (MAT) are reported in degrees Celsius. MAT: Mean Annual Temperature (° C). TAP: Total annual precipitation (mm). CMI: community moisture index reported annually and for the growing season (May to September). MAT and MAP data retrieved from McKenney *et al.* (2011), and averaged from 1981-2010. The W, C and E give the fraction of the provenance's genotype from the Western, Central and Eastern climate refugia lineages, respectively (Girardin *et al.* 2021).

No.	**Location**	**Long.**	**Lat.**	**Elev.**	**MAT**	**TAP**	**CMI**	**W**	**C**	**E**
6804	Roddickton, NL	50.90	-56.10	51	2.2	1082	6.8; 3.8	0.01	0.24	0.75
6855	Matagami, QC	49.62	-77.75	267	0.4	889	3.9; 2.0	0.02	0.70	0.29
6856	Manicouagan, QC	50.67	-68.77	437	-0.6	1031	4.6; 2.4	0.02	0.71	0.28
6859	Parc Mistassini, QC	50.45	-73.63	400	-0.4	938	4.7; 3.0	0.01	0.70	0.28
6901	Bancroft, ON	45.17	-77.17	279	5.1	905	3.1; -0.7	0.02	0.73	0.25
6907	Timmins, ON	48.53	-81.42	277	2.3	845	3.1; -0.1	0.01	0.88	0.11
6979	Rocky Mtn. House II, AB	52.37	-115.25	1125	2.8	589	1.2; 0.9	0.82	0.12	0.06

Table S3. Traits and trait combinations significantly ($p \leq 0.05$) and marginally significantly ($0.05 \leq p \leq 0.10$) under selection across the three study sites. CI = confidence intervals.

Trait	β or γ	p- value	CI	Std. Error	Site
Needle length	0.018	0.07	0,0.04	0.01	Petawawa
Needle cooling	-0.025	0.01	-0.04,-0.01	0.01	Petawawa
Water use efficiency	0.035	3.1E-5	0.02,0.05	0.008	Petawawa
Huber value	-0.025	3.6E-3	-0.04,-0.01	0.008	Petawawa
CHL:LDMC	0.023	0.06	0,0.05	0.012	Petawawa
NL:CN	-0.022	0.10	-0.05,0	0.013	Petawawa
WUE:HV	0.025	0.03	0,0.05	0.011	Petawawa
CN:HV	0.021	0.06	0,0.04	0.011	Petawawa
Huber value	-0.032	0.03	-0.06,0	0.015	Chapleau
Stomatal density	0.026	0.06	0,0.05	0.014	Chapleau
NC:LMA	-0.037	0.04	-0.07,0	0.018	Chapleau
LMA:HV	0.04	0.10	-0.01,0.09	0.024	Chapleau
Leaf mass per area	-0.058	0.01	-0.1,-0.02	0.022	Chibougamau
Twig specific density	-0.036	0.07	-0.07,0	0.019	Chibougamau
CHL:HV	-0.091	0.04	-0.18,0	0.045	Chibougamau
CHL:TSD	0.080	0.07	-0.01,0.16	0.043	Chibougamau
NL:NC	-0.067	0.04	-0.13,0	0.032	Chibougamau
NL:SD	-0.086	0.05	-0.17,0	0.043	Chibougamau
NC:TSD	0.054	0.03	0.01,0.1	0.025	Chibougamau
LDMC:HV	-0.066	0.08	-0.14,0.01	0.037	Chibougamau
WUE:HV	-0.078	0.08	-0.16,0.01	0.044	Chibougamau
WUE:TSD	-0.079	0.04	-0.15,-0.01	0.037	Chibougamau

Table S4. Pearson correlation coefficients (r) among standardized study traits calculated across all sites, color-coded to the strength or the correlation coefficient to highlight the strongest relationships (red = negative, blue = positive).

	CHL	NL	NC	LDMC	LMA	WUE	CN	HV	SD	TSD
Chlorophyll concentration (CHL)	1.00	0.29	-0.07	-0.13	-0.12	-0.11	-0.36	-0.12	-0.09	-0.06
Needle Length (NL)	0.29	1.00	0.02	-0.10	0.01	-0.05	-0.16	-0.18	-0.17	-0.04
Needle Cooling (NC)	-0.07	0.02	1.00	-0.05	-0.03	-0.05	0.09	0.02	-0.05	0.13
Lead Dry Matter Content (LDMC)	-0.13	-0.10	-0.05	1.00	0.42	0.04	0.20	0.03	0.08	0.13
Leaf Mass per Area (LMA)	-0.12	0.01	-0.03	0.42	1.00	0.09	0.30	0.03	0.26	0.03
Water User Efficiency (WUE)	-0.11	-0.05	-0.05	0.04	0.09	1.00	-0.12	0.01	0.01	-0.12
C:N ratio (CN)	-0.36	-0.16	0.09	0.20	0.30	-0.12	1.00	0.23	0.09	0.14
Huber Value (HV)	-0.12	-0.18	0.02	0.03	0.03	0.01	0.23	1.00	0.01	0.16
Stomatal Density (SD)	-0.09	-0.17	-0.05	0.08	0.26	0.01	0.09	0.01	1.00	0.03
Twig Specific Density (TSD)	-0.06	-0.04	0.13	0.13	0.03	-0.12	0.14	0.16	0.03	1.00

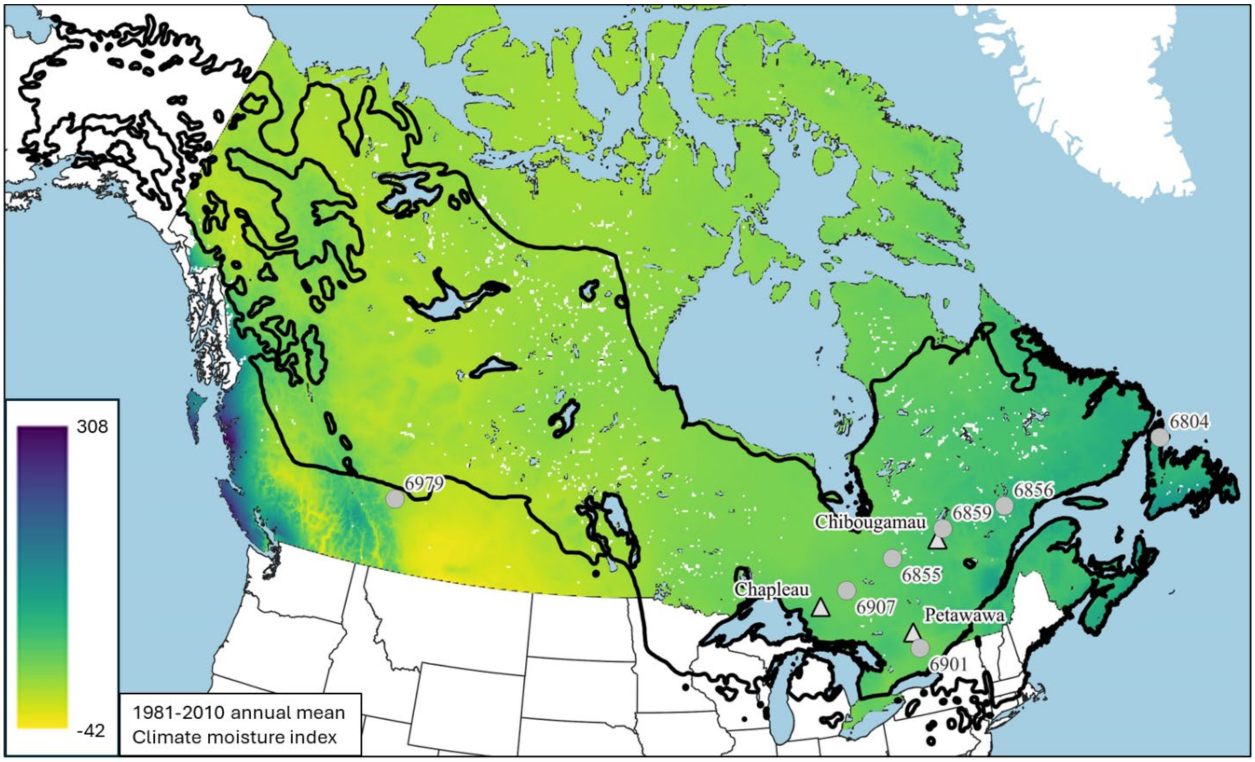
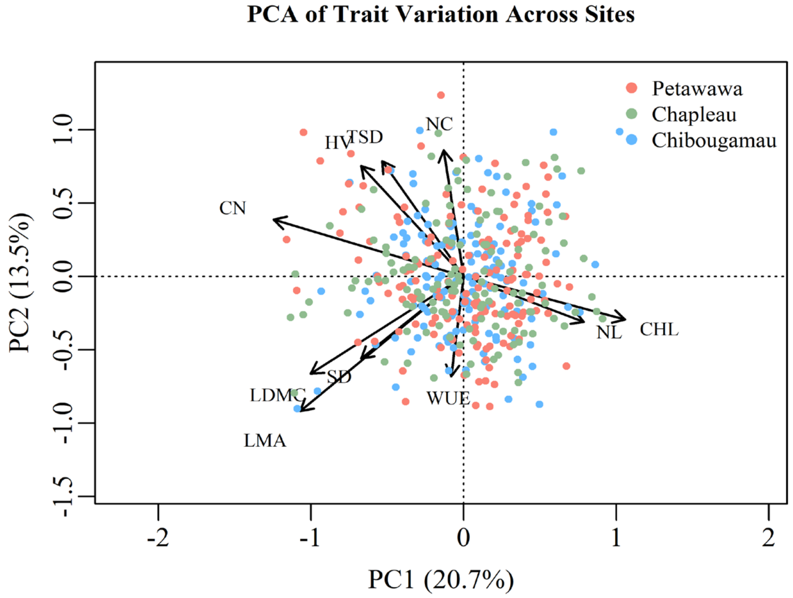


Figure S1. Geographical location of each provenance trial (grey triangles) and provenance (grey circles) used in this study. The black spruce range as define by Little (1971) is outlined in black, and the colored background represents the average annual climate moisture index (CMI) between 1981-2010. NAD83(CSRS) projection from statistics Canada was used ("NAD83(CSRS) Updates" 2024).

Panel A



Panel B

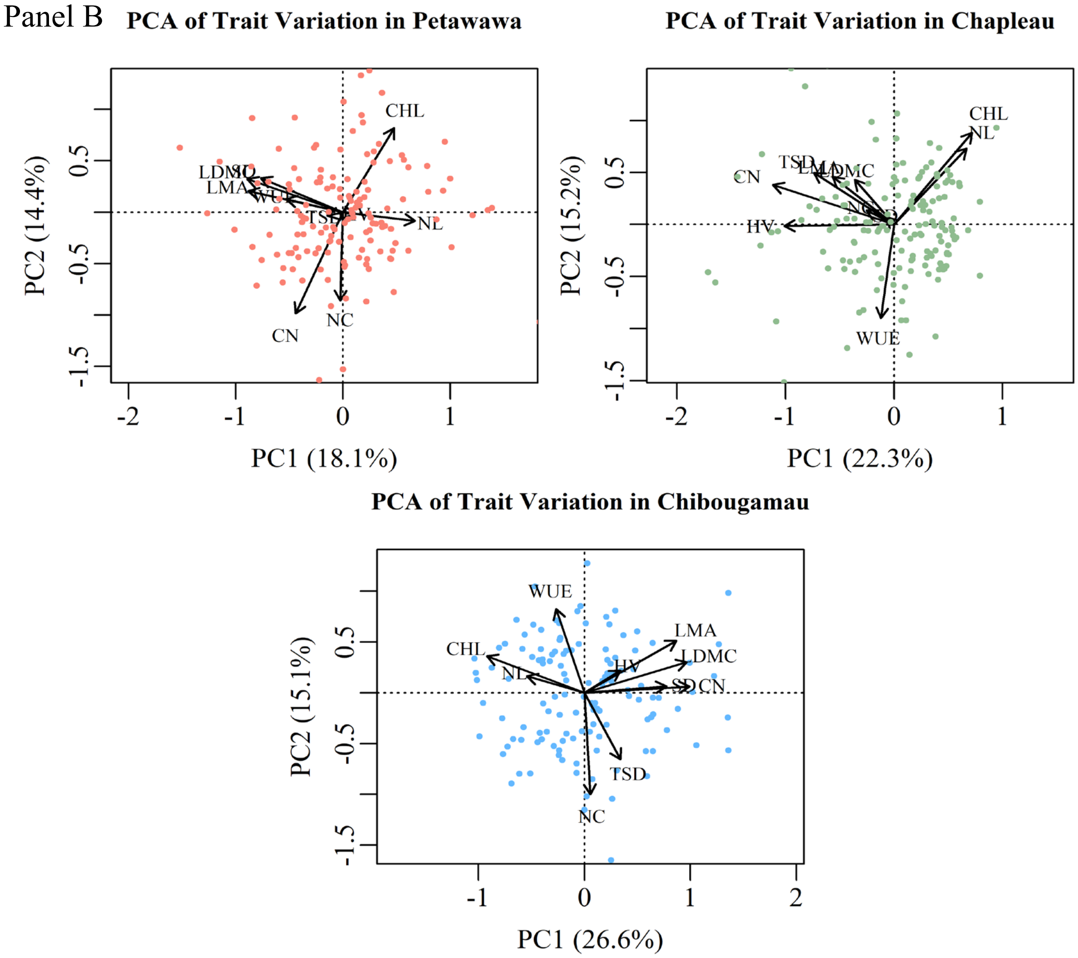


Figure S2. PCA of trait values. Panel A: Analysis on individuals from all sites. Panel B: Analyses on individuals at each site.

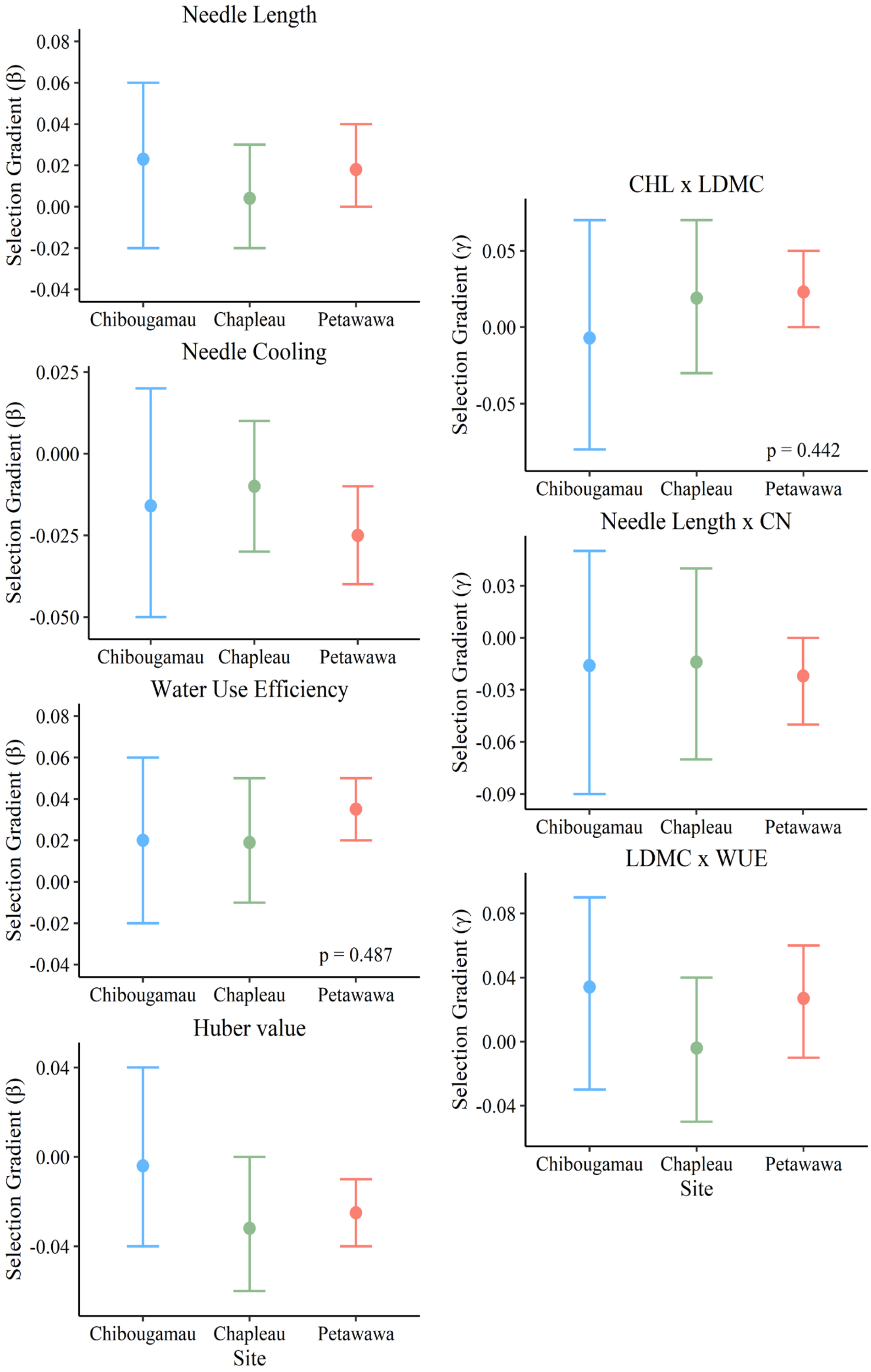


Figure S3. Selection gradients across sites for the trait and trait combinations under significant selection at Petawawa. P-values for t-tests are only shown for clinal selection gradients with climate.

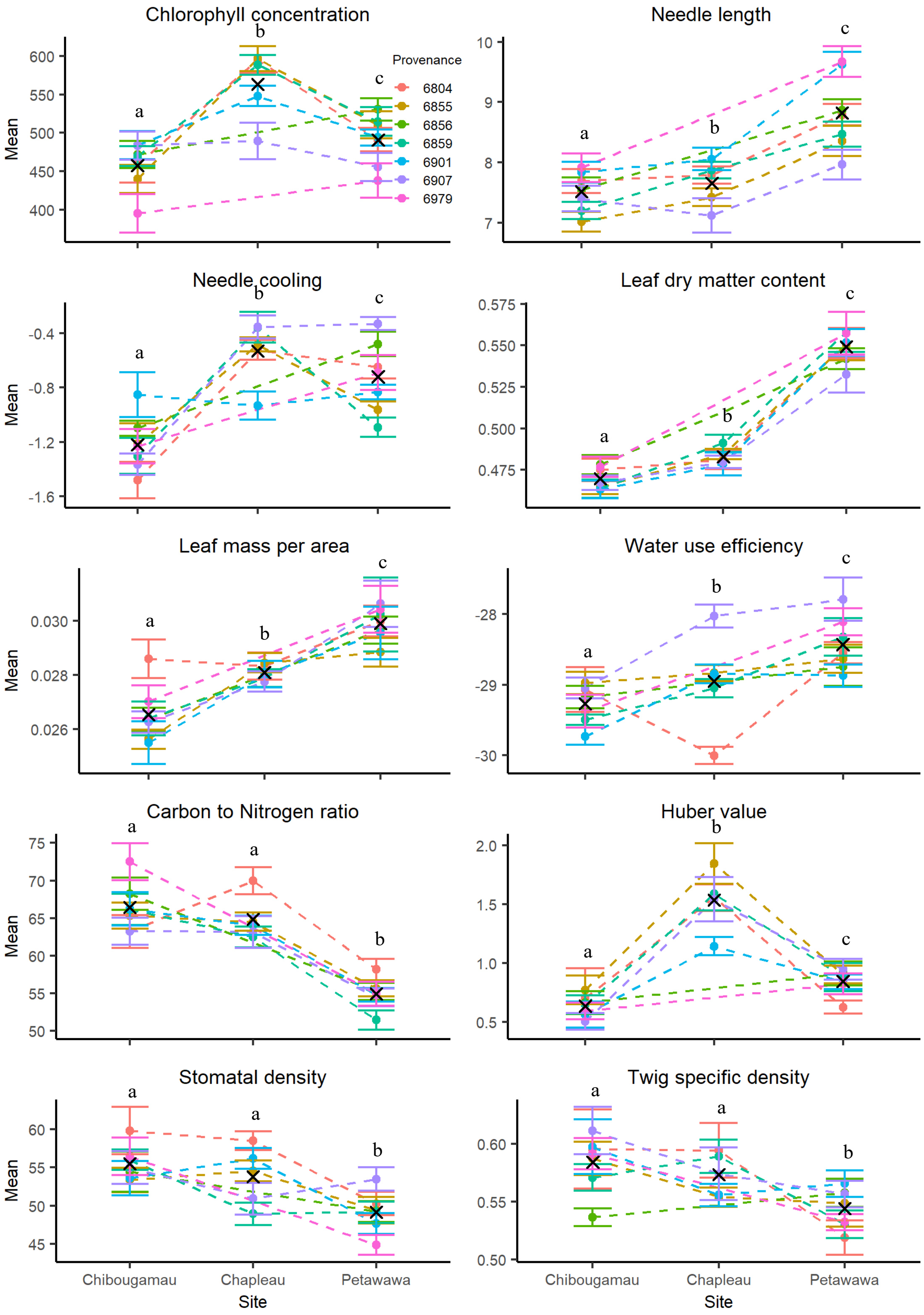


Figure S4. Comparative reaction norms of provenances across sites for all the study traits. Black crosses show site means. For a given trait, sites with different letters indicate significantly different means ($p < 0.05$). See Table 1 for units and table S1 for provenance information.

SUPPLEMENTARY METHODS

Needle Cooling

The readings from the thermal image were calibrated against the readings from a custom-built electronic thermometer with a TMP117 sensor with an accuracy of ± 0.1°C. The sensor plate of the electronic thermometer was placed inside each thermal image and the true temperature was manually recorded when each thermal picture was taken. Any difference between the true temperature and the temperature reading on the sensor plate from the thermal camera was used to correct the temperature readings of the needles within each thermal image.