

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

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Running title: Phenotypic traits are under selection from climate in black spruce

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1 **ABSTRACT**

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

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

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

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

26
27 **Keywords:** *Picea mariana* (Mill.) B.S.P.; climate change; common gardens; functional traits;
28 performance landscape; phenotypic integration; provenance trials; selection gradient analysis,
29 Huber value; water use efficiency; leaf nitrogen to carbon ratio

30 INTRODUCTION

31 As climates shift under global change, the growth, productivity and long-term persistence of tree
32 species are at risk (Allen *et al.* 2010; Choat *et al.* 2012). This threat is particularly urgent for
33 boreal tree species, which are experiencing climate change more rapidly than those at lower
34 latitudes (Peng *et al.* 2011; Aubin *et al.* 2018; Chagnon *et al.* 2022). Drought-induced tree
35 mortality has increased under climate change (Allen *et al.* 2010; Choat *et al.* 2012), suggesting
36 that plasticity has not been sufficient to keep pace with climate change (Malcolm *et al.* 2002;
37 Iverson *et al.* 2004; Brecka *et al.* 2018) and projected rates of migration are expected to be
38 insufficient (Boisvert-Marsh *et al.* 2022). Mismatches between tree species' distribution and
39 climatic niche are becoming increasingly important (Gray and Hamann 2013; Lapenis *et al.*
40 2022).

41
42 In the face of climate change, tree species can avoid extinction via migration, adaptive plasticity,
43 or adaptive evolution (Aitken *et al.* 2008). Mechanisms of adaptation increase the frequency of
44 phenotypic traits that enhance fitness in a given environment. Adaptation can occur either
45 through adaptive plasticity (where environmentally driven changes in phenotype of a given
46 genotype improve fitness), or through adaptive evolution (where heritable traits that confer a
47 fitness advantage increase in frequency across generations as a result of natural selection).
48 Although both mechanisms can contribute to population persistence, research on phenotypic
49 adaptation to climate change has predominantly focused on adaptive plasticity, and thus more
50 studies on adaptive evolution are needed to gain a more complete understanding of phenotypic
51 adaptation to climate change in trees (Lindner *et al.* 2010; Royer-Tardif *et al.* 2021). Indeed,
52 while much research has examined adaptive plasticity in populations locally adapted to warm
53 and dry environments (e.g. Blasini *et al.* 2021; Challis *et al.* 2022; Andrés-Hernández *et al.*
54 2023), we lack basic knowledge on what phenotypic traits are selected for (i.e. lead to high
55 fitness, fitness component, or performance) under warming climates in trees (Alberto *et al.*
56 2013). While research on natural selection on plant phenotypes (i.e. phenotypic selection) in
57 short-lived herbaceous species abounds (e.g. Etterson and Shaw 2001; Etterson 2004; Ludwig *et*
58 *al.* 2004; Donovan *et al.* 2007, 2009), comparatively little research has quantified phenotypic
59 selection on traits of forest tree species (e.g. Castro 2006; De La Mata *et al.* 2017; Warwell and
60 Shaw 2018). Moreover, research on phenotypic selection in trees under warm or dry climates has

61 largely focused on seed traits and early growth stages (e.g. Ramírez-Valiente *et al.* 2021; Costa E
62 Silva *et al.* 2022, 2024).

63
64 To begin addressing this gap, we examine selection on ecophysiological traits from warming
65 climates in mature trees of a dominant boreal species, black spruce (*Picea mariana* (Mill.)
66 B.S.P.). Climate change is documented to affect black spruce growth (Sniderhan *et al.* 2021;
67 Chagnon *et al.* 2022). This generalist species, which is present within the entire range of the
68 North American boreal forest, occurs in a variety of habitats and soil types. It is common in
69 habitats with wet soils such as peat bogs and swamps but also grows well on clays, loams, well-
70 drained mineral soils and boulder pavements (Viereck and Johnston 1990). Black spruce
71 generally grows in cold climate with humid to dry subhumid moisture regime. It has low drought
72 and water-logging tolerance and is shallow rooted (Viereck and Johnston 1990; Niinemets and
73 Valladares 2006). The warmer and drier conditions brought upon by climate change in much of
74 its range are expected to lead to drought stress and growth declines in parts of its range (Girardin
75 *et al.* 2016; Aubin *et al.* 2018; D'Orangeville *et al.* 2018). Climate models forecast increases in
76 temperature in Canada (Swart *et al.* 2019; IPCC, 2018), leading to decreases in water availability
77 for equivalent precipitation due to increased evapotranspiration (Dai *et al.* 2004; Barnett *et al.*
78 2005; Cholet *et al.* 2022). Indeed, climate moisture index (CMI), the difference between annual
79 precipitation and potential evapotranspiration, is predicted to decrease across most of the boreal
80 forest except for eastern-most regions (Boucher *et al.* 2020). Further, warmer air temperatures
81 result in increases in atmospheric vapor pressure deficit (Ficklin and Novick 2017; Dai *et al.*
82 2018), which increases transpiration rates and can lead to soil water limitation and decreased
83 growth (Yuan *et al.* 2019; López *et al.* 2021). Warming from climate change has affected black
84 spruce growth unevenly across its range, promoting growth in the northern boreal forest, where
85 growth is temperature limited, while reducing growth in southern regions, where water becomes
86 limiting (Gamache and Payette 2004; Beck *et al.* 2011; Sniderhan *et al.* 2021; Chagnon *et al.*
87 2022). While black spruce's growth response to climate change is documented, the phenotypic
88 traits that sustain growth under warmer and drier conditions remain unknown.

89
90 To address this gap, we test which traits are under selection for high growth rate in natural black
91 spruce populations planted in provenance trials spanning a climate gradient. We expect that

92 warmer and drier climates may select for traits related to water use, carbon economics, structural
93 support and temperature regulation (Aubin *et al.* 2016; Boisvert-Marsh *et al.* 2020; Sniderhan *et*
94 *al.* 2021). In water-limited environments, traits promoting conservative water use and durable
95 leaf construction via higher investment in structural tissues are likely to increase performance
96 and be the targets of selection. Although the relationship between wood density and hydraulic
97 transport safety is complex (Chave *et al.* 2009; Hoffmann *et al.* 2011; Pratt and Jacobsen 2017),
98 higher wood density can be associated with higher xylem cavitation resistance (Hacke *et al.*
99 2001; Hoffmann *et al.* 2011), which can result in higher performance in water limited
100 environments (Cochard *et al.* 2007). Temperature controls metabolic rates and can impede
101 photosynthetic activity above 35 °C, with the threshold varying among species (Münchinger *et*
102 *al.* 2023). Boreal spruce species have shown photosynthetic inhibition above ca. 42 °C (Bigras
103 2000; Münchinger *et al.* 2023). Some research has shown that leaves can buffer variation in air
104 temperature to maintain leaf temperatures near their metabolic optimum (Michaletz *et al.* 2015).
105 As such, we expect enhanced capacity for leaf cooling (i.e., thermoregulation) to confer higher
106 performance and thus be a target of selection in warmer climates.

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

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

127 METHODS

128 STUDY SITES

129 We studied 425 trees from three sites belonging to a set of *P. mariana* (Mill.) B.S.P. range-wide
130 provenance trials established in 1974 by the Canadian Forest service (Morgenstern and
131 Kokocinski 1976; Keable 1978). These provenance trials were established for forestry purposes
132 to determine which of various provenances (populations of different origin from the across the
133 species' range) grew the best at various geographic location. We selected three provenance trials
134 that shared seven provenances and covered a climate gradient, both in terms of temperature and
135 water availability (Supplementary Table S1, Figure 1). The warmest and driest site was located
136 in Petawawa Research Forest located near Chalk River, ON. A site with an intermediate climate
137 was located near Chapleau, and the coldest and wettest site was located near Chibougamau QC.
138 During establishment, each site was divided into three to six replicate blocks. Within each block,
139 16 trees per provenance were planted in grids of 4x4 trees, forming small plots (Morgenstern and
140 Kokocinski 1976). Since the range-wide cooperative black spruce provenance program was
141 designed to identify the optimal genetic stock for each site, tree spacing was adjusted among
142 sites to maximize productivity according to local site conditions. The sites also differ in factors
143 other than climate, such as soil texture, depth, and water holding capacity, and planting density
144 (Table S1).

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

153 and 6907 Timmins, ON. Two provenances were common to Petawawa and Chibougamau only:
154 6856 Manicouagan, QC and 6979 Rocky Mtn. House II, AB.

155 << Figure 1. here >>

156 **PERFORMANCE METRICS**

157 We used relative height growth rate of individual trees as a performance metric. Growth rate has
158 been shown to affect survival and reproduction, two fitness components (Farris and Lechowicz
159 1990; Arntz *et al.* 1998) and is broadly used as a performance metric in selection gradient
160 analyses (Geber and Griffen 2003; Caruso *et al.* 2020) and in forestry (Younginger *et al.* 2017).
161 Vegetative growth rate is also the only accessible indicator of performance in mature trees in
162 natural setting. Here, by using the phenotypic selection analyses with growth rate as a
163 performance metric, we identify the ecophysiological traits conferring a high aboveground
164 growth rate in mature black spruce. The height of each individual tree was measured in 2022 in
165 Petawawa, 2023 in Chapleau, and 2016 in Chibougamau and relative height growth rate since
166 planting was calculated by dividing tree height by the age at the time of measurement (RGR;
167 m/yr). In 2023, survival of all trees of the study provenances was measured at each site.

168 **SAMPLE COLLECTION**

169 In July and August 2022 and 2023, one branch per tree was sampled from at least three blocks at
170 each site. To standardize light exposure, in the morning of each sampling day, we collected a
171 healthy, sun-exposed, lateral branch from the top third of the tree crown using a 13.5m HV-245
172 telescopic pole pruner. It was then trimmed to contain the 0 to 5-year-old needles and an
173 additional length of branch which was placed in florist tubes filled with water to rehydrate them
174 prior to trait measurements. Branches were then placed in plastic bags with a damp paper towel
175 and stored in the dark in a cooler on ice until measurements in the afternoon (Garnier *et al.*
176 2001). On average, 27 trees per provenance per site (range of 15-41) were sampled, resulting in a
177 total of 143 trees sampled at Petawawa, 156 at Chapleau, and 132 at Chibougamau.

178 **TRAIT MEASUREMENTS**

179 We measured 10 traits associated with structural support, photosynthesis, water use, and
180 thermoregulation and known to be associated with plant response to temperature or soil moisture
181 deficit (Table 1). To characterize investment in structural support at the needle and branch levels,
182 leaf dry matter content (LDMC; g/g) and twig specific density (TSD; g/cm³) were measured.

183 LDMC, the ratio of dry to water-saturated mass, reflects needle resource investment into
184 structural support versus metabolic processes (Shipley *et al.* 2006) and has been shown to
185 increase with drought (Anderegg *et al.* 2021). TSD affects mechanical support, storage and
186 hydraulics (Pratt and Jacobsen 2017) and closely related traits such as wood density can increase
187 with water limitation (Anderegg *et al.* 2021). Leaf mass per area (LMA, g/cm²) reflects the
188 carbon cost of light interception and in global interspecific comparisons is an important indicator
189 of plant carbon-use strategies (Westoby *et al.* 2002; Wright *et al.* 2004), with high values
190 indicating high structural support and needle persistence, and low values indicating high
191 photosynthetic rate. Among species, high LMA values has also been shown to correlate with
192 tolerance to heat stress (Münchinger *et al.* 2023) and drought stress (Poorter *et al.* 2009; Scoffoni
193 *et al.* 2014; Rosas *et al.* 2019). Carbon to Nitrogen ratio (CN; g g⁻¹) reflects the nutrient
194 concentration in the leaves. High nitrogen concentration (low CN) is largely driven by high
195 concentration of RUBISCO and is therefore correlated with high photosynthetic rate (Reich *et al.*
196 1999). Water use efficiency (WUE; ‰) was measured to characterize the grams of water lost per
197 unit of carbon fixed. We used carbon isotope ratio ($\delta^{13}\text{C}$) to estimate intrinsic water use
198 efficiency (Ma *et al.* 2023). During photosynthesis, ¹²CO₂ is preferentially fixed over ¹³CO₂
199 primarily because the enzyme RuBisCO discriminates against ¹³CO₂ and only binds it when
200 ¹²CO₂ is limited, which occurs when stomata are closed (Farquhar *et al.* 1982; Lambers *et al.*
201 2008). The ratio of ¹³C to ¹²C present in plant tissue, therefore reflects the amount of time leaves
202 spent with their stomata closed. $\delta^{13}\text{C}$ gives the ¹³C isotope composition relative to the primary
203 reference scale of Vienna Pee Dee Belemnite. Within species, $\delta^{13}\text{C}$ often increases with water
204 limitation (Limousin *et al.* 2015; Hajek *et al.* 2016; Rosas *et al.* 2019; Ahrens *et al.* 2020;
205 Csilléry *et al.* 2020; Anderegg *et al.* 2021; Rabarijaona *et al.* 2022; Lochin *et al.* 2024). Stomatal
206 density (SD; number/cm) affects leaf gas exchange and is thus a strong correlate of both
207 transpiration rate and photosynthetic rate (Lambers *et al.* 2008) and has been shown to vary
208 within species with drought (Aragón *et al.* 2023). Huber value (HV; mm/g), the ratio of sapwood
209 area to needle weight, characterizes water supply relative to water demand at the branch level
210 (Carter and White 2009). High Huber values typically indicate increased hydraulic efficiency
211 and thus increased drought avoidance (Mencuccini and Grace 1995; Mencuccini *et al.* 2019). It
212 has been shown to increase within species with increasing water limitation (Rosas *et al.* 2019;
213 Anderegg *et al.* 2021). To assess thermoregulation, needle cooling (NC; °C) was measured as the

214 difference between needle temperature and ambient temperature. Leaf cooling allows a leaf to
215 partly control its temperature and stay within the optimal temperature range for photosynthesis
216 (Michaletz *et al.* 2015). Chlorophyll concentration (CHL; mg/m²) was measured because it is
217 strongly correlated with photosynthetic capacity (Lambers *et al.* 2008). Needle length (NL; mm)
218 affects total light capture and water loss of the plant, as well as needle thermoregulation by
219 modifying the thickness of boundary layer and thus passive heat loss (Lambers *et al.* 2008).

220 << Table 1 >>

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

228
229 Two time-sensitive traits (CHL and NC) were measured indoors in the afternoon immediately
230 following sample collection and while the samples were still in the water-filled tubes. Branches
231 were brought indoors and taken outside the cooler for 30 minutes to acclimate to ambient air
232 temperature. Total chlorophyll content (CHL) was measured on the adaxial side of needle
233 bundles using a CCM-300 chlorophyll content meter (Opti-Sciences; Gitelson *et al.* 1999). Two
234 measurements per needle age class were taken, except when they differed by more than 10%, in
235 which case a third measurement was taken. NC was determined from needle and ambient
236 temperature measured using a TCAM-300 thermal camera (Infrared Camera INC, 2021), a high
237 accuracy temperature sensor (TMP117 High-Accuracy Digital Temperature Sensor) and
238 FlashImagePro software. NC values are positive when the needle is warmer than the ambient
239 temperature and negative when it is below, with more negative NC values indicating stronger
240 cooling.

241 The remaining eight traits were measured in the laboratory while branches were still in
242 the florist tubes, within 7 days of sampling. Two perpendicular diameter measurements (mm)
243 were taken at the base of the fourth year of growth using digital calipers (Series 500
244 ABSOLUTE Digimatic Caliper). HV was calculated as the average branch diameter divided by

245 the dry mass (g) of needles supported by the branch. Branches were then cut and separated into
246 twigs based on growth year identified from terminal bud scars. For each growth year, 12 to 15
247 intact, healthy, mature needles were removed for trait measurements. Needle fresh weight was
248 measured using analytical balances (AG104 Metler Toledo and XSR205 Metler Toledo). The
249 needle length (mm) and fresh needle area (cm²) were then determined from scans of 600 dpi
250 (STD4800 scanner and WinSeedle V.2020). Needle dry weights were taken after drying at 60 °C
251 to constant weight. LDMC was calculated as the ratio of dry to fresh weight of each set of
252 needles. LMA for each set was calculated by dividing the dry weight by total fresh area. The
253 total dry needle weight of each growth year was used to calculate abundance-weighted needle
254 trait values at the branch level. To determine TSD, three twigs of each growth year were
255 haphazardly sampled (excluding the largest and smallest sizes to obtain median values), their
256 fresh volume was determined by water displacement and then dried at 60 °C to constant weight.
257 TSD of each growth year was then calculated as twig dry mass (g) divided by twig fresh volume
258 (cm³). Finally, branch-level TSD were determined by abundance-weighting each year's TSD by
259 their relative dry weight.

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

271 STATISTICAL ANALYSES

272 All statistical analyses were conducted in R version 4.4.0 (R Core Team 2025). Two percent of
273 all data was missing due to lost samples or measurement errors (3% in Petawawa, <1% in
274 Chapleau, <1% 3% in Chibougamau). Missing values were imputed for each site from other
275 traits from linear mixed effect models, using the *lme()* function from the *nlme* package (Pinheiro

276 *et al.* 2025). The model with the highest predictive power for missing values were identified
277 using the *dredge()* function from the *MuMIn* package (Bartoń 2025). Data were only imputed
278 when the R^2 of the predictive model was > 0.6 . Otherwise, the missing values were left as NA.
279 After imputation $< 0.01\%$ of data was missing from each site, resulting in $n = 142$ trees in
280 Petawawa, $n = 151$ trees in Chapleau, and $n = 132$ trees in Chibougamau. Differences in survival
281 among sites and among provenances within sites were tested with one-way ANOVAs using
282 *aov()* from the *stats* package. Correlations among traits were described using *rcorr()* from *Hmisc*
283 package (Harrell 2025) and principal component analyses (PCA) using the *PCA()* of the
284 *FactoMineR* package (Lê *et al.* 2008). PCA were conducted on trait data across all sites as well
285 as within each site. The statistical significance of the principal component axes was tested using
286 *PCAsignificance()* from the *BiodiversityR* package (Kindt and Coe 2005), which compares the
287 amount of variance explained by each axis to the variance explained in broken-stick null models.

288 To assess selection at each site, we used the Lande-Arnold regression approach to
289 measure directional (β) and correlational (γ) selection gradients at each site (Lande and Arnold
290 1983). Here, the partial regression coefficients in multiple linear regressions of height RGR
291 against traits represent the strength and direction of selection. We standardized trait values within
292 each site to a mean of zero and a variance of 1 (z-transformation) and relativized RGR values
293 within each site by dividing individual RGR values by the mean RGR for that site (Conner and
294 Hartl 2004). Within each site we assessed β s from multiple regressions of RGRs on all 10 traits
295 with provenance as a random effect, and we assessed γ s from multiple regressions of RGRs on
296 all 10 traits, their 2-way interactions and provenance as a random effect (Lande and Arnold
297 1983). We used the *lme()* function from the *nlme* package (Pinheiro *et al.* 2025). We confirmed
298 the absence of multicollinearity by calculating the variance inflation factor (VIF) of the fixed
299 term in the mixed model, using *vif()* for the *car* package. All VIFs were below 2.3. Following
300 Zurr & Ieno (2016), we verified the data met model assumptions by examining diagnostic plots.

301 To identify which of the selection gradients in Petawawa might be caused by climate, we
302 tested for correlations between the selection gradients and the climate among site (Wade and
303 Kalisz 1990). We used two criteria: (1) the selection gradient showed clinal variation (i.e.
304 continuous gradual change with the ecological gradient) with climate among sites, and (2) the
305 selection gradients significantly differed between Petawawa and Chibougamau, the two sites at
306 opposite ends of the climate gradient (hereafter called ‘climate-consistent selection gradients’ for

307 brevity). We used the *tsum.test()* function from the *BSDA* package to perform a two-sample t-
308 tests from the coefficient estimates and their standard errors (Arnholt and Evans 2023). While
309 specific traits may be under selection from many factors at a given site, we interpret the clinal
310 changes among sites as primarily driven by differences in climate. Indeed, other than differences
311 in tree spacing, which we do not expect to impact the results (see discussion), we are not aware
312 of any clinal differences in the environment among those sites.

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

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

328 RESULTS

329 TRAITS UNDER SELECTION AT EACH SITE

330 All 10 traits measured were under directional or correlational selection in at least one site, for a
331 total of 21 significant or marginally significant selection gradients across the three sites (eight in
332 Petawawa, three in Chapleau and 10 in Chibougamau; Figure 2, Table S3). Of the 21 selection
333 gradients, 7 were for individual trait values and (directional selection, β) and 14 were for trait
334 combinations (correlational selection, γ). The traits and trait combinations under selection largely
335 differed among sites. Specifically, in Petawawa three traits under directional selection - increased
336 needle cooling, increased needle length and increased water use efficiency – and five trait

337 combinations were under correlational selection - Chlorophyll concentration : Leaf dry matter
338 content, Needle length : Carbon to Nitrogen ratio, Leaf dry matter content : Water use efficiency,
339 Water use efficiency : Huber value and Carbon to Nitrogen ratio : Huber value.

340 << Figure 2. here >>

341 **DIFFERENCES IN SELECTION ASSOCIATED WITH CLIMATE**

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

349 << Figure 3. here >>

350 **PHENOTYPIC INTEGRATION WITHIN PROVENANCES**

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

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

364 **TRAIT PLASTICITY**

365 All traits showed a significant plastic response across sites, with most provenances showing
366 similar patterns of trait variation among sites (Figures 5 and S4). Since results indicated climate-

367 consistent selection for positive interaction among CN:HV and WUE:HV, plastic increases in
368 CN, HV and WUE would indicate adaptive plasticity. Results show that water use efficiency
369 increased from the coldest to the warmest site, following the direction of selection ($p \leq 0.0001$).
370 In contrast, CN ratio decreased from the coldest to the warmest site ($p \leq 0.0001$). Huber value
371 showed a non-linear plastic response across the climate gradient, with highest values at Chapleau
372 (the site with intermediate climate, $p \leq 0.0001$, Figure 5). The plastic response of CN ratio and
373 Huber value to the spatial gradient thus do not track the direction of selection at the warmer,
374 drier site.

375 << Figure 5. Here >>

376 DISCUSSION

377 PHENOTYPIC TRAITS UNDER SELECTION DIFFERED AMONG SITES

378 We found that all ten ecophysiological traits studied here were under selection in one of the three
379 sites, whether individually or in combination with another trait, and that the traits under selection
380 differed among sites. This finding has several implications for trait-based plant ecology. First,
381 although functional traits are defined as individual-level traits that affect performance and fitness
382 components (Violle *et al.* 2007), the adaptive nature of many commonly measured ‘functional’
383 traits often remains an untested assumption, with recurrent calls for its validation (Ackerly *et al.*
384 2000; Ackerly and Monson 2003; Shipley *et al.* 2016; Salguero-Gómez *et al.* 2018; Swenson *et*
385 *al.* 2020). Our findings that our ten study traits were associated with growth performance of
386 black spruce in at least one site thus builds on emerging evidence that commonly measured
387 functional traits indeed affect performance and fitness components (Geber and Griffen 2003;
388 Caruso *et al.* 2020). Second, most selection gradients were for trait combinations, which
389 indicates that a given trait value is only adaptive when it occurs in combination with another trait
390 value. A corollary is that measuring many traits was necessary to detect selection. These findings
391 thus suggest that assessing the individual effects of few traits could be one reason why many
392 studies fail to find relationships between traits and individual performance or demographic rates.
393 For examples, studies examined the effects of three to five traits on individual growth rate (Adler
394 *et al.* 2014; Paine *et al.* 2015) and population demographics (García Criado *et al.* 2023) found no
395 or weak relationships. Third, our finding that traits under selection differed across sites indicates
396 that natural selection differs among environments. This supports previous conclusions that the

397 environment needs to be considered at a sufficiently detailed spatial scale to properly detect
398 relationships between traits and performance (Shipley *et al.* 2016; Swenson *et al.* 2020). Lastly,
399 the fact that only some traits associated with a given function were under selection at a site
400 suggests that selection may act a specific aspect of that physiological function. This implies that
401 detecting selection on a specific function in a species may require measuring multiple traits that
402 represent different aspects of that function. For example, in Petawawa we detected selection for
403 high water use efficiency, but not for other traits directly affecting water use (such as stomatal
404 density) or drought tolerance (such as LMA). This is unfortunate as structural traits such as LMA
405 are much faster and inexpensive to measure than intrinsic WUE estimated by stable carbon
406 isotope composition. Characterizing the ecological strategies of plants by measuring a few
407 common traits is a goal of trait-based ecology (Westoby *et al.* 2002; Pérez-Harguindeguy *et al.*
408 2013). However, the need for a detailed phenotypic assessment in this intraspecific-scale study
409 suggests that meeting this goal may be limited to studies with broad phylogenetic and spatial
410 scopes, as traits may only become correlated into trait axes when they span a large range of
411 values (Funk and Cornwell 2013).

412 **TRAITS SELECTED FOR AT THE WARMER AND DRIER SITE**

413 The strength of selection for two trait combinations CN:HV and CN:WUE increased in warmest
414 and driest sites (Petawawa, Figure 3). The trees growing best in Petawawa thus had a
415 combination of high WUE, HV, and CN. To the degree that these traits are heritable, and that
416 total height growth is correlated with fitness, our results suggest that black spruce populations
417 may evolve these trait combinations in response to selection pressures exerted by the warming
418 and drying change. High intrinsic water use efficiency indicates a conservative water-use, with
419 many tree species showing higher WUE in drought-adapted populations (Hajek *et al.* 2016;
420 Rosas *et al.* 2019; Ahrens *et al.* 2020; Csilléry *et al.* 2020; Anderegg *et al.* 2021; Rabarijaona *et*
421 *al.* 2022; Lochin *et al.* 2024), or plastic increases in WUE in response to drought conditions
422 (Craven *et al.* 2013; Limousin *et al.* 2015; Forner *et al.* 2018; Schimpl *et al.* 2019). A high HV
423 indicates a superior hydraulic supply capacity per leaf area. In our study, high HV resulted
424 mainly from the presence of fewer needles because variation in HV in Petawawa was more
425 strongly correlated with total needle biomass ($r = -0.79$, $p \leq 0.0001$) than branch diameter ($r = -$
426 0.49 , $p \leq 0.0001$) and total needle biomass was more variable than branch diameter (CV = 71%
427 and 29% respectively). Lower needle biomass could result either from lower production or from

428 loss of needle during drought events. High Huber values have been found to be associated with
429 drought conditions in many other species (Mencuccini and Grace 1995; Li *et al.* 2019; Rosas *et*
430 *al.* 2019; Mencuccini *et al.* 2019; Anderegg *et al.* 2021). Fewer needles may also be beneficial
431 under water limitation by decreasing the need for transpirational cooling as a means of
432 thermoregulation. High CN reflects relatively higher investment in structural support than
433 metabolic processes. Collectively, these findings indicate that fewer water- and resource-use
434 conservative needles is a beneficial strategy for black spruce in warm, dry environments. In this
435 experiment, Petawawa was the warmest and driest site, such that we cannot separate adaptation
436 to heat from adaptation to drought. Nonetheless, the three traits under climate-consistent
437 selection are more consistent with adaptation to drought, which is consistent with literature
438 showing that black spruce are water-limited in the southern margin of its range (Sniderhan *et al.*
439 2021; Chagnon *et al.* 2022).

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

451 **WEAK PHENOTYPIC INTEGRATION WILL HAVE LITTLE EFFECT ON RESPONSE TO** 452 **SELECTION**

453 Our results suggest that response to selection for the *P. mariana* provenances under study is
454 largely unconstrained by phenotypic integration. Not only is the overall strength of trait
455 integration weak, as shown by the trait correlation matrix (Table S4) and the PCAs within sites
456 (Fig. S2), but only two of the seven provenances showed significant intra-provenance trait
457 correlations for one of the two trait combinations under climate-consistent selection (Figure 4).
458 The two trait correlations were largely perpendicular to the direction of selection, indicating that

459 phenotypic integration would hinder evolutionary response to selection. However, given the
460 rarity of these instances overall (only 2 out of 21), phenotypic integration is unlikely to
461 meaningfully affect the response to selection of *P. mariana* growing in warmer climates. Over
462 evolutionary time, given unknown future selective forces, low integration can be beneficial as it
463 provides more flexibility for a species' phenotype to respond to selection. A corollary to the
464 weak trait integration in all provenances is that their integration did not differ. Thus, except for
465 the Matagami (6855) and Rocky Mtn. House II (6979) provenances which are expected to evolve
466 more slowly than the five others in response to selection for high CHL:HV, none of the studied
467 provenances showed a particularly high potential for adaptive evolution in the face of warming
468 climates. This is consistent with Robert *et al.* (2024) who report that the productivity of all
469 populations will likely decrease by the end of the century. Provenance 6979 contains the largest
470 proportion of Western genetic lineage, which has been found to contribute to reduced population
471 productivity in some common gardens (Girardin *et al.* 2021). In contrast, the other population
472 exhibiting a pattern of trait integration (provenance 6855), is one of five with a high proportion
473 of the Central genetic lineage. Thus, there is no clear signal of climate refugia lineage in the
474 intrapopulation integration of CHL and HV. As we only studied seven provenances, it remains
475 possible that unstudied populations would have a higher adaptive potential. However, given the
476 absence of correlations for most traits under selection in most of the populations, our findings
477 suggest that trait integration is not a main factor of concerns when identifying black spruce
478 provenances best adapted to future climate.

479 **INSUFFICIENT PLASTIC RESPONSE TO MAINTAIN PERFORMANCE AT THE SITE WITH** 480 **THE WARMEST AND DRIEST CLIMATE**

481 Adaptive plasticity can help a population maintain high performance in changing environments
482 (Bradshaw 1965; Pigliucci 2001; Whitman and Agrawal 2009). Similar to other studies, we
483 found mixed results, with both adaptive and maladaptive plastic responses to the spatial climate
484 gradient (Caruso *et al.* 2006; Ramírez-Valiente *et al.* 2021). The plastic increase in WUE across
485 the spatial climate gradient was adaptive, consistent with a study reporting a temporal increase in
486 WUE in the southern, water-limited part of the black spruce range (Sniderhan *et al.* 2021), and
487 with studies in other species reporting plastic shifts in WUE with water limitation (Craven *et al.*
488 2013; Limousin *et al.* 2015; Forner *et al.* 2018; Schimpl *et al.* 2019). In contrast, the plastic
489 decrease of CN in warmer sites is in the opposite direction to selection, which could indicate a

490 maladaptive plastic response of CN to warming. However, since CN is also affected by the
491 amount of available nitrogen (Lambers *et al.* 2008), trait differences among sites might also be
492 driven by soil differences. Lastly, Huber value did not show a plastic increase along the climate
493 gradient but instead showed a humped response for reasons that elude us, as no disturbance has
494 been documented at Chapleau (the site with intermediate climate) in the last 5 years. Leaf
495 shedding in response to drought has been documented in conifers (Kouki and Hokkanen 1992;
496 Żytkowiak *et al.* 2005; Nadal-Sala *et al.* 2021), but the severity of the water limitation necessary
497 to trigger this mechanism is unclear. Alternatively, trees growing under warmer and drier
498 climates may simply produce branches with less foliage. Since the plastic response of traits
499 under selection show a combination of adaptive and maladaptive patterns and that selection for
500 both trait combinations are of similar strength, it is unclear from this specific set of traits whether
501 the net effect of plasticity on height growth rate is positive or negative. If findings from this
502 spatial climate gradient are informative of temporal trends, the abundance of black spruce will
503 likely decline at its southern edge as a result of further climate warming. This is consistent with
504 expected declines in growth and survival in the more water-limited portion of the black spruce
505 range (D'Orangeville *et al.* 2018; Sniderhan *et al.* 2021; Chagnon *et al.* 2022).

506 CONCLUSIONS

507 In this study we combined classical selection gradient analyses and used black spruce
508 provenance trials established along a spatial climate gradient to assess which phenotypic traits
509 increased height growth performance in warmer and drier climates. Provenance trials provide
510 more accurate assessment of phenotypic selection from climate in natural settings than short-
511 term studies performed under greenhouse conditions (Poorter *et al.* 2016). Selection gradient
512 analysis has rarely been used to assess phenotypic traits under selection from climate change in
513 trees (Warwell and Shaw 2018, 2019; Ramírez-Valiente *et al.* 2021), and to our knowledge this
514 is the first study to do so for ecophysiological traits in mature trees in natural settings along a
515 climate gradient. This work thus demonstrates the value of research at the intersection of
516 functional ecology and evolutionary ecology to fill pressing knowledge gaps on tree response to
517 climate change (Lindner *et al.* 2010; Alberto *et al.* 2013; Laughlin and Messier 2015; Royer-
518 Tardif *et al.* 2021). Indeed, species can use three mechanisms to avoid extinction – migration,
519 adaptive plasticity and adaptive evolution – and to our knowledge most research has focused on
520 migration and plasticity, leaving an important gap regarding the evolutionary response of tree

521 phenotypes to climate change (Lindner *et al.* 2010; Royer-Tardif *et al.* 2021). In general, this
522 approach can help assess whether some populations are better adapted to warmer climates by
523 screening for provenances with (1) phenotypes better adapted to the environment (2) phenotypic
524 integration facilitating adaptive evolution and (3) adaptive plasticity improving in situ
525 performance. Specifically, this study found that in *P. mariana*, adaptive evolution to climate
526 change may favor phenotypes with fewer needles that are conservative for water and resource
527 use, that the limited intrapopulation trait integration should minimally impede adaptive
528 evolution, and that the plastic response of black spruce to warmer and drier conditions may
529 constrain the expression of an optimally adapted phenotype. Existing long-term field
530 experiments offer considerable potential for future collaborative research on other ecologically
531 and commercially valuable species. Important directions for future research are 1. to explore
532 selection on root traits, because there is evidence they mediate response to warmer and drier
533 climates (Laughlin *et al.* 2021; Messier *et al.* 2024), 2. selection in mixed forest communities,
534 because many natural and managed forests are mixed (Alberto *et al.* 2013), and 3. separating
535 selection due to high temperature from water deficit, because some populations within a species
536 range experience water limitation whereas others do not (D'Orangeville *et al.* 2018; Sniderhan
537 *et al.* 2021; Chagnon *et al.* 2022).

538 SUPPLEMENTARY INFORMATION

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

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554 DATA AVAILABILITY

555 The trait and height growth rate data used in this study are openly available at
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567 JM, CC, IA, WP, NI, BM, WP.

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913

914 TABLES

915 Table 1. Traits measured, abbreviations, units, and associated physiological function(s). ‘B’
 916 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)	°C	Thermoregulation
Needle length (NL)	mm	Thermoregulation, Photosynthesis, Water use

917

918 **FIGURE CAPTIONS**

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

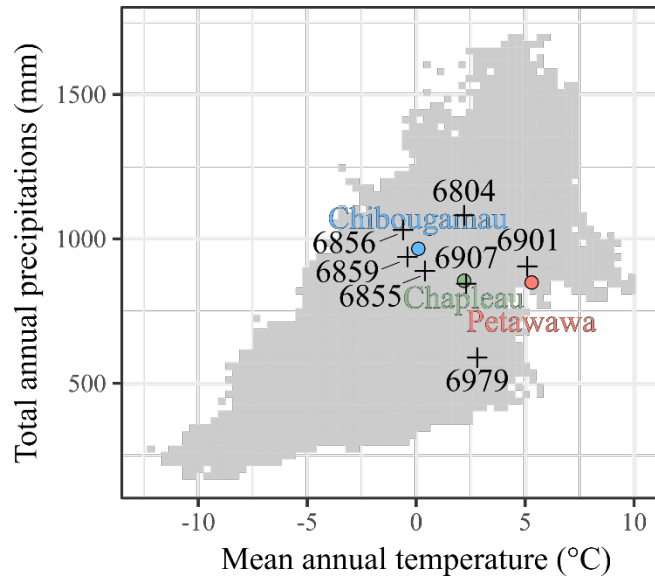
923 Figure 2. Significant and marginally significant selection gradients (β and γ) for each of the three
924 sites. Black horizontal lines represent the standard error. Asterisks indicate significance at $p \leq$
925 0.05 level. All other traits are marginally significant ($0.05 < p \leq 0.10$).

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

929 Figure 4. Significant intra-provenance trait correlations for CN:HV in 6855 (Magatami, QC) and
930 6979 (Rocky Mtn. House II, AB).

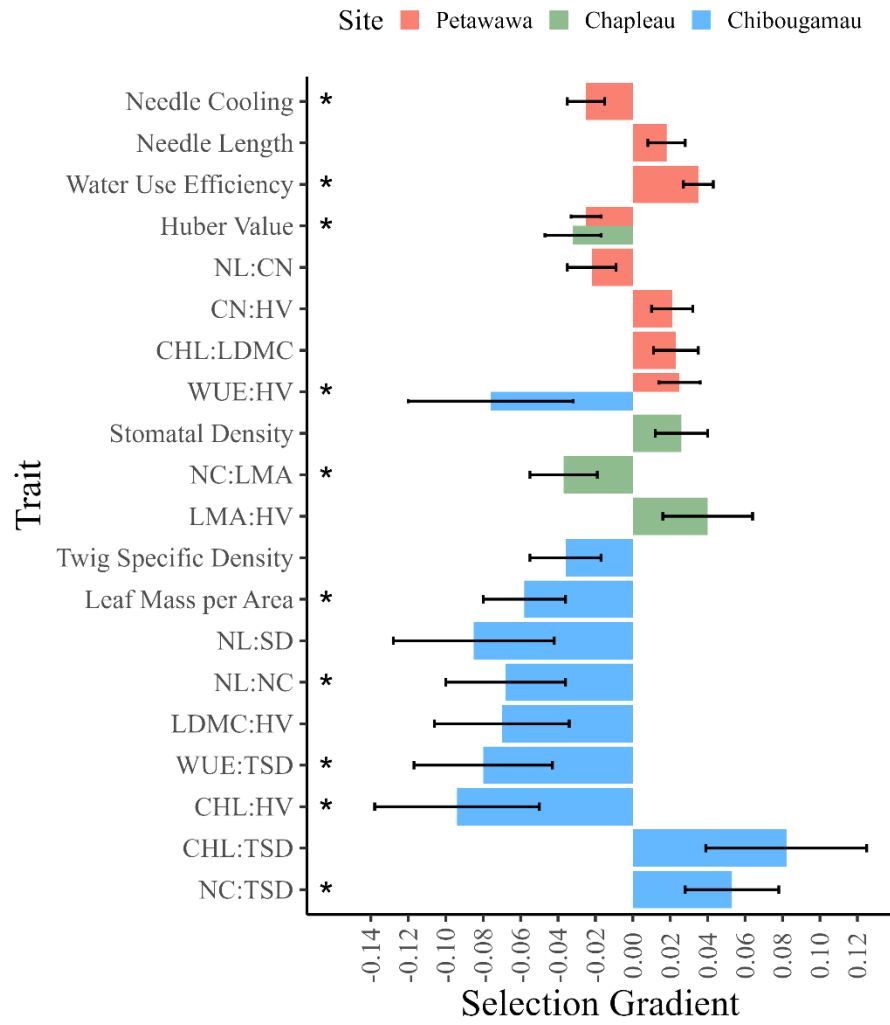
931 Figure 5. Comparative reaction norms of provenances across sites for the 3 traits under climate-
932 consistent selection. Black crosses show site means. For a given trait, sites with different
933 letters indicate significantly different means ($p < 0.05$). See table S1 for provenance
934 information.

935 FIGURES



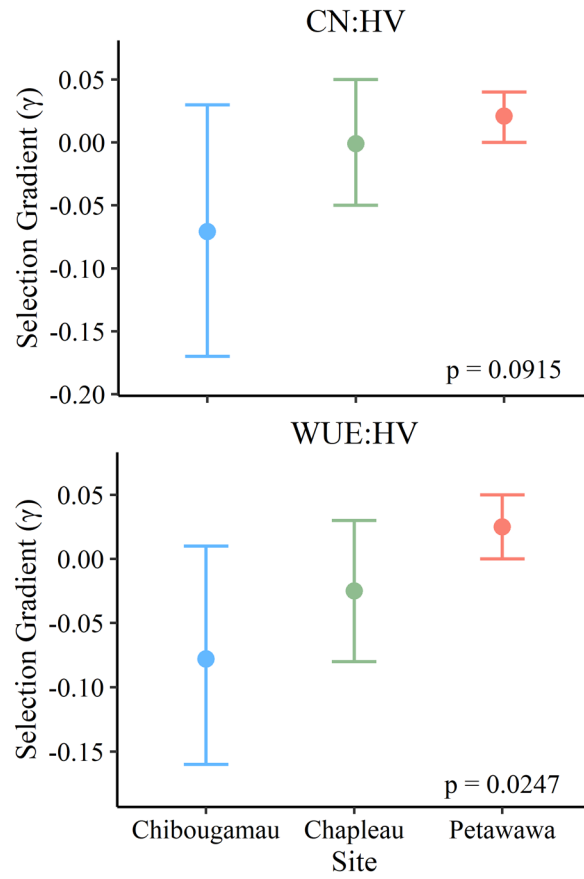
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937 Figure 1.



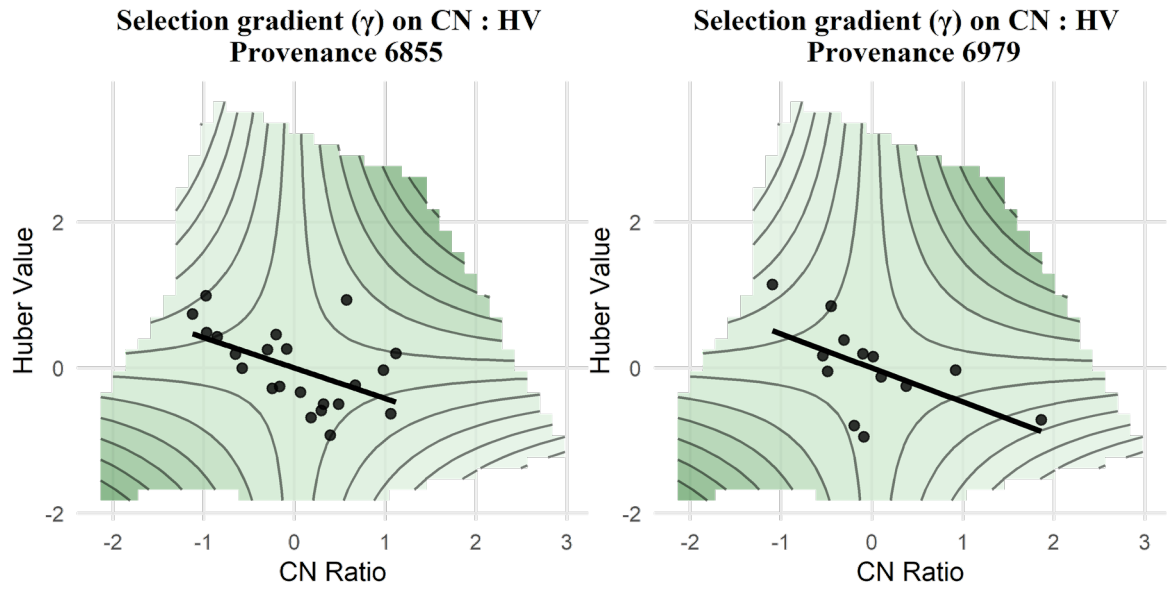
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939 Figure 2.



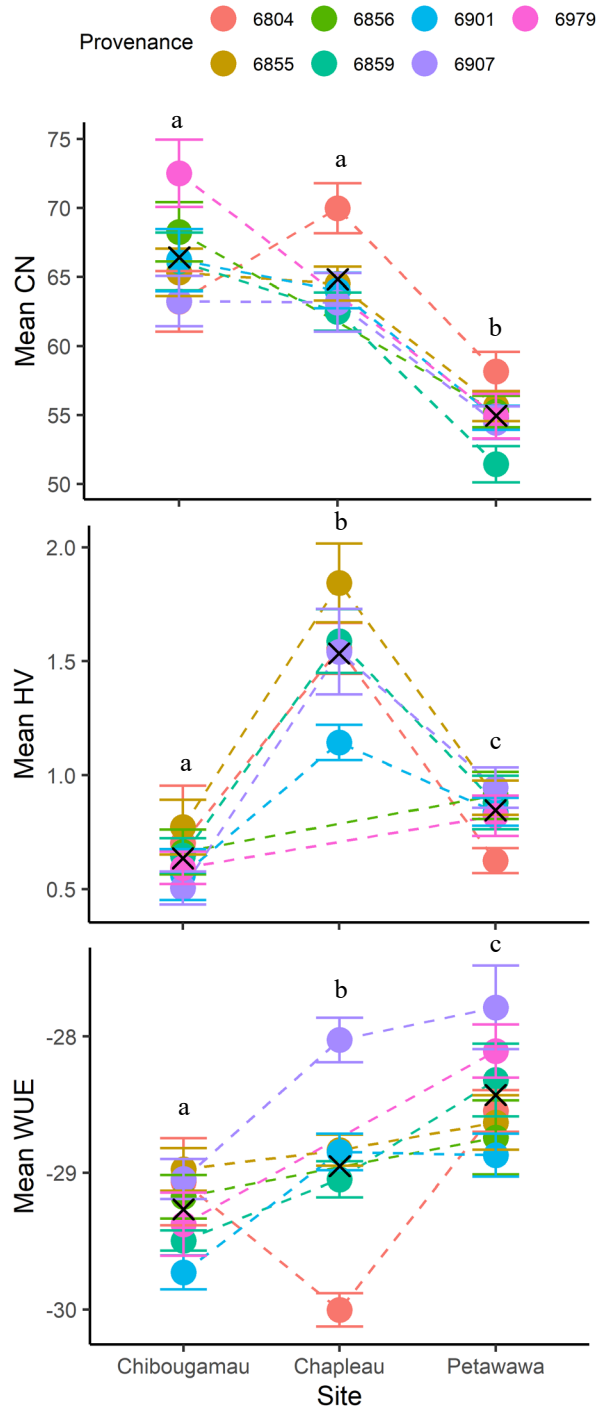
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941 Figure 3.



942

943 Figure 4.



944

945 Figure 5.

946 **SUPPLEMENTAL INFORMATION**

947 **SUPPLEMENTARY TABLES AND FIGURES**

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

Characteristic	Petawawa	Chapleau	Chibougamau
Mean annual temperature (°C)	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

953

954 Table S2. Characteristics of the seven study provenances. Latitude and Longitude are reported in
 955 decimal degrees. Elevation are reported in m above sea level. Mean Annual temperature (MAT)
 956 are reported in degrees Celsius. MAT: Mean Annual Temperature (° C). TAP: Total annual
 957 precipitation (mm). CMI: community moisture index reported annually and for the growing
 958 season (May to September). MAT and MAP data retrieved from McKenney *et al.* (2011), and
 959 averaged from 1981-2010. The W, C and E give the fraction of the provenance's genotype from
 960 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

961

962 Table S3. Traits and trait combinations significantly ($p \leq 0.05$) and marginally significantly (0.05
 963 $\leq p \leq 0.10$) under selection across the three study sites. CI = confidence intervals.

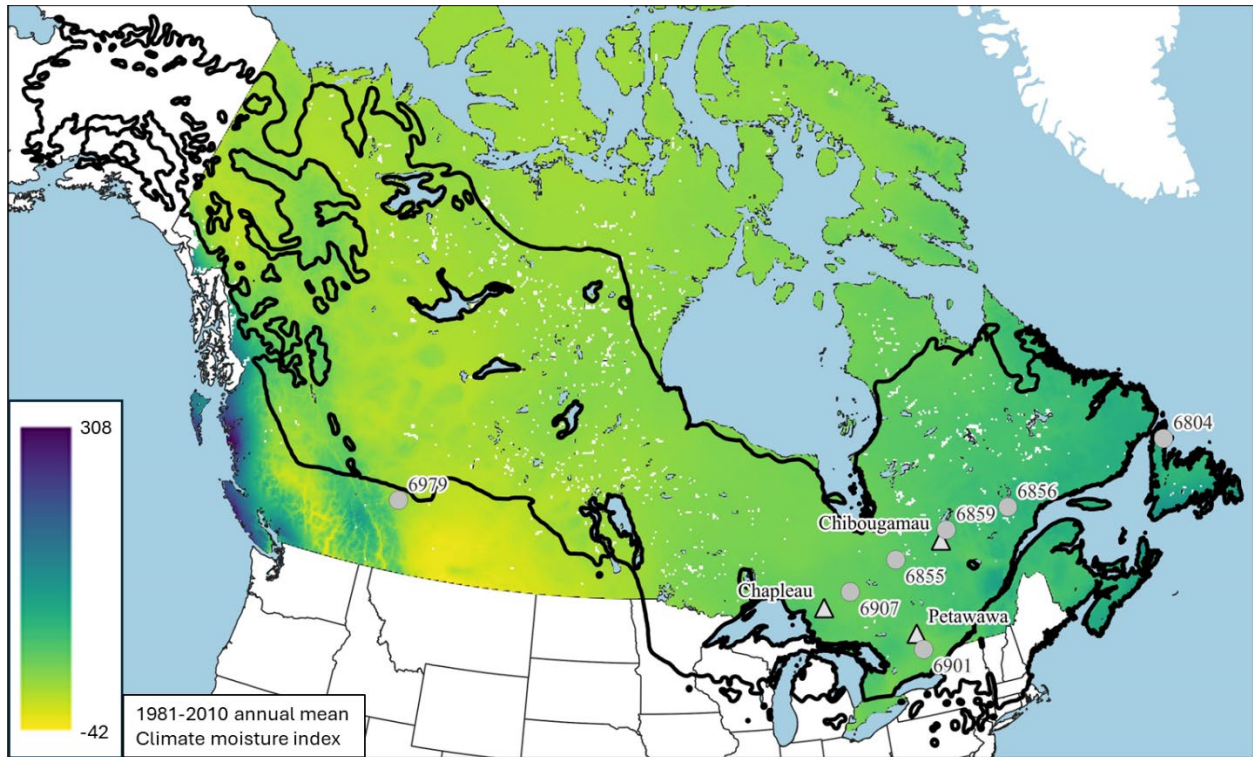
Trait	β or γ	p- value	CI	Std. Error	Site
Needle length	0.018	0.07	0,0.04	0.01	Petawawa
Needle cooling	-0.025	0.01	-0.04,-0.01	0.01	Petawawa
Water use efficiency	0.035	3.1E-5	0.02,0.05	0.008	Petawawa
Huber value	-0.025	3.6E-3	-0.04,-0.01	0.008	Petawawa
CHL:LDMC	0.023	0.06	0,0.05	0.012	Petawawa
NL:CN	-0.022	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

964

965 Table S4. Pearson correlation coefficients (r) among standardized study traits calculated across
 966 all sites, color-coded to the strength or the correlation coefficient to highlight the strongest
 967 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

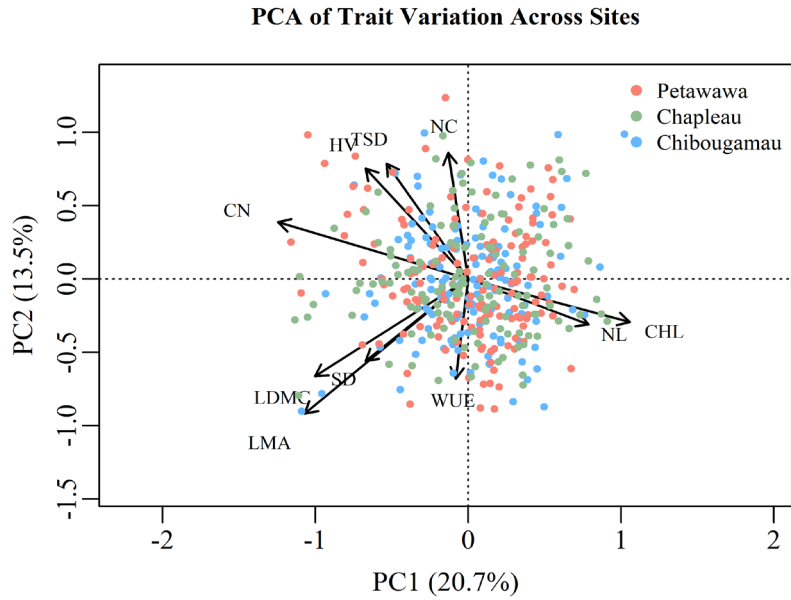
968



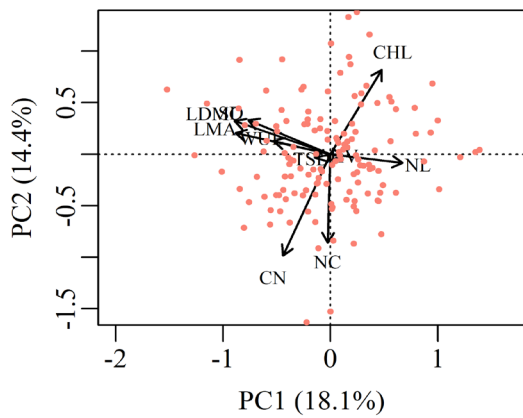
969

970 Figure S1. Geographical location of each provenance trial (grey triangles) and provenance (grey
 971 circles) used in this study. The black spruce range as define by Little (1971) is outlined in black,
 972 and the colored background represents the average annual climate moisture index (CMI) between
 973 1981-2010. NAD83(CSRS) projection from statistics Canada was used (“NAD83(CSRS)
 974 Updates” 2024).

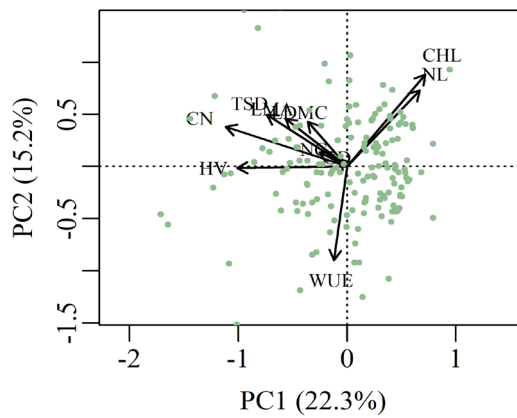
Panel A



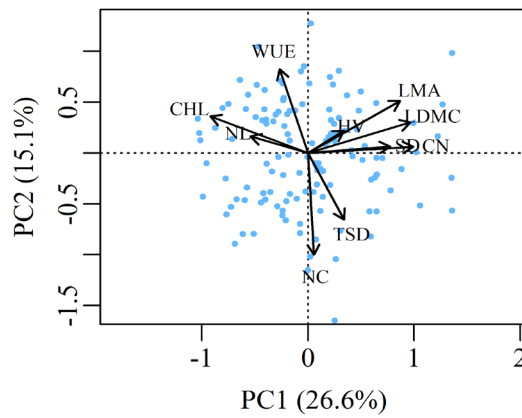
Panel B PCA of Trait Variation in Petawawa



PCA of Trait Variation in Chapleau



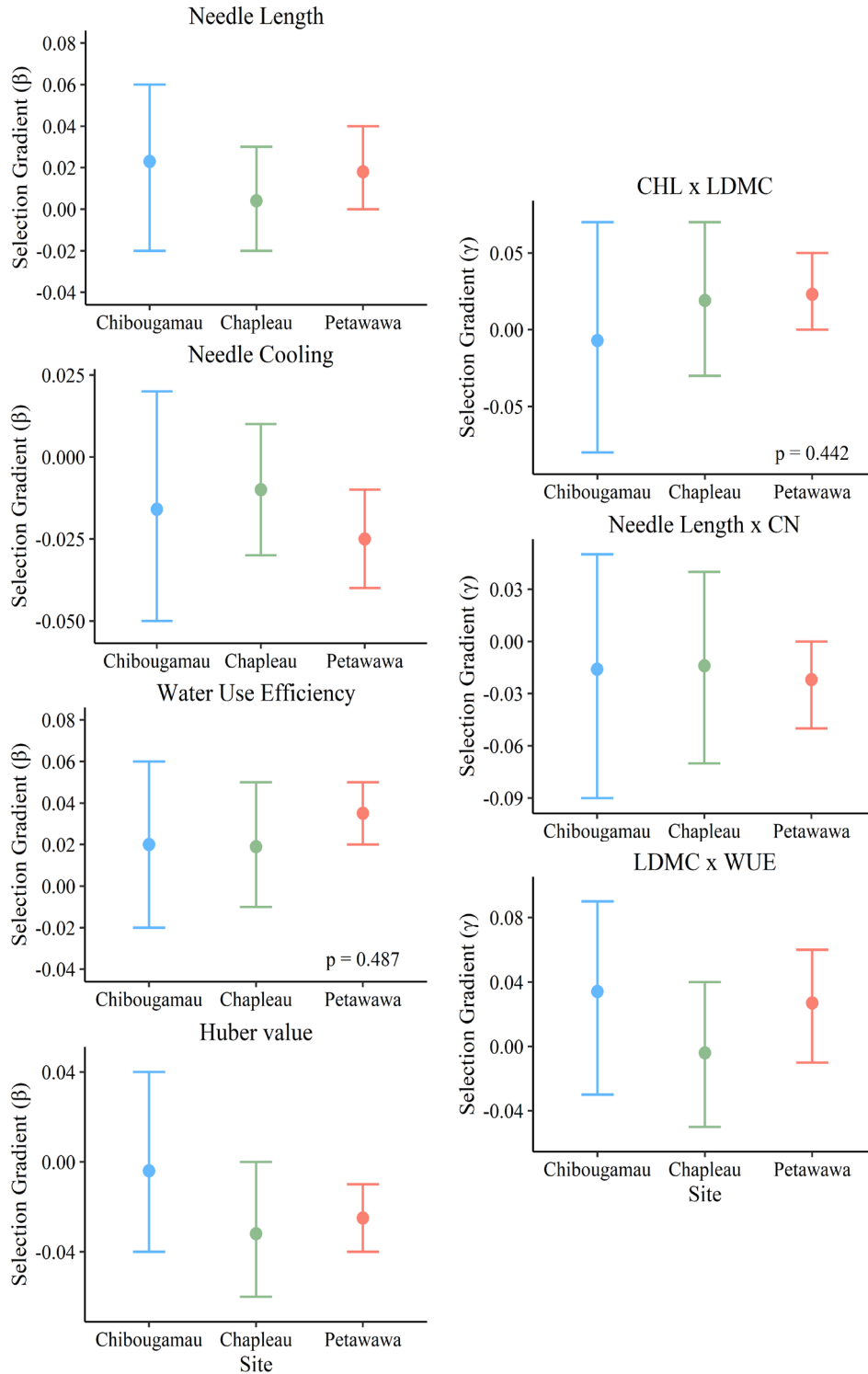
PCA of Trait Variation in Chibougamau



975

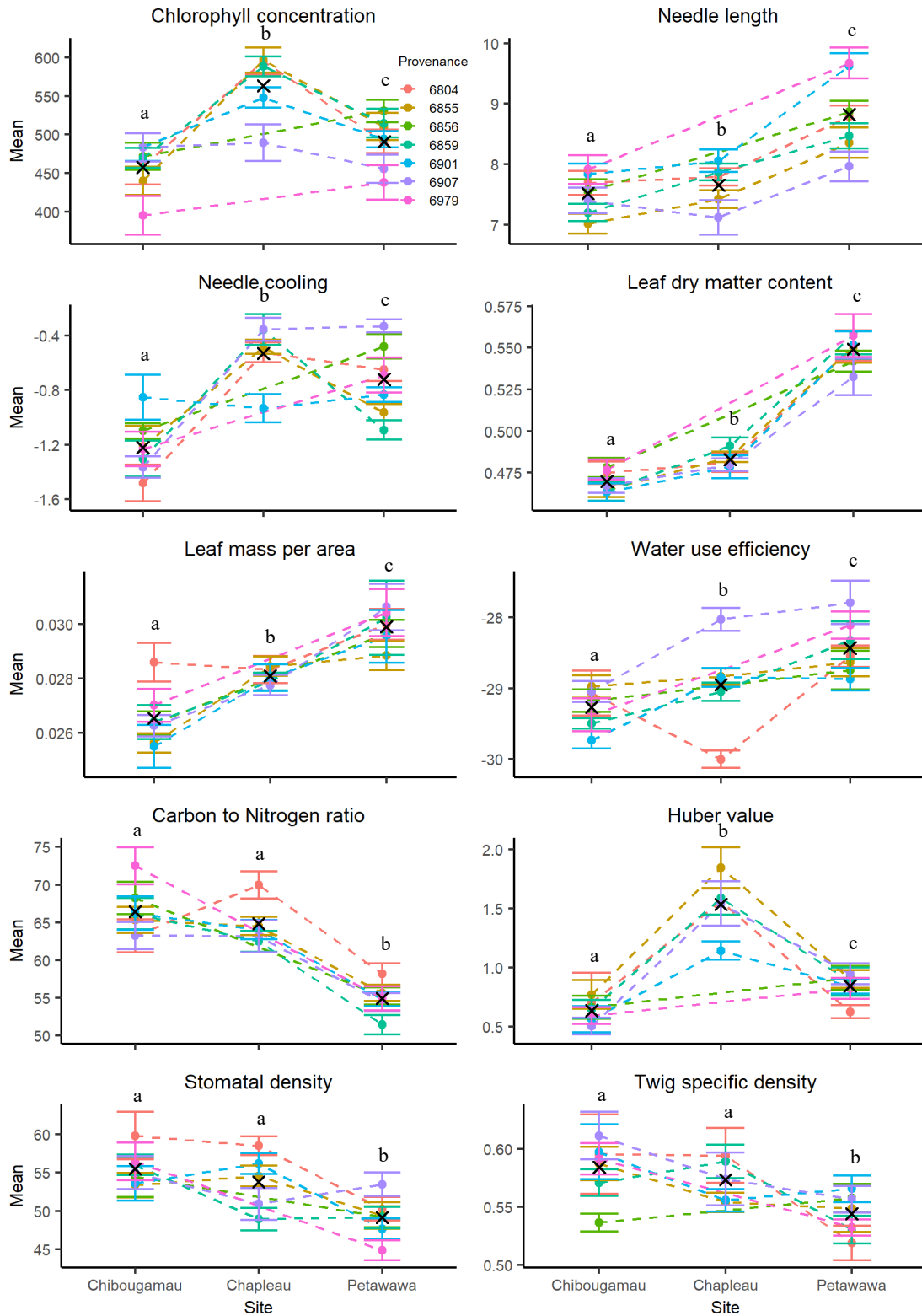
976 Figure S2. PCA of trait values. Panel A: Analysis on individuals from all sites. Panel B:

977 Analyses on individuals at each site.



978

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



982

983 Figure S4. Comparative reaction norms of provenances across sites for all the study traits. Black

984 crosses show site means. For a given trait, sites with different letters indicate significantly

985 different means ($p < 0.05$). See Table 1 for units and table S1 for provenance information.

986 **SUPPLEMENTARY METHODS**

987 Needle Cooling

988 The readings from the thermal image were calibrated against the readings from a custom-built
989 electronic thermometer with a TMP117 sensor with an accuracy of $\pm 0.1^{\circ}\text{C}$. The sensor plate of
990 the electronic thermometer was placed inside each thermal image and the true temperature was
991 manually recorded when each thermal picture was taken. Any difference between the true
992 temperature and the temperature reading on the sensor plate from the thermal camera was used to
993 correct the temperature readings of the needles within each thermal image.