



Selective breeding of lodgepole pine increases growth and maintains climatic adaptation



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ABSTRACT

Climate change is disrupting historical patterns of adaptation in temperate and boreal tree species, causing local populations to become maladapted. Tree improvement programs typically utilise local base populations and manage adaptation using geographically defined breeding zones. As climates shift, breeding zones are no longer optimal seed deployment zones because base populations are becoming dissociated from their historical climatic optima. In response, climate-based seed transfer (CBST) policies incorporating assisted gene flow (AGF) are being adopted to pre-emptively match reforestation seedlots with future climates, but their implementation requires accurate knowledge of genetic variation in climatically adaptive traits. Here we use lodgepole pine as a case study to evaluate the effects of selective conifer breeding on adaptive traits and their climatic associations to inform CBST and AGF prescriptions.

Our approach compared 105 natural stand and 20 selectively bred lodgepole pine seedlots from Alberta and British Columbia grown in a common garden of ~2200 seedlings. The effects of selection on phenotypic variation and climatic associations among breeding zones were assessed for growth, phenology and cold hardiness. We found substantial differences between natural and selected seedlings in growth traits, but timing of growth initiation was unaffected, growth cessation was delayed slightly (average 4 days, range 0.7 days to 10 days), and cold injury was slightly greater (average 2.5%, range –7% to 11%) in selected seedlings. Phenotypic differentiation among breeding zones and climatic clines were stronger for all traits in selected seedlings. Height gains resulted from both increased growth rate and delayed growth cessation, but negative indirect effects of selection on cold hardiness were weak.

Selection, breeding and progeny testing combined have produced taller lodgepole pine seedlings that are not adaptively compromised relative to their natural seedling counterparts. Selective breeding produces genotypes that achieve increased height growth and maintain climate adaptation, rather than reconstituting genotypes similar to populations adapted to warmer climates. While CBST is needed to optimise seedlot deployment in new climates, an absence of systematic indirect selection effects on adaptive traits suggests natural and selected seedlots do not require separate AGF prescriptions.

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1. Introduction

At no point in the history of reforestation has the need to establish the right trees in the right places been more acute or challenging than at present. Post-glacial recolonisation redistributed temperate and boreal trees according to species' specific ecological niches, while natural selection has shaped the distribution of genetic diversity within species, leading to local adaptation (Savolainen et al., 2007). Shifting climates and greater climatic variation are starting to disrupt historical local adaptation

(Gauthier et al., 2015; Millar and Stephenson, 2015). As a consequence, tree populations are simultaneously challenged to withstand the consequences of novel climates, and unable to adapt or migrate rapidly enough to remain locally adapted (Aitken et al., 2008). However, sustainable future timber yields depend on establishing forest stands that are productive under both the current and future climates expected during their rotation.

Most widely distributed temperate and boreal tree species are locally adapted and exhibit clines for phenotypic traits along climatic gradients (Morgenstern, 1996). Historically, this meant tree breeding programs selected and deployed local genotypes that provided relatively certain regeneration success and long-term economic returns. Within breeding programs, parental genotypes are selected from, bred, tested and deployed within a

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geographically defined zone where genotype-by-environment interactions are acceptably small or absent. This maintains adaptation by ensuring seed sources are well-matched with local site conditions. However, geographically static breeding zones are becoming obsolete as global climates shift, due to the maladaptation of local populations relative to current and future climates (St Clair and Howe, 2007; Gray et al., 2016). In response, both Alberta (AB) and British Columbia (BC), Canada, have begun shifting their reforestation policies away from fixed breeding and seed deployment zones, and towards climate-based seed transfer (CBST) to achieve sustainable future timber yields. Forests in AB and BC are the basis for a multi-billion dollar forest industry that reforests most harvesting sites with nursery-grown seedlings. Annually, 200,000–260,000 ha of public land is reforested across AB and BC with 250–325 million seedlings; lodgepole pine (*Pinus contorta* Dougl. ex Loud. var. *latifolia* Engelm.) contributes ~45% of this annual planting (Alberta Environment and Sustainable Resource Development, 2011; Forest Genetics Council of British Columbia, 2015a).

Geographic zone-based lodgepole pine breeding programs have existed in AB and BC since the 1960s. Typically these breeding programs represent continuous tree improvement progress and do not have discrete generations. At present most programs are at stages equivalent to second or third cycles of selection, breeding, and progeny testing, but currently produce seedlots from first or second generation seed orchards. By selecting for greater juvenile height growth, these programs primarily focus on producing reforestation seedlots with increased genetic worth for wood volume at rotation age, while maintaining climatic adaptation and genetic diversity (Woods et al., 1996). Trees selected for orchard production are forward or backward selections based on progeny tested on three or four climatically representative sites within each breeding program. Differing selection intensities and numbers of breeding cycles mean that genetic worth for growth varies by breeding program and province. Lodgepole pine genetic worth at rotation age has a range of 2.5–10% in AB (A. Benowicz and S. John, personal communications) and 10–22% in BC (Forest Genetics Council of British Columbia, 2015b). To ensure sustainable future yields of high quality timber, the use of selectively bred seedlots is mandatory in both provinces (British Columbia MFLNRO, 2010; Alberta Forest Genetic Resources Council, 2015).

Relatively conservative transfer distances and geographic breeding zones are used to minimise negative genotype-by-environment interactions because lodgepole pine exhibits considerable local adaptation to climate. *Pinus contorta* var. *latifolia* is the most widespread and economically important subspecies. Its range extends from high-elevation (~3500 m) populations in southern Colorado (38°N) to the southwest Yukon (64°N) where it is a component of the boreal forest (600–800 m elevation) (Critchfield, 1957). Broad-scale genetic clines in phenotypic traits reflect adaptation to regional climatic patterns, while clines are steep locally along elevational gradients (Rehfeldt, 1988). In common garden experiments, height growth is positively related to temperature and negatively related to latitude and elevation (Rehfeldt, 1983; Rehfeldt et al., 1999; Chuine et al., 2006; Wang et al., 2006b). Spring growth initiation shows little variation among provenances in common gardens (Rehfeldt and Wykoff, 1981), although Chuine et al. (2006) reported that northern provenances have slightly higher threshold temperatures for growth to avoid premature bud break. Growth cessation, terminal bud formation (bud set) and cold acclimation are initiated in response to a genetically-determined critical night length, and occur later in provenances from more southern locations and lower elevations, reflecting the *in situ* risk of fall frosts (O'Reilly and Owens, 1989; Rehfeldt, 1988). Autumn cold hardiness shows strong among-population variation in lodgepole pine (Liepe et al., 2016), which

is consistent with studies of adaptation in other temperate and boreal tree species (Alberto et al., 2013). Therefore, local adaptation to climate is conferred by genotypes that optimise trade-offs between growth and cold hardiness (Howe et al., 2003).

While there are clear trade-offs between growth, phenology and cold hardiness among natural lodgepole pine populations, the correlated responses to selection between stem growth and phenology or cold hardiness within breeding populations are less clear. This is important where seedlings from breeding programs are deployed to reforest natural landscapes with native species across regions such as western Canada, because genetic diversity and resilience to disturbance is required over long rotations to ensure timber production and ecosystem function.

Tree breeders achieve growth gains by selecting faster growing genotypes or genotypes that have a longer growing period. If selection lengthens the growing season, growth gains from selective breeding could cause unfavourable correlated selection responses in adaptive traits. Selectively bred trees would then become unsynchronised with local climates and more vulnerable to cold injury, particularly at the seedling stage (Howe et al., 2003; Aitken and Hannerz, 2001). This is a concern to forest managers because cold injury and mortality can result in understocked or failed stands that require expensive fill-planting (Hotte et al., 2016). Conversely, if selective breeding increases stem growth without negative impacts on phenology or cold hardiness, climatically adaptive synchrony to seasonal changes will be unaffected. Previous results from mature Norway spruce (*Picea abies* (L.) Karst.) (Westin et al., 2000; Hannerz and Westin, 2005) and lodgepole pine (Rehfeldt, 1989) suggest this may be possible. Selective breeding may also produce adaptive phenotypes that are equivalent to populations present elsewhere on the landscape where milder climates favour increased growth, which could result in phenology and cold hardiness phenotypes that are maladaptive when deployed in local breeding zones (Rehfeldt, 1992a; Rehfeldt, 1992b). The extent to which selective breeding actually produces this effect is unclear.

Using ClimateNA (Wang et al., 2016) we estimated that mean annual temperature was 0.58 °C higher across AB and BC during 2005–2014 than during the 1961–1990 reference period, although increases of 1.5–2 °C have been observed in northern BC (British Columbia Ministry of Environment, 2015). Lodgepole pine breeding zones in BC are predicted to generate a modest (~7%) increase in growth with warming of ~1.5 °C until the 2030s, but the current limits of climatic adaptation within breeding zones will be exceeded and productivity decreased below present levels by the late 2060s (Wang et al., 2006a). At the same time, warmer climates might be expected to diminish the risk of cold injury, but in localities with warmer autumn temperatures and weak cool temperature cues or increased climate variability, trees may actually be more vulnerable to autumn cold injury (Bansal et al., 2015).

Climate niche models suggest tree populations in western North America already lag 130 km behind their historic climates (Gray and Hamann, 2013). One potential solution for reducing the maladaptive effects of climate change on planted forests is to match pre-adapted genotypes with new climates, a form of assisted migration called assisted gene flow (AGF) (Aitken and Whitlock, 2013). British Columbia and Alberta have plans to implement AGF within new climate-based seed transfer (CBST) policies (O'Neill et al., 2008; Gray and Hamann, 2011). Changing the deployment of ~300 million seedlings per year to a CBST system, and implementing AGF is predicated on detailed knowledge of local adaptation (Aitken and Whitlock, 2013). An implicit assumption of AGF is that when deployed, seedlings will be shifted towards the margins of their adaptive climatic niche, but this creates a trade-off between early rotation risk from cold injury, and mid- to late-rotation growth gains under suitable climates.

To manage this trade-off, knowledge of adaptive variation is needed for both natural stands and selectively bred seedlings, as currently ~22% of planted lodgepole pine seedlings originate from breeding programs and governments in both provinces have mandates to increase this percentage in coming years.

Here we use reforestation in AB and BC as a case study to assess how selective breeding modifies adaptive phenotypes, their relationships with climate, and the implications for CBST and AGF. We address three objectives. (1) to quantify the direct effects of selection on climatically adaptive traits; (2) to evaluate the indirect effects of selection and trade-off among traits; and (3) to determine the effects of selection on adaptive phenotype–climate associations. Using operational lodgepole pine seedlots sourced from breeding programs across AB and BC, because they are the basis of current and future reforestation, our study samples the breadth of standing genetic variation in traits that are relevant to developing CBST and AGF policies. By establishing a seedling common garden in a mild coastal environment, we decompose the components of phenotypic adaptation to climate that may respond to selective breeding. This approach allows us to evaluate the effects of selective breeding on seedling traits, and shifts in phenotype–climate associations relative to natural (wild-stand) seedlings within and among breeding zones. From a single common garden test site, we cannot determine the composite fitness of selectively bred and natural populations in field environments, but our research compliments long-term field trials by assessing the relative effects of selection on the growth, phenology and cold hardiness traits of populations from different source climates. Finally, we address whether selective breeding produces phenotypes similar to the climatypes of natural populations adapted to warmer climates that favour faster growth.

2. Methods

2.1. Experimental sampling & establishment

Open-pollinated, selectively-bred orchard seedlots were obtained from 12 lodgepole pine breeding zones across AB and BC (Table 1). Where available, more than one selectively bred seedlot was included from each breeding zone, for a total of 20 seedlots. Seedlots with the highest available genetic worth from the most recent growing season available were selected. The number of parent tree clones contributing to each selectively bred seedlot ranged from 36 to 117 (Table 1). For each geographic breeding zone, 4 to 16 open-pollinated wild stand (natural) seedlots were obtained, for a total of 105 natural seedlots (Table 1, Fig. 1). The number of selectively bred seedlots is less than natural seedlots because the

latter are collected from single stands within a breeding zone, while parent trees in each breeding program originate from across the respective zone, and in a few cases also include clones of highly performing parent trees from other breeding zones.

Seed was stratified using a modified version the BC Ministry of Forests, Lands and Natural Resource Operations seed stratification protocol (Kolotelo, 1994). Seeds were soaked in distilled water for 24 h, washed briefly in 2% bleach to reduce pathogens, rinsed in distilled water, surface dried, then chilled at 4 °C for 3 weeks. Stratified seeds were sown in early May 2012 into two adjacent outdoor raised beds filled with double-screened topsoil on the UBC campus in Vancouver, Canada. The common garden was split into 12 blocks, with seeds sown in a randomised incomplete block design developed using a custom R script (R Core Team, 2016). Each block contained 240 seedlings established as single-seedling plots, surrounded by a row of buffer seedlings. Seeds were initially triple-sown into planting positions at 8 cm spacing, then systematically thinned post-germination by position to leave one healthy seedling.

A total of 2176 seedlings (natural $n = 976$; selected $n = 1200$) were established. For each breeding zone there was a minimum of 60 and maximum of 112 natural seedlings (Table 1), with 12 to 16 seedlings from each of six randomly selected natural seedlots, and at least four seedlings from each remaining natural seedlot. Each selectively bred seedlot was represented by 60 seedlings.

The experiments were maintained and measured over three growing seasons. They were well watered and received two or three fertiliser applications per growing season (Peters Excel 15-5-15 NPK water soluble fertiliser applied at a manufacturer recommended N concentration of 200 ppm). Some damage to seedlings in the common gardens was caused by an unidentified fungal infection of unignified new shoots that was treated with an appropriate systemic fungicide, or by shoot boring larvae which were removed manually. Seedlings were excluded from analyses ($n \leq 115$) if they incurred damage that compromised their data for a given trait.

2.2. Phenotypic measurements and data collection

During the second and third growing seasons (2013 and 2014 respectively), phenotypic data were collected for six growth, phenology and cold hardiness traits that often show local adaptation to climate in conifers (Savolainen et al., 2007). Height (cm) was measured repeatedly during season 3 and final height measurements were made after growth cessation and bud set. Growth rate (cm day^{-1}) was interpolated from growth curves (Section 2.4) fitted to the height growth time series data. Shoots were destructively sampled after the third growing season, and shoot dry

Table 1
Breeding zones sampled for natural and selected seedlots, their elevational range, number of seedlots and seedlings per breeding zone. The number of clones contributing cones to selected seedlots is given, with the total number of clones in their respective seed orchards in brackets. Alberta's breeding zones are formally identified as A, B1, B2, C, J and K1. British Columbia's breeding zone abbreviations are BV = Bulkley Valley, CP = Central Plateau, EK = East Kootenay, NE = Nelson, PG = Prince George, and TO = Thompson - Okanagan.

Province	Breeding zone	Elevation range (m)	Natural		Selected		
			Seedlots	Seedlings	Seedlots	Clones per Seedlot	Seedlings
AB	A	1050–1350	6	72	1	36 (36)	60
AB	B1	800–1200	5	60	2	117 (118), 117 (118)	120
AB	B2	1200–1600	4	60	1	108 (111)	60
AB	C	800–1200	5	60	1	113 (113)	60
AB	J	600–1000	8	80	2	50 (60), 38 (60)	120
AB	K1	1100–1500	8	80	1	60 (60)	60
BC	BV low	700–1200	11	92	2	67 (71), 73 (88)	120
BC	CP low	700–1100	7	76	3	55 (61), 72 (72), 65 (67)	180
BC	EK low	800–1500	13	100	1	48 (49)	60
BC	NE low	700–1400	11	92	2	38 (42), 40 (46)	120
BC	PG low	600–1200	11	92	2	45 (64), 84 (86)	120
BC	TO low	700–1400	16	112	2	72 (77), 48 (65)	120

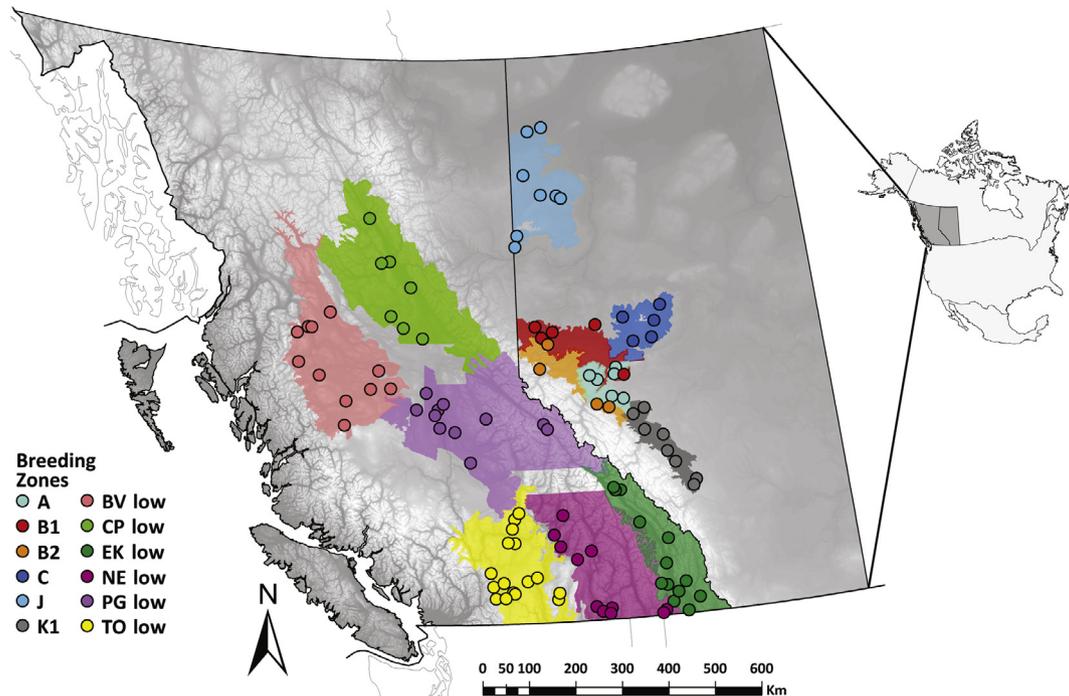


Figure 1. Geographic origins of the natural (filled circles) and selected (filled polygons) seedling populations sampled from breeding zones across the range of lodgepole pine in Alberta and British Columbia.

mass (g) above the root collar was measured after drying samples at 70 °C for a minimum of 48 h. Growth initiation and cessation in pines are not discrete processes, and these two traits were also interpolated from seedling growth curves (Section 2.4).

Autumn cold hardiness testing was performed on needles formed during the preceding summer's growth. We used a slightly modified version of the artificial freeze testing and electrolyte leakage measurement protocol described by Hannerz et al. (1999) to estimate damage using the ratio of cellular electrolytes leaked after freezing relative to total electrolyte leakage after heat killing. Cold hardiness testing was performed over three consecutive weeks to accommodate the large number of seedlings. Three samples of five, 5 mm long needles segments were collected from each seedling; two samples were subjected to different freeze test temperatures for a one-hour period, and the third sample served as an unfrozen control. Timing and test temperatures were determined by pre-testing to identify the temperature at which approximately 50% cold injury occurred. Cold hardiness testing commenced on October 14th 2013 (season 2) using -14 °C and -18 °C test temperatures. Control samples were placed in a fridge at 4 °C and test samples were frozen using a Tenney T20C-3 programmable temperature chamber. Electrical conductivity measurements were made on test and control samples after freezing, and again after heat killing at 95 °C in a laboratory oven, using Amber Science Inc. Model 2052 Digital Electrical Conductivity meters. The cold injury damage incurred by each seedling at both test temperatures was calculated relative to unfrozen control samples using Flint et al.'s (1967) index of cold injury (I). Lastly, we averaged the values of I between test temperatures, and used this mean value for our analyses. Seedlings with I values of zero were undamaged, while values of 100 indicate maximum freezing damage.

2.3. Climatic data

We analysed relationships between phenotypic traits and 19 climatic and three geographic variables (collectively referred to

as climatic variables) (Table S1). Climatic variables for natural seedlot provenances were estimated for the 1961–1990 climate normal period using ClimateNA version 5.21 available from <http://cfcg.forestry.ubc.ca/projects/climate-data/climatebcwna/#ClimateNA>, based on the methodology of Wang et al. (2016). This climate normal period is appropriate because it more closely reflects the historical conditions populations are likely to be locally adapted to, preceding climate warming of the last ~25 years.

Selectively bred seedlots used here are the product of open-pollination in seed orchards and bulking of seeds from multiple parent tree clones. To obtain representative climate estimates for selected seedlots, the latitude, longitude and elevation of all parent trees in each seed orchard were obtained, and their climatic variables estimated using ClimateNA. Mean climatic variables for selectively bred seedlots were averages of their respective parent tree climate data, weighted by the maternal contribution of each parent to the seedlot. Maternal contributions to a given selected seedlot are determined from the number of cones collected from each seed orchard clone. Data on paternal contributions of each clone are not consistently available among breeding programs, and were not used to weight our climatic estimates. Each natural or selected seedling was assigned the average climatic data of its respective seedlot. Climate variables were then summarised as PCA scores for each seedling and these were used as additional climatic variables. Lastly, for both seedling types, breeding zone-specific estimates of every climatic variable, including PC scores, were calculated as the mean of all natural or selectively bred seedlings within a given breeding zone.

2.4. Growth curve analysis

Pines have compound long-shoot buds that elongate rapidly early in the growing season well before needle fascicles rupture their bud scales (Owens, 2006). As a result, bud break and bud set phenology is more difficult to phenotype directly in lodgepole pine than in many other conifers. Instead, we derived pine growth

initiation and cessation phenotypes from growth curve analyses (e.g., [Chuine et al., 2001](#)).

Seedling height was measured 19 times in the third growing season to characterize rapid early season growth and phenology accurately. We fit individual seedling height growth time series data to the sigmoid four-parameter logistic regression model of ([Chuine et al., 2001](#)) (Eq. (1)) using the nls function of the 'stats' package in R.

$$H_{(t)} = a + \frac{b}{1 + e^{-c(t-d)}} \quad (1)$$

where $H_{(t)}$ = predicted height on day t , the time in days since January 1st, a is the previous growing season's final seedling height, b is the current season's height growth increment, c is a component of the maximum growth rate, and d is the day since January 1st that half of the current season's growth increment was attained. Growth initiation and growth cessation timing in each pine seedling were estimated as the day that 5% and 95% of the growing season's height increment was completed. We chose these 5% and 95% values as a trade-off between the sensitivity to detect growth initiation or cessation and the possibility of height measurement error. Maximum growth rate was estimated as the tangent of the sigmoidal growth curve at its inflection point.

2.5. Comparison of breeding zone \times seedling type means

To test the effects of selective breeding on each trait, we used a linear mixed effects model (Eq. (2)) that accommodates the unbalanced experimental design, implemented in ASReml-R version 3.0 ([Butler, 2009](#)).

$$Y_{ijklm} = \mu + S_j + Z_k + (S * Z)_{jk} + B_l + L(B)_{lm} + e_{ijklm} \quad (2)$$

where Y_{ijklm} is the phenotypic observation of a trait made on individual i from the j^{th} seedling type (S) and k^{th} breeding zone (Z), grown in the l^{th} block (B), at the m^{th} seedling location (L) nested within block ($L(B)_{lm}$). $S * Z$ denotes the seedling type by breeding zone interaction. μ is the experimental mean and e is the residual error of individual i . Seedling type (natural stand or selectively bred) and breeding zone were fixed effects in the model; block and location within block were random effects.

Residual values from the linear mixed-models of seedling traits were assessed using Shapiro-Wilk normality tests and F-test for homogeneity of variances. All traits except shoot dry mass met normal distribution and homogeneity of variance assumptions for large sample sizes; shoot dry mass data was quarter-root transformed to meet these assumptions. Best linear unbiased estimates (BLUEs) of the fixed effects were extracted using ASReml-R for each seedling type by breeding zone combination as the means of seedlings pooled across seedlots within breeding zones. These means were used to test the effects of selection within specific breeding zones, and for clinal analyses described in Section 2.7. We tested for significant pairwise differences between seedling type BLUEs within breeding zones using two-sample t-tests.

2.6. Breeding zone variance partitioning

Our experimental comparisons are designed to use the same bulk seedlots that are used in operational reforestation in western Canada. The lack of family-level population structure prevents the estimation of additive genetic variance within and between seedlots. Instead we estimated V_{POP} , the ratio of among-breeding zone variance to total phenotypic variance within breeding zones, to estimate how phenotypic variation is partitioned across breeding zones for natural and selectively bred reforestation populations. V_{POP} is analogous to Q_{ST} , a quantitative genetic estimate of additive genetic variance among versus within populations ([Alberto et al., 2013](#)).

A modified version of Eq. (2) with seedling type excluded and all factors set as random was used to estimate the among and within breeding zone variance components, whereby each breeding zone is represented by individual phenotypes pooled from across its respective seedlots. Models for each seedling type were run separately and breeding zone differentiation (V_{POP}) was calculated using Eq. (3).

$$V_{POP} = \frac{\sigma_p^2}{\sigma_p^2 + \sigma_e^2} \quad (3)$$

where V_{POP} is the phenotypic differentiation among breeding zones, σ_p^2 is the variance among breeding zones (populations), and σ_e^2 is the model's residual error approximating the variance within breeding zones.

2.7. Clinal analysis

We used a multiple linear regression model (Eq. (4)) to compare phenotypic clines along gradients for nine climatic variables (indicated in [Table S1](#)) as well as PC1 and PC2 climate variable scores, between natural and selectively bred seedlings. Clines were estimated using mixed-model BLUEs of each breeding zone and seedling type combination as the dependent variable, and mean climatic values of breeding zones as the independent variable. Seedling type was included in the model as a categorical variable.

$$y_{ij} = \beta_0 + \beta_1(x_1) + \beta_2(x_2) + \beta_3(x_1x_2) + e_{ij} \quad (4)$$

where y_{ij} is the BLUE of seedling type i in breeding zone j , x_1 is a continuous climatic variable, x_2 is the categorical covariate 'seedling type', β_0 is the intercept, β_1 and β_2 are the climatic variable and seedling type coefficients respectively, β_3 is the coefficient of the seedling type \times climatic variable interaction, and e_{ij} is the residual error of y_{ij} . We tested for the fit and significance of each seedling type cline independently, and for significant differences between slopes of seedling type clines.

2.8. Trait - trait correlations

To identify differences in trade-offs among adaptive traits in the selected versus natural seedlings, seedling type-specific correlations between the mixed-model BLUEs for seedling height and the other five traits were calculated. For each trait we also calculated the difference between natural and selected seedling BLUEs of every breeding zone. Difference values for height were then correlated with difference values of each remaining trait, so that we could identify differences between selected and natural seedlings which co-varied between traits.

2.9. Climatic biases in breeding programs

For each breeding zone we calculated the temperature difference between mean source MAT of natural seedlings and the weighted mean MAT of selected seedlings. We also calculated height gains from natural to selected seedlots in each zone, and then tested how much variation in height gains among breeding zones was explained by within-zone MAT differences.

Lodgepole pine embryo development occurs between June and late August ([Owens, 2006](#)). To test for potential epigenetic effects of seed orchard environments during seed development on seedling traits, we calculated differences between mean summer temperature (MST) (June–August) of breeding zones and their respective seed orchard locations in the years our seedlots were produced. We then regressed the height gains of each zone upon these MST differences.

3. Results

3.1. Breeding zone × seedling type means

After germination failure, subsequent mortality, and damage were accounted for, 89% of seedlings remained for analysis after three growing seasons. Individual seedling growth curves were successfully modelled for the height time series data of all but two seedlings. The average height growth curves for both seedling types (analysed separately) had an R^2 value of 0.98.

BLUEs of seedling height were greater, in some cases up to ~50% greater, for selected seedlings than for wild stand seedlings in all but one breeding zone (Fig. 2a, Table S4). The differences between seedling types, reflecting genetic gain from selective breeding for faster growth, were significant in 10 of 12 breeding zones in pairwise t-tests. Growth rate and transformed shoot dry mass exhibited very similar results to seedling height (Fig. 2b and c, Table S4), and gains in growth traits were consistently greater in BC breeding zones that have older, more advanced breeding programs than in AB.

Unlike growth traits, selective breeding had only minor effects on growth initiation timing. The day of growth initiation varied by no more than 1.4 days between seedling types in any breeding zone, and varied by only 3.5 days across all breeding zones × seedling type combinations (Fig. 2d, Table S4). Among breeding zones, the direction of change was inconsistent. Differences between seedling types were statistically significant only in BV low and CP low, but were in opposite directions in these two zones. In contrast to growth initiation, differences between seedling types for growth cessation timing were relatively consistent across breeding zones. On average across breeding zones, growth cessation occurred 4 days later in selected seedlings. Growth cessation was delayed in 11 of 12 breeding zones, and significantly so in nine of these cases (Fig. 2e, Table S4), with a maximum growth cessation delay of 10 days in the TO low breeding zone.

On average across breeding zones, selectively bred seedlings had 2.5% greater cold injury. Selectively bred seedlings exhibited slightly greater cold injury in 9 of 12 breeding zones (Fig. 2f, Table S4), but the only statistically significant difference between seedling types was 11% in the CP low breeding zone. All differences between seedling types in the remaining breeding zones were <7%.

3.2. Phenotypic differentiation among breeding zones (V_{POP})

Differentiation among breeding zones varied substantially among traits, but was always stronger in selected than in natural seedlings (Table 2). V_{POP} for growth rate was 2.4× greater in selected than in natural seedlings ($V_{POP} = 0.124$ and 0.052 respectively), while seedling height V_{POP} was ~2× and shoot mass 1.5× greater. V_{POP} was slightly greater in selected versus natural seedlings for growth initiation (selected $V_{POP} = 0.086$, natural $V_{POP} = 0.052$), and 2× greater for growth cessation (selected $V_{POP} = 0.345$, natural $V_{POP} = 0.178$). Cold injury showed the strongest breeding zone differentiation, with V_{POP} values of 0.545 and 0.415 in selected and natural seedlings respectively, but the smallest difference between seedlot types of any trait.

3.3. Clinal analysis

PCA of seedling climate variables found PCs 1, 2, 3 & 4 cumulatively accounted for 54%, 71%, 85% and 93% of climatic variation (Table S2). PC1 loadings were dominated by temperature and frost-related variables, while PC2 loadings were dominated by precipitation as well as derived variables combining temperature and precipitation (Table S3).

Most traits showed significant clinal variation along gradients of latitude, mean annual temperature, summer heat-moisture index, degree days above 5 °C, number of frost-free days, extreme minimum temperature and climate PC1, after Bonferroni adjustment for multiple climatic comparisons (Table S5). Clines in longitude, elevation, log mean annual precipitation and climatic PC2 were moderate to weak for all trait-by-seedling type combinations. Here we use clines in mean annual temperature (MAT) to illustrate differences between selected and natural stand seedlings. Second only to climatic PC1 scores, clines in MAT are consistently the strongest and show the greatest differences between natural and selected seedlings. Consistent with estimates of V_{POP} , patterns of clinal variation for the three growth traits were markedly stronger for selected than for natural seedlings (Fig. 3a–c). However, after correcting for multiple comparisons ($\alpha = 0.0045$), slope differences between seedling type clines were not statistically significant for any growth trait. Growth initiation had a significant cline in MAT (Fig. 3d) only for selected seedlings, while growth cessation clines with MAT (Fig. 3e) are strong but not significantly different between seedling types. Consistent with the high V_{POP} values, clines in cold injury for MAT are the strongest of any trait and highly significant in both seedling types, but differences between seedlot type slopes are small and not significant (Fig. 3f).

3.4. Trait – trait correlations

Correlations among traits were uniformly strong ($r \geq 0.74$) and statistically significant ($p \leq \alpha = 0.0033$) in all but one case: height versus growth initiation (Table 3). Correlations with height were also slightly stronger for selected seedlings in all traits. Corresponding with our clinal analyses, seedling height was positively associated with growth initiation and growth cessation, indicating that taller seedlings broke and set bud later (although the actual magnitude of this delay is greater for growth cessation, described in Section 3.1).

Difference correlations of height versus growth rate (Fig. 4a), and shoot dry mass were moderate to strong and statistically significant ($p \leq 0.01$). Differences in height were not strongly correlated with differences in growth initiation (Fig. 4b), but height – growth cessation differences were strongly and significantly correlated ($r = 0.94$) (Fig. 4c). Lastly, height differences between seedling types were neither strongly nor significantly correlated with cold injury differences (Fig. 4d).

3.5. Climatic biases in breeding programs

Mean source MATs of natural seedlings differ from weighted mean MATs of selected seedlings within breeding zones by –0.09 °C to +1.6 °C, but this explained little variation in height gains among breeding zones ($r^2 = \sim 0.12$; $p = 0.27$). Likewise, differences in mean summer temperature (MST) (June–August) between breeding zones and seed orchards had a range of 0.81 to 5.95 °C, but the variation in height gains these difference explain is negligible ($r^2 \leq 0.14$, $p = 0.11$). Equivalent regressions for the remaining five traits were also weak; cold injury had the strongest relationship ($r^2 = 0.22$; $p = 0.035$).

4. Discussion

Lodgepole pine breeding programs in western Canada have achieved substantial growth gains over wild-stand seedlings that are reflected in our common garden. Our breeding zone-level analyses showed that in addition to growing up to 50% taller, selectively bred seedlings expressed greater phenotypic differentiation

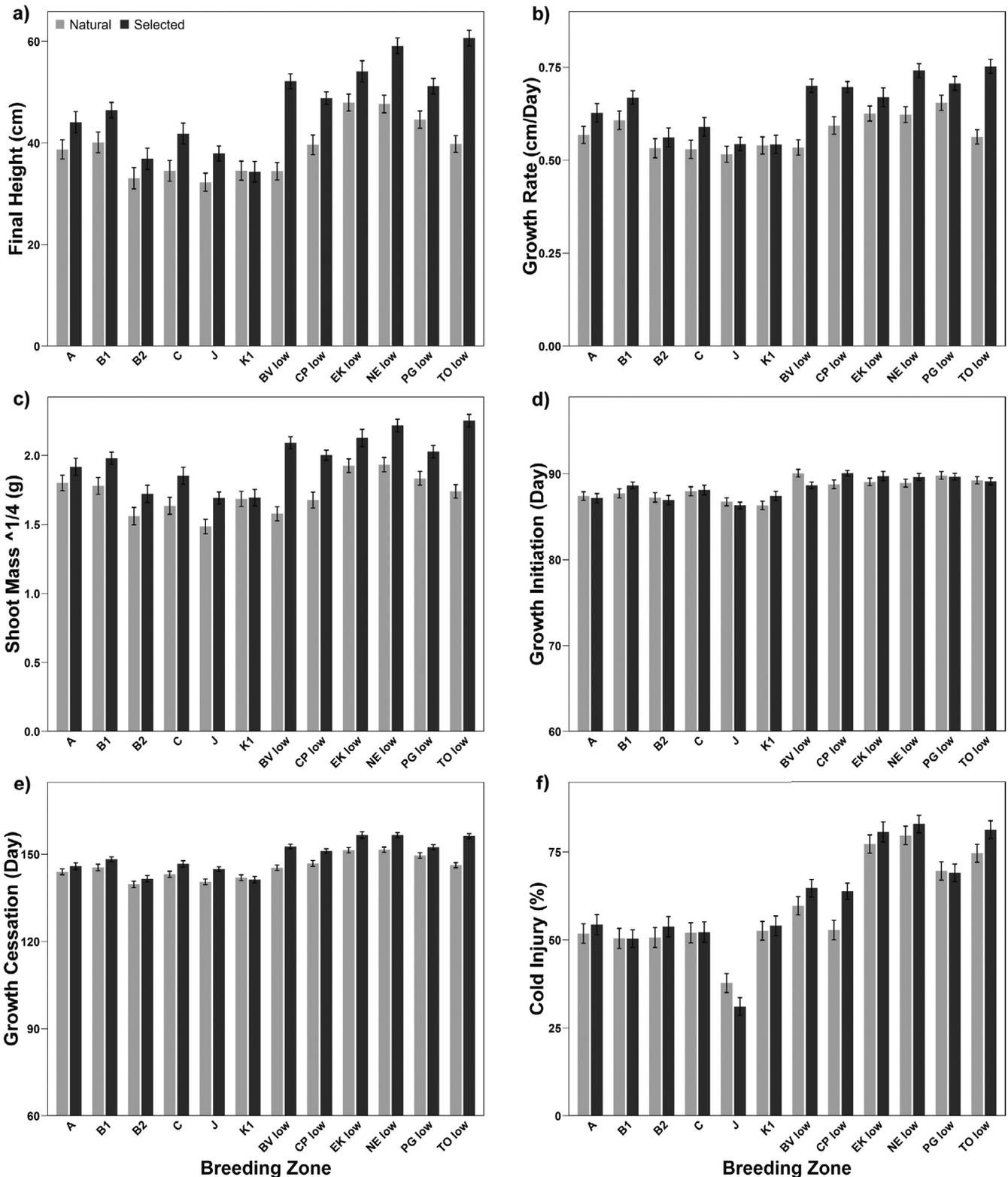


Figure 2. Bar plots of breeding zone level means (BLUEs) including standard error bars, for seedling (a) height; (b) growth rate; (c) shoot dry mass (quarter-root transformed); (d) growth initiation; (e) growth cessation; and (f) cold injury.

among breeding zones and stronger climatic clines for all traits we assessed. The increased height of selected seedlings was underlain by stronger correlations across breeding zones with faster growth rate and slightly later growth cessation. Crucially, autumn cold hardiness remained similar between natural and selected seedlings

and was not significantly correlated with increased height growth resulting from selection. This indicates that selection and breeding for greater growth within local populations has not compromised the cold hardiness of seedlings used in operational reforestation compared to natural seedlings.

Table 2

Proportion of phenotypic variance among (σ_p^2) and within (σ_e^2) breeding zones (i.e. populations), and population differentiation among breeding zones (V_{POP}) for the six phenotypic traits of both natural and selectively bred seedling types. Standard errors of all estimates are given in brackets.

	Natural					Selected						
	σ_p^2		σ_e^2		V_{POP}	σ_p^2		σ_e^2		V_{POP}		
Seedling height	27.519	(12.945)	205.89	(9.958)	0.118	(0.056)	70.004	(31.223)	248.08	(10.828)	0.220	(0.101)
Growth rate	0.002	(0.001)	0.031	(0.001)	0.052	(0.028)	0.005	(0.002)	0.037	(0.002)	0.124	(0.058)
Shoot mass	0.018	(0.009)	0.186	(0.009)	0.088	(0.043)	0.035	(0.016)	0.222	(0.01)	0.135	(0.063)
Growth initiation	1.071	(0.573)	19.465	(0.944)	0.052	(0.028)	1.403	(0.676)	14.904	(0.652)	0.086	(0.042)
Growth cessation	14.068	(6.393)	65.012	(3.172)	0.178	(0.082)	30.116	(13.175)	57.131	(2.501)	0.345	(0.16)
Cold injury	169.05	(73.381)	238.53	(11.218)	0.415	(0.195)	234.62	(100.99)	196.24	(8.254)	0.545	(0.267)

4.1. Effects of selection on adaptive traits

Seedling growth trait differences between natural and selected seedlings from the same geographic areas in our study reflect height gains achieved by selective breeding programs. Broadly, these gains are greater in BC where breeding programs have applied a greater selection intensity, and have undergone an additional breeding cycle relative to AB. Growth traits have relatively low phenotypic differentiation among breeding zones for both seedling types. Estimates of V_{POP} (Table 2) fall into the bottom third of Q_{ST} ranges for height and growth traits summarised by Savolainen et al. (2007) and Alberto et al. (2013), but they are congruent with those of Liepe et al. (2016) who estimated V_{POP} from >250 natural populations, including all those in our study, in multiple growth chambers rather than outdoor common garden experiments. Selected seedlings have greater V_{POP} values for growth traits and steeper slopes of clines with MAT (Fig. 3a–c), albeit not significantly. This indicates that population differentiation and climatic associations are stronger in selected seedlings, which corresponds to similar findings from selectively bred progeny of other conifers in BC (O'Neill et al., 2014).

Natural seedlings showed modest relationships between MAT and growth initiation timing among breeding zones (Rehfeldt and Wykoff, 1981; Chuine et al., 2006). Selected seedlings had slightly stronger relationships between MAT and growth initiation, but across all breeding zones and both seedling types, timing of mean breeding zone growth initiation spanned only 3.5 days (Figs. 2d and 3d). This suggests, consistently with a number of other temperate-boreal conifers, that timing of bud break and growth initiation is under strong genetic control in response to heat sum accumulation but varies little among populations (Bigras et al., 2001; Cooke et al., 2012). In contrast, growth cessation had the second largest breeding zone differentiation (V_{POP}) out of all traits, as expressed by V_{POP} values, and had the greatest V_{POP} difference between seedling types (Table 2). Clines in temperature-related climatic variables for growth cessation (Fig. 3e and Table S5) were also stronger for selected seedlings. In natural populations, our growth cessation results are consistent with the bud set V_{POP} values of Liepe et al. (2016), and previously reported clines for lodgepole pine in temperature-related climatic variables (Rehfeldt, 1988; O'Reilly and Owens, 1989). However, for growth cessation in both seedling types there were only weak to moderate clines associated with latitude, and therefore photoperiod, as the primary cue for growth cessation in woody plants (Bigras et al., 2001; Petterle et al., 2013).

In line with other conifers from this region (Bansal et al., 2015; Rehfeldt, 1983; Hannerz et al., 1999), cold hardiness showed the strongest association of any trait to local climate (Fig. 3f, Table S5), reflecting the importance of cold hardiness as an adaptive trait (Howe et al., 2003). Both V_{POP} and clines were slightly stronger for selected seedlings, but the differences are relatively small compared to the effects of selection on growth traits and growth cessation. For cold hardiness, we did not observe clines

in elevation identified by other studies of natural populations, likely because our seedlings are selected from within breeding zone elevational limits (Table 1) that are narrow relative to the species range in elevation. Therefore, clines in cold hardiness and the remaining traits are more likely to reflect broad regional gradients, principally in latitude, rather than steep local gradients mediated by topography.

4.2. Correlated responses to selection

Strong correlations between average population seedling height and the other traits we measured conform to expectations of how traits co-vary across natural environments in lodgepole pine and other temperate conifers (Rehfeldt and Wykoff, 1981; Rehfeldt, 1988; Howe et al., 2003; Savolainen et al., 2007). Differences in the strength of correlations between seedling types are small (Table 3), suggesting that negative trade-offs between height gains and phenology or cold hardiness within populations are weak or non-existent. As a result, selection within populations for growth has not compromised local adaptation to low temperatures.

Although changes in the strength of trait-trait correlations resulting from selective breeding are small, among-population height differences between seedling types correlate strongly with differences in both growth rate ($r = 0.98$, $p < 0.0001$) (Fig. 4a) and growth cessation timing ($r = 0.94$, $p < 0.0001$) (Fig. 4c). Therefore, our selected seedlings attained greater height by growing both faster and longer. We cannot truly separate the respective contributions of these effects, although our results support those of Chuine et al. (2001) who found more of the among-provenance height differences are associated with growth rate than with phenology in lodgepole pine. Correlations of the differences between seedling types for height vs growth initiation and height vs cold injury were weak and not significant (Fig. 4b and d). This implies that height gains achieved by extending the growing season are mostly derived from delayed growth cessation, rather than earlier growth initiation, while negative trade-offs in phenology and cold hardiness attributable to breeding for increased height are weak, conforming to the predictions of Rehfeldt (1989).

Cold hardening and shoot dormancy follow growth cessation and bud set in what typically is thought of as a sequential process with two or three steps. Growth cessation is triggered when night length exceeds a genotype's critical value (Petterle et al., 2013). This stimulates bud formation and initial cold hardening (Cooke et al., 2012). Critical night length is a cue for climatically adaptive bud set and autumn cold acclimation that reflects the local timing and severity of low temperatures which are the selective agents. Relationships between growth cessation and latitude (a proxy for photoperiod) are relatively weak across the 9.5° of latitude sampled in this study (natural $r^2 = 0.39$, selected $r^2 = 0.35$) (Table S5). Growth cessation has much stronger relationships with extreme minimum temperature (EMT) (natural $r^2 = 0.62$, selected $r^2 = 0.85$) (Table S5). Similarly, cold hardiness has strong relationships with latitude, MAT and EMT that differ little between natural and selected seed-

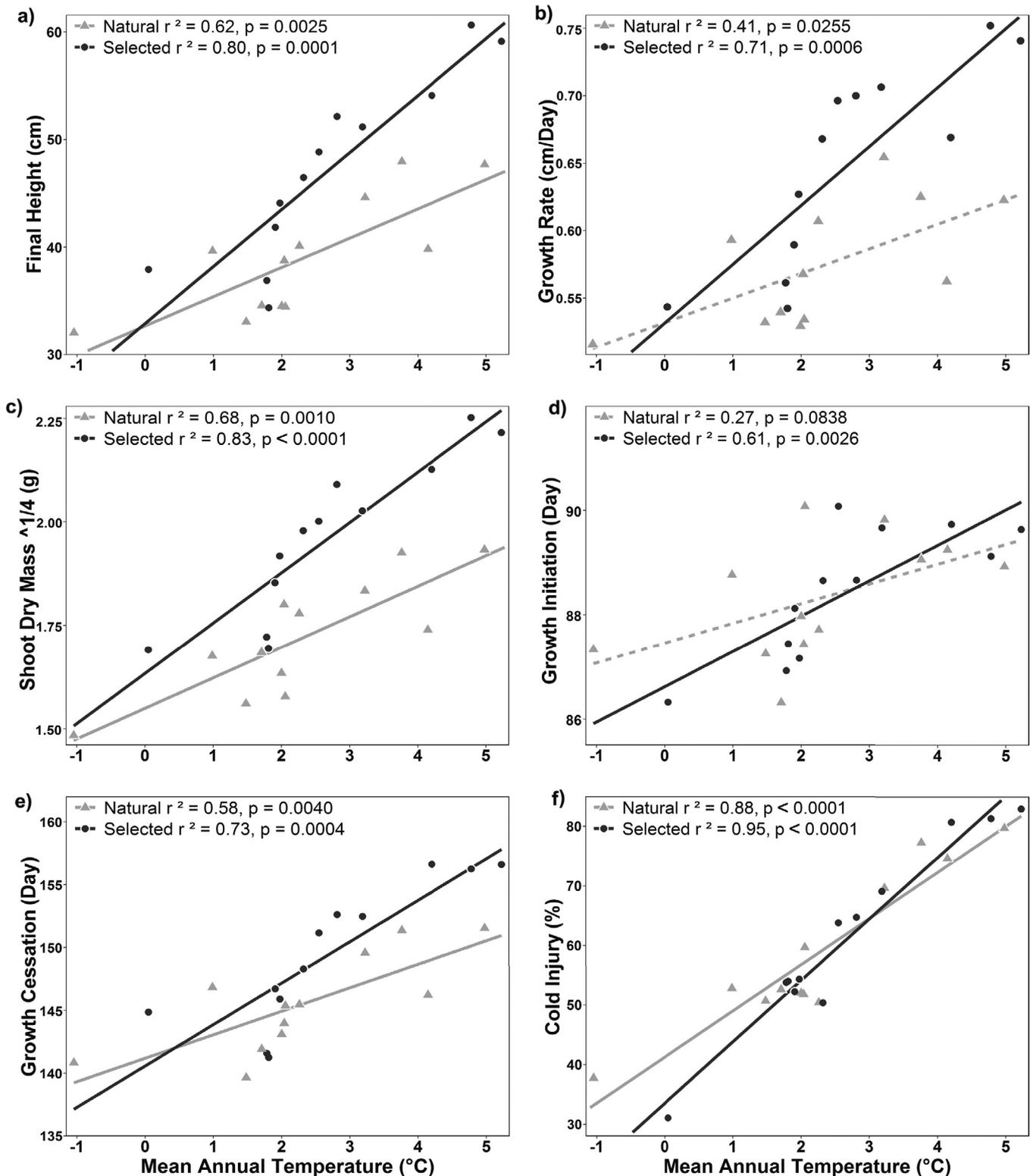


Figure 3. Clines with MAT for seedling (a) height; (b) growth rate; (c) shoot dry mass (quarter-root transformed); (d) growth initiation; (e) growth cessation; and (f) cold injury. Points represent trait means displayed in Fig. 2 and source MAT means for each of 12 breeding zones as the dependent and independent variables respectively. Cline r^2 values are significant at an $\alpha = 0.0045$ cut-off value after correction for multiple comparisons across 11 climate variables per seedling type.

lings, and the correlation between growth cessation and cold hardiness is almost identical between seedling types ($\Delta r = 0.01$) (Table 3). Therefore, selectively bred seedlings have greater growth and similar cold hardiness to natural seedlings, despite slightly delayed growth cessation, and breeding does not appear to compromise adaptive relationships with low temperatures.

Our finding that selective breeding programs increase the strength of climatic clines across breeding zones in growth traits might initially seem like a paradox; gains from selective breeding in growth might be expected to result from trade-offs with phenology or cold hardiness traits. However, this is not the case. Breeding programs sampled here test progeny over three or more typical

Table 3

Pairwise correlation coefficients between all six traits for natural and selected seedlings. Correlations are calculated using breeding zone means of each trait. p-values are statistically significant at an adjusted $\alpha = 0.0033$ cut-off value for 15 correlations per seedling type.

Comparison	Seedling type	r	p-value
Height - Growth Rate	Natural	0.92	<0.0000
	Selected	0.96	<0.0000
Height - Shoot Mass	Natural	0.94	<0.0000
	Selected	0.99	<0.0000
Height - Growth Initiation	Natural	0.52	0.0827
	Selected	0.80	0.0019
Height - Growth Cessation	Natural	0.95	<0.0000
	Selected	0.97	<0.0000
Height - Cold Injury	Natural	0.83	0.0009
	Selected	0.86	0.0004
Growth Rate - Shoot Mass	Natural	0.86	0.0003
	Selected	0.96	<0.0000
Growth Rate - Growth Initiation	Natural	0.49	0.1021
	Selected	0.85	0.0005
Growth Rate - Growth Cessation	Natural	0.87	0.0002
	Selected	0.91	<0.0000
Growth Rate - Cold Injury	Natural	0.66	0.0191
	Selected	0.81	0.0014
Shoot Mass - Growth Initiation	Natural	0.33	0.2985
	Selected	0.82	0.0012
Shoot Mass - Growth Cessation	Natural	0.85	0.0005
	Selected	0.96	<0.0000
Shoot Mass - Cold Injury	Natural	0.78	0.003
	Selected	0.87	0.0002
Growth Initiation - Growth Cessation	Natural	0.71	0.0092
	Selected	0.84	0.0007
Growth Initiation - Cold Injury	Natural	0.67	0.0177
	Selected	0.81	0.0016
Growth Cessation - Cold Injury	Natural	0.85	0.0005
	Selected	0.84	0.0007

field sites within a breeding zone to estimate the genetic value of parent trees and make selections. To perform successfully, families must be well adapted to climatic conditions on all of the test sites. Thus, selection and breeding within populations produces faster-growing phenotypes, and progeny testing in natural environments within breeding zones constrains phenotypes within the limits of local climates. Selected genotypes grow taller by growing faster and for slightly longer, while refining trade-offs between growth, growth cessation and dormancy to maintain cold hardiness and climate adaptation.

4.3. Mechanisms of growth responses to selective breeding

The increased height growth and stronger height-climate associations in selected seedlings could be achieved by several indirect or direct mechanisms, including the following. (1) Selection may favour genotypes equivalent to those from warmer adapted populations. (2) Parent trees in breeding programs may be selected from parts of the breeding zone where climates are most favourable to growth. (3) Pollen contamination may originate from warmer-adapted populations local to seed orchards. (4) Seed size and conditions during seed development could result in epigenetic effects on seedling phenotypes. (5) Selected genotypes may have more phenotypic plasticity than unselected genotypes. Here we address each of these possibilities.

Artificial selection has not resulted in growth cessation and cold hardiness phenotypes equivalent to those of faster growing but less cold-adapted natural populations (Rehfeldt, 1992a, 1992b). On a trait-by-trait basis, mean phenotypes may be equivalent to those of other breeding zones. However, considering all traits in combination and the correlations among them, it appears that provenance testing, selection, breeding and subsequent progeny testing have not reproduced pre-existing phenotypes similar to

those from warmer provenances, but produced phenotypes well suited to their progeny test environments.

Microgeographic phenotypic and genetic variation contributes within-stand genetic structure of forest trees (reviewed in Scotti et al. (2016)). Genetic gain could result from simply selecting productive parent trees from warmer sites that are more favourable for growth within topographically and climatically heterogeneous breeding zones. This hypothesis is not supported by our results, because differences between mean source MATs of natural seedlings and weighted mean MATs of selected seedlings explain a negligible proportion of the variation in observed height gains.

Seed orchards in AB and BC are typically located on warmer, drier sites that are optimal for intensive seed production, but may be geographically distant from their breeding zone of origin. Using neutral molecular markers, pollen contamination in two of the interior BC lodgepole pine seed orchards where we obtained seed was previously estimated to be 8% and 14.5% (Funda et al., 2014; Stoehr and Newton, 2002). It is possible that pollen contamination from nearby warm-adapted populations or other seed orchards could contribute to greater height growth in selected seedlings, but this effect would be greatest in seedlings from cooler breeding zones and would reduce rather than increase the slopes of clines in height with MAT for selected seedlings.

Conditions during seed development are also known to contribute to differences in growth among provenances. Larger seeds are expected to confer greater seedling growth and survival (Castro, 1999 and references therein). Cultural practices in lodgepole pine seed orchards favour the production of relatively large seeds that germinate and grow rapidly. Larger average seed size could contribute to observed differences between natural and selected seedlings, particularly for growth traits. However, weighting our linear mixed models (Eq. (3)) by average seedlot seed mass had no tangible effect on BLUEs of trait means, and correlations between seed weight and means of all six phenotypic traits were weak ($r < 0.25$, except growth initiation $r = -0.39$, $p = 0.06$).

Temperature effects on seed development have also been associated with epigenetic changes in some conifers (Bräutigam et al., 2013). Extensive studies on Norway spruce show that progeny from cold-adapted provenances grown using seed produced in warmer environments grow taller, cease growth later and are less cold hardy than progeny from seeds with the same genetic background collected in the respective cold adapted wild stands (Johnsen et al., 2005; Skråppa et al., 2007). This is because temperature regulates differential gene expression during embryo development and seed maturation, leaving a long-term epigenetic memory that affects the progeny's phenotype. Similar effects have been reported in *Pinus* species, although they only lasted for a few years (Dormling and Johnsen, 1992; Schmidting and Hipkins, 2004). The epigenetic effects of temperature on seed maturation and selected seedling phenotypes are not directly quantifiable in our study, but the evidence we have suggests they are likely to be weak by the third year of growth. Differences between breeding zone and seed orchard MSTs explained little variation in height gains, or in the remaining traits.

By using a single common garden site in a mild climate we were able to make many repeated growth, phenology and cold hardiness measurements, as the basis to quantify and test differences among populations and between seedling types in the absence of stress-related seedling mortality (Campbell, 1986). With this experimental design, we cannot rule out the possibility that phenotypic plasticity varied among breeding zones or between seedlot types. A plastic response to the mild climate may have contributed to height gains of selected seedlings, especially for seedlings from warmer breeding zones. This could contribute to the stronger relationship we observe between growth and climate in selected seed-

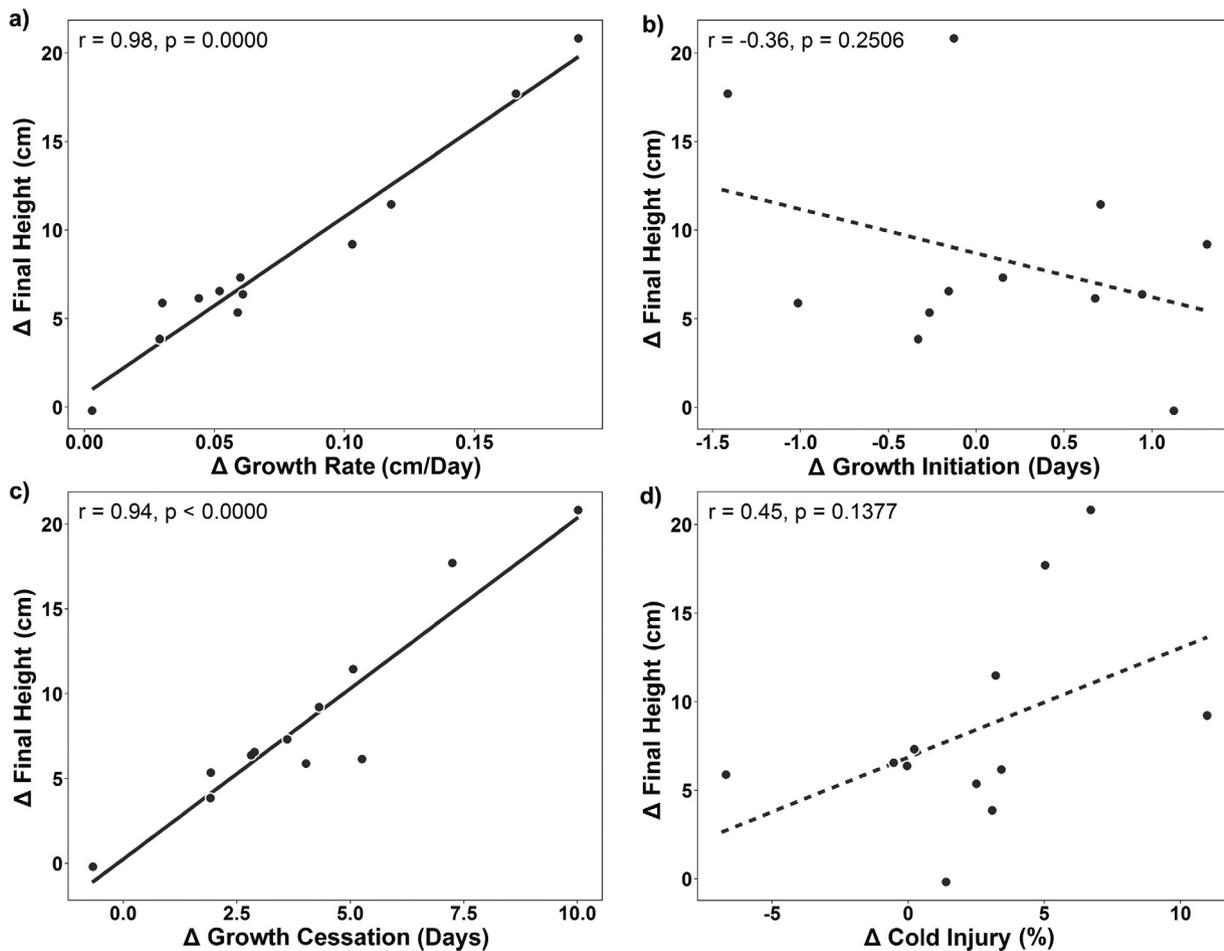


Figure 4. Correlations of differences in the values of height between natural and selected seedlings of each breeding zone with equivalent differences in phenology and cold injury traits. Difference correlations are plotted for height versus (a) growth rate; (b) growth initiation day; (c) growth cessation day; and (d) cold injury. Correlations are significant at an $\alpha = 0.0125$ cut-off value after Bonferroni correction for four comparisons.

lings. However, if this effect occurs we think it is likely to be small, because our observed height gains are consistent with estimates of genetic gain for growth from field progeny tests in provincial breeding programs.

The primary reason for the stronger climatic associations with height and stronger phenotypic differentiation among breeding zones in selected versus natural seedlings is the greater genetic gain that has been achieved in the warmer breeding zones. Even if breeding values for height were equal among breeding zones, zones with taller seedlings would have greater absolute height gains because breeding values are estimated relative to the base population mean. Climatically favourable breeding zones have the tallest seedlings, and the highest field-based breeding value estimates for growth, while the need for adaptation to extreme low temperatures constrains genetic gains in growth in the coldest zones. Breeding program history also varies among zones, and the oldest most advanced programs have achieved the greatest gains. Similarly, greater population differentiation (V_{POP}) of selected seedlots reflects stronger growth climate relationships. For all traits, variance among breeding zones (σ_p^2) increased with selection relative to variance within breeding zones (σ_e^2). Greater population differentiation corresponds to stronger clinal variation and a narrowing of the climatic niche within breeding zones. These effects mean that the greater realised growth gains in climatically favourable breeding zones account for most of the greater phenotypic differentiation among breeding zones and stronger growth clines in selected versus natural seedlings. The strong correlations

between growth and other adaptive traits in selected seedlings result in stronger clines in phenology and cold hardiness traits of selected seedlings. However, clinal responses are variable among traits because trait-trait correlations are imperfect and do not change consistently between seedling types.

5. Selective breeding and assisted gene flow in a changing climate

AGF is a promising, proactive strategy to mitigate the negative impacts of climate change on the health and productivity of planted temperate and boreal forests (Gauthier et al., 2014; Gray et al., 2016). Strong phenotypic clines among populations of both natural and selected seedlings support adopting CBST and AGF policies to accurately redeploy seedlots for future climates. Selective breeding of lodgepole pine should be compatible with CBST and AGF because it produces seedlings that grow vigorously and are well adapted to recent local climatic conditions. Within breeding zones, the faster growth of selected seedlots should buffer some of the short-term negative impacts of climatic change on forest productivity for as long as their growth exceeds that of natural populations. This runs contrary to concerns that selective breeding and the increased deployment of selected seedlings might negatively impact climatic adaptation and future AGF.

Artificial selection for greater height growth has strengthened associations between adaptive traits and climate in lodgepole pine. Cold hardiness has far stronger relationships with climate than any

other trait, but artificial selection for greater growth has not substantially decreased cold hardiness. While selectively bred seedlings grow faster than natural populations from the same geographic areas, they are not the adaptive equivalents of natural populations from warmer climates because their tolerance of cold injury is largely maintained. By extension, differences in growth observed among natural populations in provenance trials should not be considered a proxy for the effects of selective breeding on other adaptive traits. In future, the suitability of selectively bred populations for reforestation must be assessed in relation to the complete testing, selection and breeding process, rather than just the isolated effects of selection.

Growth differences among breeding zones in our seedling common garden reflect similar patterns of variation among natural populations identified from long-term lodgepole pine field trials. By decomposing the relationships between growth, phenology and cold hardiness, we found that selective breeding within zones has balanced these phenotypic components of adaptive variation. The growth potential of selected seedlots is increased under favourable conditions, yet adequate phenological synchronisation and autumn cold hardiness is retained. Through replicated long-term provenance and progeny tests in a range of field test site conditions, lodgepole pine breeding programs in AB and BC have effectively avoided negative trade-offs between growth and adaptive traits. Climatic adaptation of selected lodgepole pine seedlings has not been compromised in terms of phenology or cold hardiness relative to natural seedlings, and on this basis different AGF prescriptions for natural stand and selectively bred seedlots are not warranted. Assisted gene flow of selectively bred seedlots is a valid mechanism for increasing the productivity of lodgepole pine under future climates in western Canada.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.foreco.2017.02.008>.

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