esa
ECOSPHERE

Decline in the strength of genetic controls on aspen environmental responses from seasonal to century-long phenomena

I. Drobyshev [1,2,†] S. Picq [1] E. Ganivet [1] F. Tremblay [1] and Y. Bergeron [1]

[1]*Institut de recherche sur les forêts, Université du Québec en Abitibi-Témiscamingue, 445 Boulevard de l'université, Rouyn-Noranda, Canada J9X 5E4*
[2]*Southern Swedish Forest Research Centre, Swedish University of Agricultural Sciences, PO Box 49, SE-230 53 Alnarp, Sweden*

Citation: Drobyshev, I., S. Picq, E. Ganivet, F. Tremblay, and Y. Bergeron. 2019. Decline in the strength of genetic controls on aspen environmental responses from seasonal to century-long phenomena. Ecosphere 10(9):e02869. 10.1002/ecs2.2869

Abstract. Understanding intra-specific variation in climate sensitivity could improve the prediction of tree responses to climate change. We attempted to identify the degree of genetic control of tree phenology and growth of trembling aspen (*Populus tremuloides* Mchx.) in a natural stand of this species in northwestern Quebec. We mapped and genotyped 556 aspen trees growing within the plot, using seven nuclear microsatellite loci for clone identification. We selected 13 clones (*n* of trees per clone >5, in total 350 trees) and evaluated the explanatory power of clone identity in (a) variability of spring leaf phenology and (b) short- and long-term growth responses. The clone's identity explained 43% of the variability in spring leaf phenology, between 18% and 20% of variability in response to monthly climate variables significantly affecting growth, between 8% and 26% of growth response to insect outbreaks, and 12% in the long-term growth rates. Strong clonal control of aspen phenology and moderate control of growth responses to monthly weather do not result in an equally large impact on long-term growth rates. The result suggests an important role of environmental extremes and within community interactions as factors averaging aspen growth performance at the stand level.

Key words: clonal structure; genetic controls; genotypic diversity; genotyping; growth dynamics; growth resilience; phenotypic plasticity; selection pressure.

Received 3 December 2018; revised 22 July 2019; accepted 24 July 2019. Corresponding Editor: Nancy C. Emery.

† **E-mails:** igor.drobyshev@uqat.ca; igor.drobyshev@slu.se

Introduction

Boreal forests face rapid changes in climatic conditions (IPCC 2014), which raises concerns about the ability of this biome to maintain its structure and ecosystem functions. By the middle of the 21st century, the mean annual temperature in the eastern boreal forests is projected to rise by 2.5–5°C and precipitation by 10–25% (De Elia and Cote 2010). In southern Canada, for example, the mean annual temperature has risen between 0.5°C and 1.5°C and annual total precipitation has increased by 5–35% during the 1900–1998 period (Zhang et al. 2000). The ability of dominant tree species to couple with these changes will ultimately control the degree and rate of resulting vegetation succession toward new stable states.

A better understanding the future growth performance of tree species calls for the analysis of both population-level responses to environmental stresses and their within-population variability (Kremer et al. 2014). The existing research has focused primarily on the population-level effects, assuming a marginal effect of genetic, age, and size differences among trees. Classic dendrochronological research provides an excellent example of such an approach, commonly based

on the analyses of 10–30 dominant trees per stand, to capture a climatic signal in growth dynamics (Fritts 2001, Schweingruber and Nogler 2003). However, recent studies have shown a large variability of growth patterns within tree populations (Nehrbass-Ahles et al. 2014) and indicated that climatic variables identified as significant with the mean chronology may affect only a proportion of the individuals within the population (Ettl and Peterson 1995, Rozas and Olano 2013, Galvan et al. 2014). Understanding the drivers of this variability should help minimize biases in historical (dendrochronological) reconstructions of past climate and better project growth responses to future climate changes (Carrer 2011, Galvan et al. 2014).

Genetic differences among trees may be potentially an important control of within-population variability in growth patterns, especially considering that it is the within-population variability, which accounts for a large part of the total genetic variability in boreal trees (Beaulieu and Bousquet 2010). For example, boreal conifers with a large geographical distribution such as white spruce (*Picea glauca* (Moench) Voss) and white pine (*Pinus strobus* L.) maintain more than 80% of the total genetic variability in growth and phenological characters as within-population variability (Beaulieu et al. 1996, 2006, Li et al. 1997). Since within-species variability in genotypes can influence the growth response to climate (Alberto et al. 2013), it can affect their ability to respond to climate change. It has been hypothesized that during interglacial periods, tree populations have repeatedly colonized northern areas due to large genetic variability in the frontier populations, in particular, the presence of cold-adapted sub-populations in ice-free refugia (De Carvalho et al. 2010, Savolainen et al. 2011).

Quantification of the genotype effects involves strict control of the genetic makeup of trees. Experimental studies have shown that genetic makeup controls 10–30% of the variability in tree size in forest trees growing in plantations (Cornelius 1994). In natural settings, the analysis of genotype effects would involve a clonal species with trees of different clones growing intermingled in a homogeneous environment. In the Canadian boreal forest, the tree species fitting this setup would be trembling aspen (*Populus tremuloides* Michx). This species is the most widely distributed deciduous tree in North America, extending from southern Mexico to northern Alaska and Newfoundland (Little 1971, Perala 1990). Aspen is shade-intolerant, fast growing, and relatively short-lived, colonizing areas after disturbances, such as fires or large windfalls. Aspen seedlings can produce suckers within 4–6 yr following seed germination (Shepperd and Mata 2005). The number of suckers varies according to tree genotype (Farmer 1962, Zufa 1971, Schier 1974). Genetic analyses have revealed large clonal diversity in natural aspen populations (Wyman et al. 2003, Namroud et al. 2005, Mock et al. 2008, De Woody et al. 2009). The emerging picture indicates that aspen populations consist of intermingled clones represented by more than 10 ramets and single ramet genets homogeneously distributed among more abundant clones.

We studied the variation in climate sensitivity of even-aged trembling aspen clones growing in a fire-initiated natural population in western Quebec. We hypothesized that the trees' clone identity affects their growth responses to spring phenology, monthly weather and also affected their absolute growth rates. To quantitatively test this hypothesis, we mapped and genotyped all aspen trees within a one ha plot, and measured their growth using dendrochronological methods. We then assessed the interaction between genotype, on one side, and phenological and growth dynamics, on the other, through ordinary and generalized linear regression models.

Materials and Methods

Study area

The study site is located within the Lake Duparquet Research and Teaching Forest (FERLD) in northwestern Quebec (79°1′ W, 48°30′ N). The FERLD is encompassed in the Eastern Canadian boreal mixedwood zone that is dominated by balsam fir, paper birch, white spruce, trembling aspen, and eastern white cedar (Saucier et al. 2003). The climate is cold temperate with a mean (1971–2000) annual temperature of 0.7°C and mean annual precipitation of 890 mm (Environment Canada 2006). The topography of the area is typically flat, characterized by low elevation hills and a mean altitude

between 250 and 400 m above sea level. The most common soil types on well-drained clay deposits are luvisols and gleysols (Soil Classification Working Group 1998). The forest tent caterpillar (FTC, *Malacosoma disstria*) is a major defoliator of trembling aspen (Bergeron 2000) in the area. A total of six outbreaks have been reported in the region since 1938 (see cluster 8 in fig. 4 in the Cooke and Lorenzetti 2006).

Climatic data

Climate data were obtained for the 1953–2009 period using BioSIM, a set of spatially explicit bioclimatic models using a network of available meteorological stations to generate climate data for a set of user-selected geographical locations (Regniere and Bolstad 1994). We used the spatial regression method, which fits a multiple regression between a climatic variable in question, latitude, longitude, and elevation. The climatic variables included the monthly mean temperature (in °C) and the monthly total precipitation (in mm).

Study plot measurements and topographical data

The studied trees belong to an aspen-dominated stand that originated from the 1923 fire. Since 1998, this stand has been a control treatment (SAFE 1 block 1) of the SAFE (Sylviculture et Aménagement Forestiers Écosystémique) project, which tests stand-level silvicultural treatments emulating different aspects of natural forest dynamics (Brais et al. 2004). In 2005, we established a 1-ha plot in a natural control area of the stand and divided it into a hundred 10×10 m^2 subplots. We tagged and mapped all of the trees with the diameter at breast height (dbh) above 5 cm. We collected samples of root cambium tissue and cored trees at dbh level (two cores per tree) to obtain chronologies of ring width growth from the 556 living and standing aspen trees. We used the Hegyi index to quantify competition or facilitation interactions between trees within the stand (Hegyi 1974; Appendix S1).

DNA extraction, microsatellite amplification, and estimation of genetic diversity

Root cambium tissue samples were dried with silica gel and stored at room temperature. DNA was extracted from the dried tissue using the Extract-N-Amp™ Plant kit (Sigma-Aldrich, St Louis, MO, USA). Individuals were genotyped at seven microsatellite loci, PTR1, PTR2, PTR3, PTR4, PTR6, PTR14, and VVPSM16 (Dayanandan et al. 1998, Rahman et al. 2000, Smulders et al. 2001). We selected these loci due to their high power of genotype definition in trembling aspen (Rahman et al. 2000, Cole 2005, Namroud et al. 2005). See further details in Appendix S1. On the 556 trembling aspen sampled in the 1-ha plot, 62 trees were eliminated from genetic analysis, due to unsuccessful amplification for one or more microsatellites.

Genotypic diversity (R) was estimated by calculating observed clonal diversity (R_o), which is the proportion of different genotypes within a population, using the following formula:

$$R_o = \frac{G_o - 1}{N - 1}$$

where G_o is the number of observed genets and N is the total number of individuals (Dorken and Eckert 2001).

The Simpson's D (D) is the complement of the Simpson index corrected for finite sample size (Pielou 1969). It was calculated as

$$D = 1 - \frac{\sum ni(ni - 1)}{N(N - 1)},$$

where ni is the number of plants with microsatellite phenotype i, and N is the number of plants analyzed. D equals 1 if every plant in a population represents a distinct microsatellite phenotype; D equals 0 if all plants in a population have identical microsatellite phenotypes.

Records of leaf phenology

We used drone-assisted observations of leaf phenology during the period of spring leaf development between the second week of May and the first week of June between 2014 and 2016. This timing, which generally corresponded to the mid-point of leaf development in the stand, allowed us to observe variability in crown development among trees and to use differences in crown coloration as a proxy for differences in the degree of leaf development. For each year, we classified stand canopy into different leaf density classes, using ArcGis 10.2 (ESRI 2011) in four

main steps: (1) geo-referencing drone-derived layers, (2) unsupervised image classification using ArcMap Classification toolbar, (3) supervised image classification using the same tool, and (4) correcting spatial mismatch between tree coordinates, as mapped with the help of a total station theodolite on the ground, and tree crowns as mapped by the drone. See additional detail in the Appendix S1.

Development of tree ring chronologies

All tree cores were mounted on wooden supports, polished, and cross-dated using the visual pointer year method (Stokes and Smiley 1968). Using scanned images of dated samples, we measured the ring widths to the nearest 0.01 mm with the CooRecorder and CDendro software package ver. 7.3 (Larsson 2010). Ring dating was statistically validated with a R package *dplR* (Bunn 2008).

Analyses of growth patterns

We partitioned growth patterns into low- and high-frequency components. To study the former, we used tree diameters measured at breast height in 2010 and regarded them as a proxy of absolute growth rates over the tree lifespan since the stand regeneration. To study the high-frequency component, we analyzed (1) growth response to monthly weather and (2) growth response to outbreaks of FTC. Prior to dendroclimatic analyses, the chronologies were detrended using a spline function with 50% frequency cutoff at 32 yr. Natural persistence (temporal autocorrelation) in chronologies was removed by modeling each curve as an autoregressive process with the order selected by the first-minimum Akaike Information Criterion (AIC; Akaike 1974).

To assess clone-level differences in growth response to monthly weather, we averaged the single tree series into clone-specific chronologies and detrended them using the protocol outlined above. We then calculated response function coefficients between growth indices, on one hand, and average monthly temperature and the sum of monthly precipitation from May of the previous growing season to August of the current year, on the other. Response coefficients, in contrast to the ordinary correlation coefficients, are generated using principal component analysis, which removes intercorrelation present in the climatic data (Biondi and Waikul 2004). We tested coefficients for their statistical significance, using the bootstrap method (Efron and Tibshirani 1993) by random re-sampling with the replacement of the original dataset, generating a statistical distribution of a response coefficient, and calculating its 2.5% and 97.5% distribution limits. For the analysis of growth responses to extreme environmental conditions, we first used the Cropper (Cropper 1979) algorithm to select negative growth anomalies. The algorithm involves z-transformation of non-detrended tree ring chronology value in year i within a symmetric moving window of n years and identifying the years with values below the product of the window-specific mean and its standard deviation multiplied by 0.75 (Appendix S1).

Analyses of clonal effects on phenological and growth responses

In this paper, we use the term genetic control to refer to the predictive power of clone identity in explaining variability in phenology and growth, as estimated by R^2 in the respective models. The term therefore does not imply a particular mechanistic relationship between clone identity and the model's predictands. We used an array of spatially implicit and explicit models to test the effect of genotype (i.e., clonal identity) on tree phenology and growth. The statistical setup was similar in all cases but differed in the type of the dependent variables. These were (1) phenology classes, (2) response function correlations between growth indices and monthly weather, and (3) metrics of growth resilience to two outbreaks of FTC. We used a generalized linear (for phenology, which were counts) or an ordinary linear (for other response/dependent variables) regression model:

$$DepVar = \alpha + \beta_1 \cdot Genotype + \beta_2 \cdot Topography + \beta_3 \cdot Competition + error$$

where *Genotype* was a variable representing clone identity, and *Topography* was represented by altitude classes and *Competition* by Hegyi index (see *Study plot measurements and topographical data* section above). In all cases, the dependent variable was resolved at tree level. For dendroclimatic analyses, it implied recalculation of response coefficients for single tree detrended chronologies.

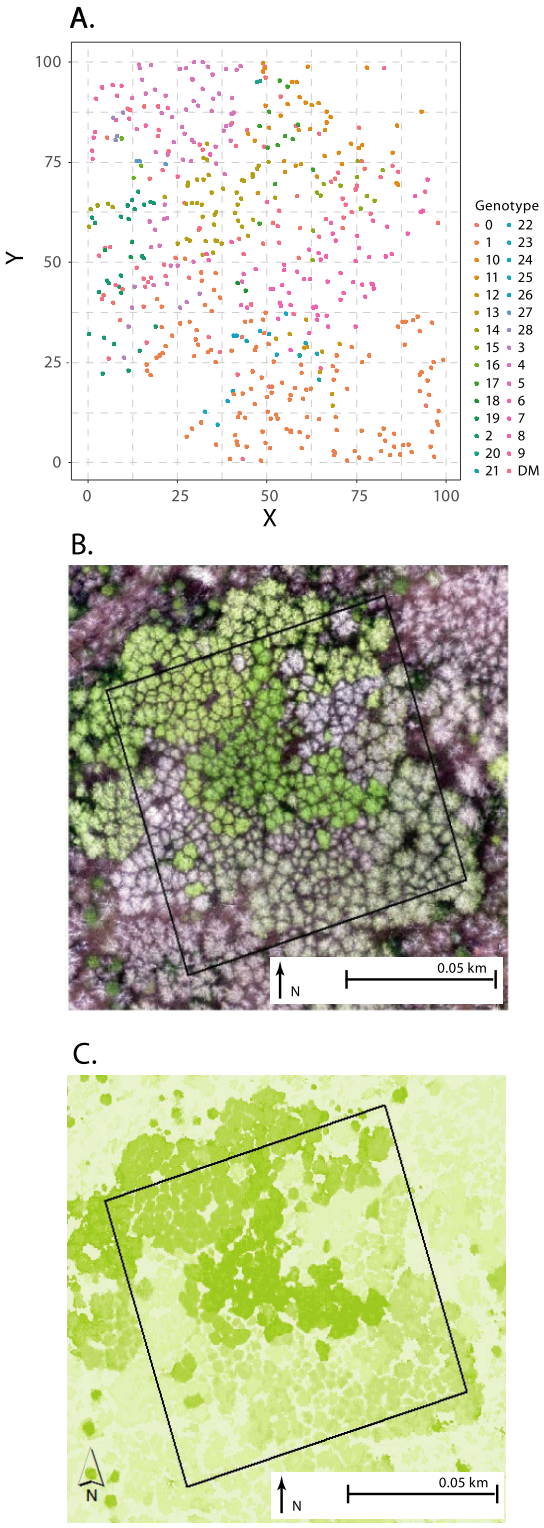

Fig. 1. Map of trembling aspen clones in the studied 1-ha post-fire regenerated stand in Western Quebec (A) and their spring leaf phenology, as revealed by drone-aided photography (spring 2014, B) and resulting canopy classification representing degrees of leaf flashing (C). Darker areas on B and C indicate trees with higher degree of leaf development in the crowns.

The significance of spatial autocorrelation in spatial variability of the studied dependent variables was assessed with the Mantel test calculated with the R function *mantel.rtest* of the R package *ade4* (Dray and Dufour 2007). We also used three spatially explicit models: spatial conditional autoregression model (CAR), spatial simultaneous autoregressive model (SAR), and spatial eigenvector mapping model (SEVM; Dormann et al. 2007), realized in the R package *spdep* (Bivand et al. 2013). Conditional autoregression model is formulated in its general form as:

$$Y = X \cdot \beta + \rho \cdot W(Y - X\beta) + \varepsilon$$

where Y is predictand matrix, X is the predictor matrix, β is the vector describing the slopes associated with predictors in the original predictor matrix X, ρ is a spatial autoregressive parameter, W is the spatial weights matrix, and ε is the error term.

For the SAR model, the general equation is

$$Y = \rho \cdot W \cdot Y + X \cdot \beta + \varepsilon$$

where ρ is the autoregressive parameter, W is the matrix of spatial weights, and β is a vector reflecting the slopes corresponding to predictors in the initial predictor matrix X.

Partial regression was used to partition the variability of the dependent variable due to clone identity, since all models also included topography and competition index (Hegyi index, Hegyi 1974) as explanatory variables. We used the function *varpart* of the R package *vegan* (Oksanen et al. 2013) for the partial regression. The clones with both genetic and dendrochronological data available for at least 5 ramets were selected for further analysis, with the number of analyzed clones reaching 13.

Results

Genotypic and genetic diversity

The stand was composed of several large aggregated clones partially or completely intermingled,

small clones distributed among large clones, and single ramet genets (Fig. 1). Among the 494 trees successfully genotyped, 56 genotypes were identified resulting in a genotypic diversity (R) equal to 0.11. Exactly 50% of the genotypes were composed of only one tree. For the multi-ramet genotypes, the number of ramets varied greatly from 2 to 137 and 43% of the clones had more than 10 ramets. The mean and the median number of ramets per genotype were 8.82 and 1.50, respectively. The mean clonal dimension, that is, the mean distance between ramets within clone, was 23 m with values from 1.02 m for the smallest clone to 86.7 m for the largest one. The variogram of the Simpson's diversity D revealed that at distances inferior to 30 m, the probability of sampling two trees of same genotypes is significantly higher than expected with an absence of clonal spatial structure (Appendix S5).

Effect of genotype on aspen phenology and growth

The analyses of all dependent variables revealed statistically significant effects of clone identity (Appendix S3). Clone identity explained between 8% and 26% of growth response to insect outbreaks, and 12% in the long-term growth rates. The generalized least squares regression models that were used accounted for competition and topography-related effects. The Mantel test revealed a moderate amount of spatial autocorrelation (Appendix S3), although spatial explicit versions of the model (Appendix S6) did not overperform a non-spatial version, which was selected to produce the final results.

Clone identity alone explained 43% of spring leaf phenology, with R^2 values in particular years varying between 35% and 44% (Fig. 2, Appendix S4). Years 1980 and 2001 were identified as the most common negative pointer years, with the largest proportion of trees, which exhibited more than 75% decline in growth in the three years following the event year (65.8% and 92.3% for years 1980 and 2001, respectively). Both years were earlier identified as outbreak years in the studied landscape (Huang et al. 2008).

Aspen growth was strongly and negatively influenced by precipitation in February of the current year and temperature in August of the current year, the pattern being generally consistent across the clones (Fig. 3A). For these months,

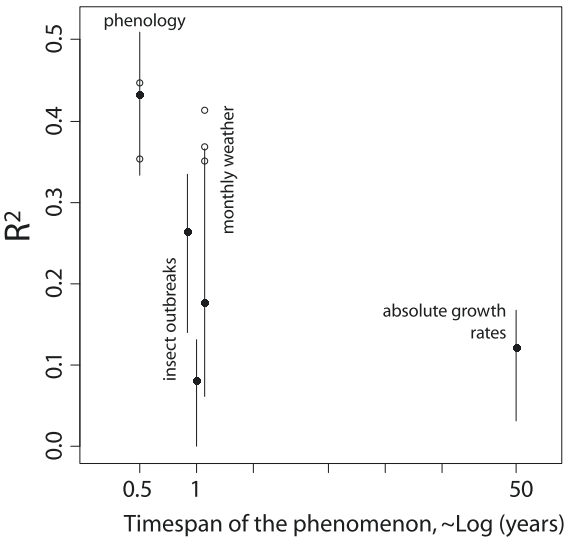

Fig. 2. Genetic control of phenological and growth variability in trembling aspen along a time gradient reflecting typical temporal frequency of respective phenomenon or environmental interactions. Dark dots and vertical lines represent means of R^2 and their respective confidence limits for variable *genotype*, as obtained in generalized least square regression (phenology variable) and ordinary linear regressions (other variables). Bootstrapping was used to generate 95% confidence envelops for all variables, with the exception of monthly climate variables, where we used the quantiles of the R^2 distribution originating from the analyses of all monthly variables. For the phenology variable, values for the three years (2014 through 2016) were averaged. Empty circles indicate R^2 values for the years 2014 through 2016 (phenology variable), and the highest values obtained in the analyses of monthly climate variables. The *x*-axis approximates the temporal scale of the respective effects. For the monthly weather, three empty circles indicate climate–growth correlations with the strongest genetic control. These involve the mean monthly temperature in April and May, and total March precipitation.

clone identity explained between 18% and 20% of the variability in response to coefficients linking growth and weather variables. The total sums of monthly precipitation generally showed a tendency to have higher R^2 values, compared to the average temperatures of the respective months (Fig. 3B). Although R^2 values reached 35–42% for spring temperature and precipitation (March through May; Fig. 3B), these months did not

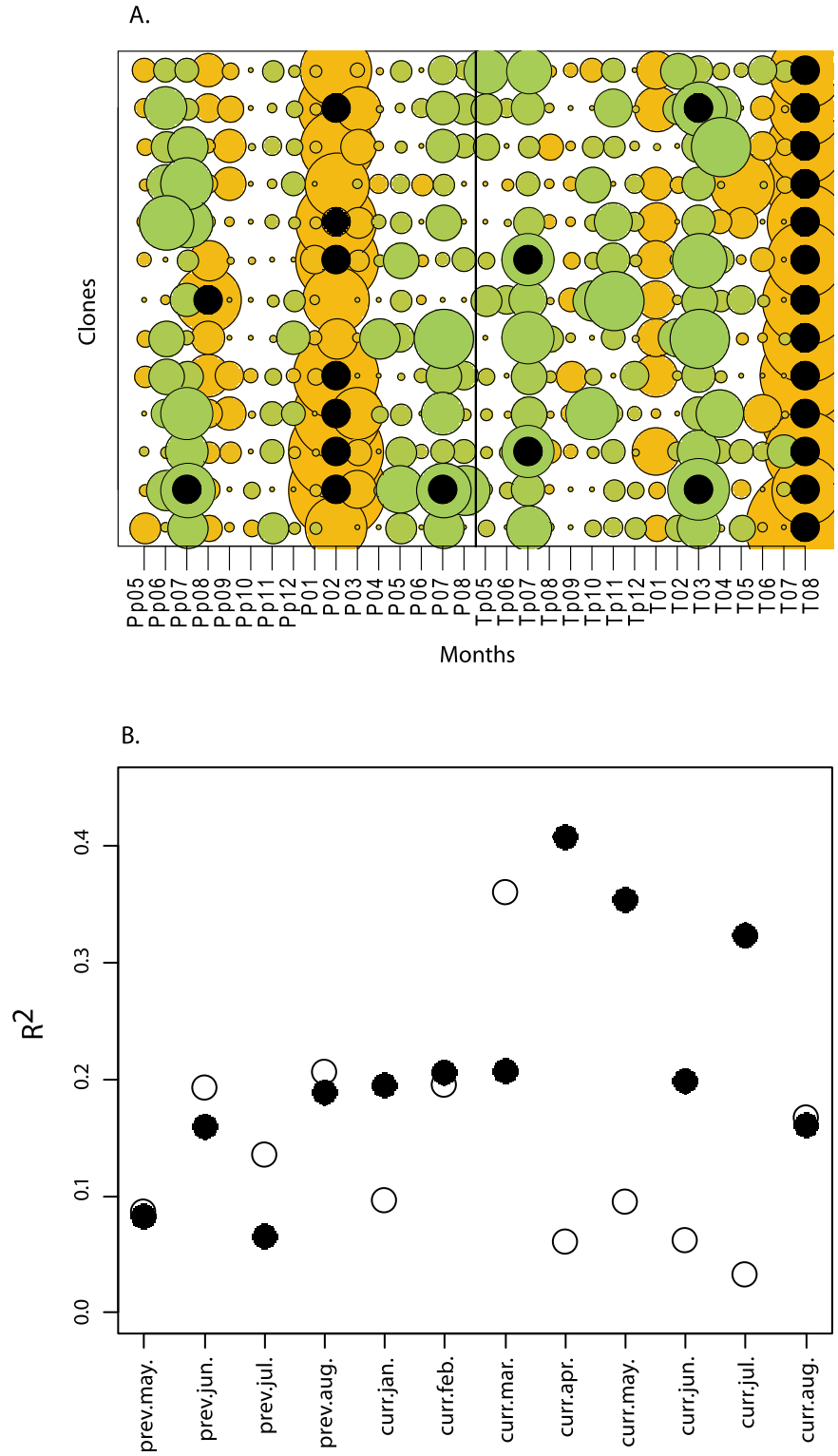

Fig. 3. Influence of aspen clone identity on the growth response to monthly weather. (A) Results of correlation analysis between monthly climate variables and clone-specific master chronologies. Yellow and green and circles

(Fig. 3. *Continued*)
indicate negative and positive coefficients, respectively. Black dots within colored circles refer to significant ($P < 0.05$) response function coefficients. For horizontal axis labels, P and T refer to the total sums of precipitation and average monthly temperature of the months indicated by the respective numbers. Lower-case p indicates months of the previous growing season. (B) Dynamics of explanatory power of clone identity (R^2) in the variability of correlation coefficients between growth and monthly climate variables. Black and white points refer to the total sum of monthly precipitation and average monthly temperature, respectively. "Prev" prefix stands for "previous calendar year."

reveal significant correlation with growth in response function analyses (Fig. 3A).

Clonal effects to studied phenological and growth characteristics appeared non-correlated in most of the pairwise comparisons of residuals from respective regression models (Appendix S7). Out of 15 tested correlations, significant association was observed in only one case and involved growth response following the 2001 insect outbreak. This association, however, was not replicated following the 1980 outbreak.

To assess variability in predictands directly representing growth dynamics, we calculated coefficient of variation (CV), that is, a ration between distribution mean and its standard deviation, for the clone-specific means. Coefficient of variation for growth responses to tent caterpillar outbreaks was 0.23 and 0.33 for the years 1980 and 2010, respectively. For the long-term growth rates, the CV value was 0.07.

Discussion

The studied natural population of trembling aspen exhibited a high level of clonal diversity, which exercised significant yet various levels of controls on tree phenology and growth (this paper and Latutrie et al. 2015). Our results suggest that the strength of these controls generally decreased from high- to low-frequency phenomena (Fig. 2), spring phenology being the process with the strongest genetic control and long-term growth rates—the process with the lowest control. Importantly, we found no correlation between clone-specific responses across a range of studied phenological and growth metrics, therefore, providing no evidence of possible trade-offs or presence of clone-specific growth strategies. We speculate, therefore, that selection pressure was stronger on the absolute growth rates than other phenological and growth metrics. We discuss these findings below in more detail.

Diversity of natural aspen populations

The majority of clones in the post-fire stand of trembling aspen were represented by just few ramets with an average of 8.8 ramets per genotype. The total amount of identified genets was 56, of which 50% was present as a single ramet. Two natural aspen populations established after a fire in the early 19th century and located in the vicinity of the studied stand indicated equally higher genotypic diversity with 75% of the genets composed of only one ramet and only, on average, four ramets per clone (Namroud et al. 2005). Overall, the clonal diversity revealed in our study is comparable with the one found in western Canada, where aspen stands are similarly composed of several intermingled larger clones mixed together with both smaller clones and unique stem genotypes (Mock et al. 2008). However, we did not observe triploidy, that is, the presence of three copies of the genome, which has been earlier reported as a common feature of the aspen population in western Canada, southern Utah, and western Colorado (Mock et al. 2012). However, the proportion of triploids was negligible in the eastern aspen populations compared to what was observed in the southwestern USA (Mock et al. 2012).

Effect of clones on phenological and growth responses

The effect of higher genetic diversity has been traditionally linked to the improved resilience of the tree population to environmental changes (Jump and Penuelas 2005, Bettinger et al. 2009, Latutrie et al. 2015), although conclusive empirical support for this view is still missing. Direct measurements of physiological processes in trees of different genotypes have shown considerable differences in their timing and efficiency (Aspinwall et al. 2011). This is a result, which renders indirect support for the idea of better adaptive capacity of more diverse populations. In the

study of the hybrid poplar (*Populus* spp.), the greater aboveground growth and lower root-to-shoot ratios were observed in polyclonal stands as compared to monoclonal stands, suggesting a certain degree of resource use partitioning among clones (Elferjani et al. 2014).

Clone identity accounted for only 12% of the variability in the long-term growth rates, while it explained 43% of the variability in spring leaf phenology (Fig. 2). We propose three non-exclusive mechanisms that may reduce the effects of genotypes along the gradient from short-term (phenological) to long-term (decadal growth rates) responses. These include physiological integration among clones, effect of environmental extremes, and weak connections between phenology and xylogenesis.

First, physiological integration of trees within the studied plot could partially compensate for genetically controlled differences in spring leaf phenology and, in the long term, lower effects of selection over time and space (Jelinkova et al. 2014). A study of another *Populus* species, *P. balsamifera*, has demonstrated physiological integration of connected ramets, resulting in water sharing (Adonsou et al. 2016*a*). During the periods of insect outbreaks, the ramets of this species with the roots connected to their neighbors have shown 16% better growth, as compared to non-connected ramets (Adonsou et al. 2016*b*). Connected stems do not necessarily belong to the same genotype (Jelinkova et al. 2014). This may further obscure genotypic responses, since connected stems may share photosynthates, impacting each other's radial growth or responses to environmental signals (Baret and Desrochers 2011). Root connections among trees may act as physical signal carriers to synchronize tree physiological performance at the stand scale (Baret and Desrochers 2011).

Second, environmental extremes may downplay the long-term effect of strong genetic control on spring phenology. Growth performance of aspen during two outbreaks of FTC was controlled by genetics by only ~10– 20%, which was ~20% less than genetic control of spring phenology. Moreover, we observed no correlation between clone-specific phenology and the response to the extreme conditions (Appendix S7). Our results point to the absence of clone-specific strategies, suggesting that environmental extremes may reduce potentially large differences in long-term growth rates resulting from differences in leaf phenology among the clones. The finding is consistent with the suggestion that environmental disturbances act as one of the principal controls of productivity in trembling aspen (Hogg et al. 2005), overriding the genetic effects at stand, regional, and probably also sub-continental scales.

Third, studies in other deciduous species have indicated that a connection between phenology and xylogenesis may be generally weak. For example, a study of pedunculate oak has revealed a lack of consistent association between the leaf phenophases and xylogenesis (Puchalka et al. 2017). Research on coniferous species supported the idea that phenology may be considerably more sensitive than xylogenesis to environmental changes, probably due to more "hard-coded" controls of wood production exercised by day length in boreal trees (Rossi et al. 2006, Lupi et al. 2012).

Growth of aspen was negatively affected by February precipitation, due to a likely association between increased winter precipitation and the delay in the onset of the growing season. Consistent with this interpretation were generally high and positive, yet largely insignificant, correlations between growth and March temperatures (Fig. 3A), reflecting a positive response of aspen growth to early snow melt and the onset of the growth season. In *Populus* trees, the highest levels of physiological activity are observed in late spring and early summer (Deslauriers et al. 2009) and one would expect genetic control of climate–growth relationships, particularly for this period of the year. In our study, however, high levels of variability (~30–40%) in climate–growth relationship during spring months, accounted for by clone identity (Fig. 3B), were not accompanied by the significance of response coefficients themselves (Fig. 3A), indicating a limited effect of early season weather variability on aspen growth. The negative effect of August temperatures on growth implied that aspen growth was negatively affected by increased transpiration demand. Clone identity explained ~20% of both effects. In the case of August temperature effect, the result suggested some degree of genetic control over photosynthesis efficiency along temperature gradient (Weston and Bauerle 2007, Weston et al. 2007, Hozain et al. 2010). Such variation in

photosynthesis efficiency may arise due to genetically controlled differences in the thermal properties of RuBisCo activase (Hozain et al. 2010), an enzyme playing an important role in carbon fixation (Portis 2003). Effects of temperature on tree stomatal closure, transpiration rates, and CO_2 availability might be also at play in controlling for this relationship.

Clone identity explained about 12% in variation in aspen long-term growth rates, contradicting a result of a previous study where clone identity had not revealed effects on aspen absolute growth rates nor the variability in its interannual growth along a Canada-wide transect (Latutrie et al. 2015). Our results were generally consistent with estimates obtained for growth variability (e.g., North American willows had R^2 of 12–23%, Mosseler et al. 2017), but was somewhat lower than estimates of genetic control of morphological traits of tree species (~20%, Cornelius 1994). The indirect evidence of limited effect of genetic identity on growth performance of aspen could be an observation that no consistent geographical differences in clonal diversity were found along a continent-wide transect of aspen stands in Canada, stretching over generally wet conditions in eastern Canada to dry conditions in the West (Latutrie et al. 2015). In contrast to our results, a study on *Eucalyptus globulus* has suggested clone-specific strategies of response to environmental extremes, with the different physiological metrics being strongly correlated (Granda et al. 2014).

Would the decline in predictive skill of clone identify not be fully accounted for by factors discussed earlier (see *Effect of clones on phenological and growth responses* subsection above), we propose two non-exclusive explanations of the decline in the predictive skill: (1) past selection pressure and (2) phenotypic plasticity in growth integrating over longer time periods due to microsite differences. However, one can argue that the second interpretation (phenotypic plasticity) does not necessarily contradict the first one (selection pressure). Indeed, we demonstrate that the variability in long-term growth rates is poorly explained by the clone identity. Aspen long-term growth rates showed a rather limited level of variability (CV = 0.07), whatever its nature—phenotypic or genotypic. We consider these results as more consistent with the interpretation operating with the notion of selection pressure and also as an indication that the alleles we studied were not coding for the long-term growth rates.

Speculation on clonal diversity and stand growth resilience

Clonal diversity may have a positive, yet apparently limited, effect on growth resilience at the stand scale. It remains unclear how strong this effect is at larger spatial scales. At stand scale, we found no evidence of clone-specific physiological strategies, as approximated by the respective growth reactions, in response to non-extreme (seasonal phenology) and extreme (insect outbreaks) conditions. Further, lack of correlation between clone-specific growth reactions following two defoliation events pointed to a stochastic nature of this response. A low amount of variability in the long-term growth rates accounted for by clone identities suggests that natural selection at the clonal level was likely driven by long-term climate conditions, that is, long-term environmental predictability (Reed et al. 2011, Chevin et al. 2013), rather than by maximization of the growth rates in respect to growth season onset or avoidance of large nutrient losses during the insect-caused defoliations. Assuming this hypothesis holds, it is easy to understand why such climatic extremes act as the principal drivers of aspen productivity (Hogg et al. 2005). A considerable amount of phenological and annual growth variability was likely unrelated to variability in clone fitness (Mitton and Grant 1984), making these traits escape from selection pressure. It is unclear whether these conclusions hold for larger spatial scales at which variability in climate conditions would exercise an increasingly stronger control on clonal selection. Climate change lowers climate predictability at multiple temporal scales (Hurrell et al. 2006, Latif et al. 2006) and may lead to climate exercising a stronger selection pressure on clonal fitness. Would it be the case, the local variability in fitness-related traits should be reduced as selection pressure filters out unfit genotypes.

The relationship between the level of selection pressure and the level of genetic control apparently depends on a chosen temporal scale (more aligned to the scale of ecological processes as in this study or, alternatively, to species evolution)

and the study spatial scope. Since we did analyses on a very local scale, our study operated with pool of genotypes that were probably preselected at larger (both spatial and temporal) scales. It follows that the effect of stronger genetic controls of traits controlling the fitness of the individuals should be increasingly more detectable in the analyses operating at such large scales.

Acknowledgments

The study was supported by FRQNT (grant PR-191194 to FT) and CRSNG (grant 150680937 to YB). We thank Prof. Annie Desrochers (UQAT) for many useful comments on an earlier version of the MS, Tatiana Khakimulina for help with the measurement of tree rings, and two anonymous reviewers for providing insightful comments on an earlier version of the paper.

Literature Cited

Adonsou, K. E., A. Desrochers, and F. Tremblay. 2016*a*. Physiological integration of connected balsam poplar ramets. Tree Physiology 36:797–806.

Adonsou, K. E., I. Drobyshev, A. Desrochers, and F. Tremblay. 2016*b*. Root connections affect radial growth of balsam poplar trees. Trees-Structure and Function 30:1775–1783.

Akaike, H. 1974. A new look at the statistical model identification. IEEE Transactions on Automatic Control 16:716–723.

Alberto, F. J., et al. 2013. Potential for evolutionary responses to climate change evidence from tree populations. Global Change Biology 19:1645–1661.

Aspinwall, M. J., J. S. King, J. C. Domec, S. E. McKeand, and F. Isik. 2011. Genetic effects on transpiration, canopy conductance, stomatal sensitivity to vapour pressure deficit, and cavitation resistance in loblolly pine. Ecohydrology 4:168–182.

Baret, M., and A. Desrochers. 2011. Root connections can trigger physiological responses to defoliation in nondefoliated aspen suckers. Botany-Botanique 89:753–761.

Beaulieu, J., and J. Bousquet. 2010. Facteurs genetiques affectant la variabilité des cernes annuels chez les espéces arborescentes nordiques. Pages 137–163 *in* S. Payette and L. Filion, editors. La dendroecologie. Principes, methodes et applications. Presses de l'Université, Laval, Quebec, Canada.

Beaulieu, J., A. Plourde, G. Daoust, and L. Lamontagne. 1996. Genetic variation in juvenile growth of *Pinus strobus* in replicated Quebec provenance-progeny tests. Forest Genetics 3:103–112.

Beaulieu, J., S. Y. Zhang, Q. Yu, and A. Rainville. 2006. Comparison between genetic and environmental influences on lumber bending properties in young white spruce. Wood and Fiber Science 38:553–564.

Bergeron, Y. 2000. Species and stand dynamics in the mixed woods of Quebec's southern boreal forest. Ecology 81:1500–1516.

Bettinger, P., M. Clutter, J. Siry, M. Kane, and J. Pait. 2009. Broad implications of southern United States pine clonal forestry on planning and management of forests. International Forestry Review 11:331–345.

Biondi, F., and K. Waikul. 2004. DENDROCLIM2002: A C++ program for statistical calibration of climate signals in tree-ring chronologies. Computers and Geosciences 30:303–311.

Bivand, R., J. Hauke, and T. Kossowski. 2013. Computing the Jacobian in Gaussian spatial autoregressive models: an illustrated comparison of available methods. Geographical Analysis 45:150–179.

Brais, S., B. D. Harvey, Y. Bergeron, C. Messier, D. Greene, A. Belleau, and D. Par. 2004. Testing forest ecosystem management in boreal mixedwoods of northwestern Quebec: Initial response of aspen stands to different levels of harvesting. Canadian Journal of Forest Research 34:431–446.

Bunn, A. G. 2008. A dendrochronology program library in R (dplR). Dendrochronologia 26:115–124.

Carrer, M. 2011. Individualistic and time-varying tree-ring growth to climate sensitivity. PLOS ONE 6: e22813.

Chevin, L., S. Collins, and F. Lefevre. 2013. Phenotypic plasticity and evolutionary demographic responses to climate change: taking theory out to the field. Functional Ecology 27:967–979.

Cole, C. T. 2005. Allelic and population variation of microsatellite loci in aspen (*Populus tremuloides*). New Phytologist 167:155–164.

Cooke, B. J., and F. Lorenzetti. 2006. The dynamics of forest tent caterpillar outbreaks in Quebec, Canada. Forest Ecology and Management 226:110–121.

Cornelius, J. 1994. Heritabilities and additive genetic coefficients of variation in forest trees. Canadian Journal of Forest Research 24:372–379.

Cropper, J. P. 1979. Tree-ring skeleton plotting by computer. Tree-Ring Bulletin 39:47–59.

Dayanandan, S., O. P. Rajora, and K. S. Bawa. 1998. Isolation and characterization of microsatellites in trembling aspen (*Populus tremuloides*). Theoretical and Applied Genetics 96:950–956.

De Carvalho, D., P. K. Ingvarsson, J. Joseph, L. Suter, C. Sedivy, D. Macaya-Sanz, J. Cottrell, B. Heinze, I.

Schanzer, and C. Lexer. 2010. Admixture facilitates adaptation from standing variation in the European aspen (*Populus tremula* L.), a widespread forest tree. Molecular Ecology 19:1638–1650.

De Elia, R., and H. Cote. 2010. Climate and climate change sensitivity to model configuration in the Canadian RCM over North America. Meteorologische Zeitschrift 19:325–339.

De Woody, J., T. H. Rickman, B. E. Jones, and V. D. Hipkins. 2009. Allozyme and microsatellite data reveal small clone size and high genetic diversity in aspen in the southern Cascade Mountains. Forest Ecology and Management 258:687–696.

Deslauriers, A., A. Giovannelli, S. Rossi, G. Castro, G. Fragnelli, and L. Traversi. 2009. Intra-annual cambial activity and carbon availability in stem of poplar. Tree Physiology 29:1223–1235.

Dorken, M. E., and C. G. Eckert. 2001. Severely reduced sexual reproduction in northern populations of a clonal plant, *Decodon verticillatus* (*Lythraceae*). Journal of Ecology 89:339–350.

Dormann, C. F., et al. 2007. Methods to account for spatial autocorrelation in the analysis of species distributional data: a review. Ecography 30:609–628.

Dray, S., and A. B. Dufour. 2007. The ade4 package: implementing the duality diagram for ecologists. Journal of Statistical Software 22:1–20.

Efron, B., and R. J. Tibshirani. 1993. An introduction to the bootstrap. Chapman and Hall/CRC, New York, New York, USA.

Elferjani, R., A. Desrochers, and F. Tremblay. 2014. Effects of mixing clones on hybrid poplar productivity, photosynthesis and root development in northeastern Canadian plantations. Forest Ecology and Management 327:157–166.

Environment Canada. 2006. Canadian climate normals 1971–2000. Available at www.climate.weatheroffice.ec.gc.ca

ESRI. 2011. ArcGIS Desktop: Release 10.2. Environmental Systems Research Institute, Redlands, California, USA.

Ettl, G. J., and D. L. Peterson. 1995. Extreme climate and variation in tree growth: individualistic response in subalpine fir (*Abies lasiocarpa*). Global Change Biology 1:231–241.

Farmer, R. E. J. 1962. Aspen root sucker formation and apical dominance. Forest Science 8:403–410.

Fritts, H. C. 2001. Tree rings and climate. The Blackburn Press, Caldwell, New Jersey, USA.

Galvan, J., J. Camarero, C. Ginzler, and U. Buentgen. 2014. Spatial diversity of recent trends in Mediterranean tree growth. Environmental Research Letters 9:1–11.

Granda, V., C. Delatorre, C. Cuesta, M. L. Centeno, B. Fernandez, A. Rodriguez, and I. Feito. 2014. Physiological and biochemical responses to severe drought stress of nine *Eucalyptus globulus* clones: a multivariate approach. Tree Physiology 34:778–786.

Hegyi, F. 1974. A simulation model for managing Jackpine stands. Pages 74–90 *in* J. Fries editor. Crown model for tree and simulation. Forest Research Notes 30. Royal College of Forestry, Stockholm, Sweden.

Hogg, E. H., J. P. Brandt, and B. Kochtubajda. 2005. Factors affecting interannual variation in growth of western Canadian aspen forests during 1951-2000. Canadian Journal of Forest Research 35:610–622.

Hozain, M., M. E. Salvucci, M. Fokar, and A. S. Holaday. 2010. The differential response of photosynthesis to high temperature for a boreal and temperate *Populus* species relates to differences in Rubisco activation and Rubisco activase properties. Tree Physiology 30:32–44.

Huang, J. G., J. Tardif, B. Denneler, Y. Bergeron, and F. Berninger. 2008. Tree-ring evidence extends the historic northern range limit of severe defoliation by insects in the aspen stands of western Quebec, Canada. Canadian Journal of Forest Research 38:2535–2544.

Hurrell, J. W., et al. 2006. Atlantic climate variability and predictability: a CLIVAR perspective. Journal of Climate 19:5100–5121.

IPCC. 2014. Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, R. K. Pachauri and L. A. Meyer, editors.]. p. 151. IPCC, Geneva, Switzerland.

Jelinkova, H., F. Tremblay, and A. Desrochers. 2014. The use of digital morphometrics and spring phenology for clone recognition in trembling aspen (*Populus tremuloides* Michx.) and its comparison to microsatellite markers. Trees-Structure and Function 28:389–398.

Jump, A. S., and J. Penuelas. 2005. Running to stand still: adaptation and the response of plants to rapid climate change. Ecology Letters 8:1010–1020.

Kremer, A., B. M. Potts, and S. Delzon. 2014. Genetic divergence in forest trees: understanding the consequences of climate change. Functional Ecology 28:22–36.

Larsson, L.-Å. 2010. Cybis dendro dating program v. 7.3. Cybis Elektronik and Data AB, Saltsjöbaden, Sweden.

Latif, M., M. Collins, H. Pohlmann, and N. Keenlyside. 2006. A review of predictability studies of Atlantic

sector climate on decadal time scales. Journal of Climate 19:5971–5987.

Latutrie, M., P. Merian, S. Picq, Y. Bergeron, and F. Tremblay. 2015. The effects of genetic diversity, climate and defoliation events on trembling aspen growth performance across Canada. Tree Genetics and Genomes 11.

Li, P., J. Beaulieu, and J. Bousquet. 1997. Genetic structure and patterns of genetic variation among populations in eastern white spruce (*Picea glauca*). Canadian Journal of Forest Research 27:189–198.

Little, E. L. 1971. Atlas of United States trees, volume 1, conifers and important hardwoods. Miscellaneous Publication 1146. U.S. Department of Agriculture, Washington, D.C., USA.

Lupi, C., H. Morin, A. Deslauriers, S. Rossi, and D. Houle. 2012. Increasing nitrogen availability and soil temperature: effects on xylem phenology and anatomy of mature black spruce. Canadian Journal of Forest Research 42:1277–1288.

Mitton, J. B., and M. C. Grant. 1984. Associations among protein heterozygosity, growth-rate, and developmental homeostasis. Annual Review of Ecology and Systematics 15:479–499.

Mock, K. E., C. A. Rowe, M. B. Hooten, J. Dewoody, and V. D. Hipkins. 2008. Clonal dynamics in western North American aspen (*Populus tremuloides*). Molecular Ecology 17:4827–4844.

Mock, K. E., et al. 2012. Widespread triploidy in western North American aspen (*Populus tremuloides*). PLOS ONE 7:e48406.

Mosseler, A., J. Major, and D. Ostaff. 2017. Distribution of genetic variation in five coppice growth traits among natural populations of seven North American willow (*Salix*) species. Canadian Journal of Forest Research 47:36–46.

Namroud, M. C., F. Tremblay, and Y. Bergeron. 2005. Temporal variation in quaking aspen (*Populus tremuloides*) genetic and clonal structures in the mixedwood boreal forest of eastern Canada. Ecoscience 12:82–91.

Nehrbass-Ahles, C., F. Babst, S. Klesse, M. Notzli, O. Bouriaud, R. Neukom, M. Dobbertin, and D. Frank. 2014. The influence of sampling design on tree-ring-based quantification of forest growth. Global Change Biology 20:2867–2885.

Oksanen, J., F. G. Blanchet, R. Kindt, P. Legendre, P. R. Minchin, R. B. O'Hara, G. L. Simpson, P. Solymos, H. H. Stevens and H. Wagner 2013. vegan: Community Ecology Package. version 2.0-10.

Perala, D.A. 1990. *Populus tremuloides* Michx. Silvics of North America, 2. Harwoods. USDA Forest Service, Washington, D.C., USA

Pielou, E. 1969. An introduction to mathematical ecology. Wiley, New York, New York, USA.

Portis, A. R. 2003. Rubisco activase - Rubisco's catalytic chaperone. Photosynthesis Research 75:11–27.

Puchalka, R., M. Koprowski, J. Gricar, and R. Przybylak. 2017. Does tree-ring formation follow leaf phenology in pedunculate oak (*Quercus robur* L.)? European Journal of Forest Research 136:259–268.

Rahman, M. H., S. Dayanandan, and O. P. Rajora. 2000. Microsatellite DNA markers in *Populus tremuloides*. Genome 43:293–297.

Reed, T. E., D. E. Schindler, and R. S. Waples. 2011. Interacting effects of phenotypic plasticity and evolution on population persistence in a changing climate. Conservation Biology 25:56–63.

Regniere, J., and P. Bolstad. 1994. Statistical simulation of daily air-temperature patterns in Eastern North-America to forecast seasonal events in insect pest management. Environmental Entomology 23:1368–1380.

Rossi, S., A. Deslauriers, T. Anfodillo, H. Morin, A. Saracino, R. Motta, and M. Borghetti. 2006. Conifers in cold environments synchronize maximum growth rate of tree-ring formation with day length. New Phytologist 170:301–310.

Rozas, V., and J. M. Olano. 2013. Environmental heterogeneity and neighbourhood interference modulate the individual response of *Juniperus thurifera* tree-ring growth to climate. Dendrochronologia 31:105–113.

Saucier, J.-P., P. Grondin, A. Robitaille, and J.-F. Bergeron. 2003. Vegetation zones and bioclimatic domains in Quebec. Resources Naturelle Québec (MRNFP), Québec, Quebec, Canada. https://mern.gouv.qc.ca/english/publications/forest/publications/zone-a.pdf

Savolainen, O., S. T. Kujala, C. Sokol, T. Pyhajarvi, K. Avia, T. Knurr, K. Karkkainen, and S. Hicks. 2011. Adaptive potential of northernmost tree populations to climate change, with emphasis on Scots pine (*Pinus sylvestris* L.). Journal of Heredity 102:526–536.

Schier, G. A. 1974. Vegetative propagation of aspen: clonal variation in suckering from root cuttings and in rooting of sucker cuttings. Canadian Journal of Forest Research 4:565–567.

Schweingruber, F. H., and P. Nogler. 2003. Synopsis and climatological interpretation of Central European tree-ring sequences. Botanica Helvetica 113:125–143.

Shepperd, W. D., and S. A. Mata. 2005. Planting aspen to rehabilitate riparian areas: a pilot study. U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, Fort Collins, Colorado, USA.

Smulders, M. J. M., J. Van Der Schoot, P. Arens, and B. Vosman. 2001. Trinucleotide repeat microsatellite markers for black poplar (*Populus nigra* L.). Molecular Ecology Notes 1:188–190.

Soil Classification Working Group. 1998. The Canadian system of soil classification. Third edition. Agriculture and Agri-Food Canada, Ottawa, Ontario, Canada.

Stokes, M. A., and T. L. Smiley. 1968. An introduction to tree-ring dating. University of Chicago Press, Chicago, Illinois, USA.

Weston, D. J., and W. L. Bauerle. 2007. Inhibition and acclimation of C3 photosynthesis to moderate heat: a perspective from thermally contrasting genotypes of *Acer rubrum* (red maple). Tree Physiology 27:1083–1092.

Weston, D. J., W. L. Bauerle, G. A. Swire-Clark, B. Moore, and W. Baird. 2007. Characterization of Rubisco activase from thermally contrasting genotypes of *Acer rubrum* (*Aceraceae*). American Journal of Botany 94:926–934.

Wyman, J., A. Bruneau, and M. F. Tremblay. 2003. Microsatellite analysis of genetic diversity in four populations of *Populus tremuloides* in Quebec. Canadian Journal of Botany 81:360–367.

Zhang, X. B., L. A. Vincent, W. D. Hogg, and A. Niitsoo. 2000. Temperature and precipitation trends in Canada during the 20th century. Atmosphere-Ocean 38:395–429.

Zufa, L. 1971. A rapid method for vegetative propagation of Aspens and their hybrids. Forestry Chronicle 47:3–9.

Supporting Information

Additional Supporting Information may be found online at: http://onlinelibrary.wiley.com/doi/10.1002/ecs2.2869/full