

# WHAT COMMON-GARDEN EXPERIMENTS TELL US ABOUT CLIMATE ADAPTATION IN PLANTS

## Research Article

Journal of Ecology



# Common-garden experiment reveals clinal trends of bud phenology in black spruce populations from a latitudinal gradient in the boreal forest

Xiali Guo<sup>1,2,3</sup> | Marcin Klisz<sup>4</sup> | Radosław Puchałka<sup>5,6</sup> | Roberto Silvestro<sup>3</sup> | Patrick Faubert<sup>3</sup> | Evelyn Belien<sup>3</sup> | Jianguo Huang<sup>1</sup> | Sergio Rossi<sup>1,3</sup>

<sup>1</sup>Key Laboratory of Vegetation Restoration and Management of Degraded Ecosystems, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou, China; <sup>2</sup>University of Chinese Academy of Sciences, Beijing, China; <sup>3</sup>Département des Sciences Fondamentales, Université du Québec à Chicoutimi, Chicoutimi, QC, Canada; <sup>4</sup>Department of Silviculture and Genetics, Forest Research Institute, Raszyn, Poland; <sup>5</sup>Department of Ecology and Biogeography, Nicolaus Copernicus University, Torun, Poland and <sup>6</sup>Centre for Climate Change Research, Nicolaus Copernicus University, Toruń, Poland

### Correspondence

Jianguo Huang

Email: huangjg@scbg.ac.cn

### Funding information

National Natural Science Foundation of China, Grant/Award Number: 41861124001; Direction général du secteur nord-est, région du Saguenay–Lac-St-Jean, Ministère des Forêts, de la Faune et des Parcs du Québec, Forêt d'Enseignement et de Recherche Simoncouche, the Fondation de l'Université du Québec à Chicoutimi,

**Handling Editor:** Susan Schwinning

### Abstract

1. Climate warming is modifying the movement of air masses over Northern latitudes, producing warming and cooling events across the boreal regions. These new conditions changes may mismatch plant phenology from weather conditions, and affect the growing period of trees. Understanding the processes of local adaptation in bud phenology can help to predict the response of plants to these rapid and unexpected environmental changes.
2. Our study monitored bud burst and bud set weekly during four growing seasons in black spruce [*Picea mariana* (Mill.) B.S.P.] saplings planted in a common garden and originating from five provenances representing the whole latitudinal distribution of the closed boreal forest in Quebec, Canada. We compared the variance in bud phenology among populations and years, and analysed the relationships with temperatures at the origin sites.
3. Bud burst and bud set occurred in mid-May and mid-July, respectively, with a large variability among provenances and between the study years. A delayed bud phenology was observed in the provenances from warmer sites, with bud burst and bud set being 1.1 and 1.4 days later for every additional degree in mean annual temperature at the origin site respectively. Populations with earlier bud bursts also showed earlier bud sets, thus the growing season was similar among provenances. The heritability of bud set was higher than that of bud burst, with estimates of 0.26 and 0.21 respectively. On average, variance in bud phenology among provenances reached 5.3%, which was higher than that within provenances (2.6%). The factor year explained 37.7%–69.7% of the variance in bud phenology.
4. **Synthesis.** The findings demonstrate the evolutionary response to climate variations in the boreal biome. Endogenous and environmental components have different effects on the phases of bud burst and bud set. This complex interaction among drivers of bud phenology may prevent reliable predictions of changes in

the growing season under changing climate. An earlier growth reactivation due to higher spring temperatures could result in higher frost risks to the developing buds. Despite a longer period for photosynthesis expected for the evergreen black spruce under warmer conditions, the period spent for bud development could remain unchanged.

#### KEYWORDS

bud burst, bud set, ecotype, frost, global warming, *Picea mariana*, temperature

## 1 | INTRODUCTION

One of the central questions in ecology is how species are able to survive along wide environmental ranges, especially in the boreal forest, where the low temperature is limiting plant development. Trees can adapt locally to match the growing conditions, thus maintaining populations under specific climates (Anderson et al., 2012; Kim & Donohue, 2013). Local adaptation allows genotypes to exhibit higher fitness in their local environment as a result of directional natural selection (Kawecki & Ebert, 2004). This phenomenon occurs in species spreading over large and diverse areas, in the case of temporal or spatial barriers to gene flow, and when selection is stronger than gene flow (Kremer et al., 2012). According to clinal variations in the environmental factors, species with broad distributions or living under heterogeneous conditions can form populations with specific adaptations to the local climate (Rossi, 2015; Salmela et al., 2013).

Timing of bud phenology, one of the functional traits that to a large extent determine fitness (Chuine & Beaubien, 2001), is used as a useful indicator to study the mechanisms of adaptation in plants. The optimal timing of bud phenology is based on a trade-off between long growing season and frost avoidance (Allevato et al., 2019), especially in temperate and boreal climates. Species with earlier bud burst could avoid competition and benefit from the longer growing season (Ren et al., 2020), thus achieving growth advantage. Different timings in bud phenology have been observed among populations originating along gradients of latitude, altitude and continentality, reflecting strong adaptation to the local sites (Guo, Khare, et al., 2020). Compared with spring phenology, the adaptation mechanism of autumn phenology in trees is less studied due to the complex signals involved in this process (Gallinat et al., 2015). Given the associated relationship between autumn phenology and plant growth, combined studies are needed to illustrate the adaptive mechanism over the whole growing season. In fact, despite the variance among populations, a high genetic variance within populations was reported for other functional traits, such as growth rate (Yamahira & Takeshi, 2008), frost hardness (Savolainen et al., 2004) and browsing tolerance (Byington et al., 1994). This variability within provenances can help populations to survive under changing environmental conditions (Jump et al., 2009). Bud phenology is reported to be regulated by multiple genes (Wilczek et al., 2010), however, the genetic variance within populations is still poorly understood.

Northern ecosystems will experience the greatest changes under the current global warming (Bronson et al., 2009), with a rate estimated at +0.5°C/decade (Gauthier et al., 2015). However, this warming shows a heterogeneous pattern across the boreal belt. The warmer air masses moving north force the polar vortex to expand towards lower latitudes, leading to zonally asymmetric temperature variance, and cooling down some boreal regions. Colder springs or later warmings in spring have recently been recorded in south-eastern Canada, northeastern Siberia and northern Fennoscandia as a climatic consequence of global warming (Wang et al., 2011; Zhang et al., 2016). As a result, populations of boreal species with broad distributions can face site-specific changes in local conditions, that is, warming or cooling events, or both. These events may mismatch plants' phenology from the optimal growing period (Chmura et al., 2019; Richardson et al., 2013), and represent a challenge for the persistence of some local populations of boreal forests. Therefore, evaluation of the adaptation mechanism of species to local conditions and their potential to deal with environmental changes is needed to predict the fate of boreal tree species.

Black spruce [*Picea mariana* (Mill.) B.S.P.] is a dominant tree species of the boreal forest, with a broad transcontinental distribution from Alaska to Labrador. Due to its heterogeneous growing conditions, black spruce represents a model species to investigate the variability among and within populations. Black spruce seedlings originating from five provenances representing the whole latitudinal distribution of the closed boreal forest in Quebec, Canada, were planted in a common garden, and bud phenology was monitored weekly during four growing seasons. In this study, we aim to compare the variance in bud phenology among years and provenances, and assess the drivers of bud phenology along a latitudinal gradient. Two hypotheses were raised that (1) the variance in bud phenology among populations is higher than that within populations, and (2) the temperature of the original sites affects bud phenology of black spruce provenances.

## 2 | MATERIALS AND METHODS

### 2.1 | Study area and provenance characteristics

Black spruce provenances originated from five natural stands located along a latitudinal gradient ranging between the 48th and 53rd

parallels in the coniferous boreal forest of Quebec, Canada (Table 1; Figure 1). The stands SIM and BER were located in the balsam fir [*Abies balsamea* (L.) Mill.]–white birch (*Betula papyrifera* Marsh.) bioclimatic domain. MIS and DAN belonged to the black spruce–moss bioclimatic domain. MIR was located in the black spruce–lichen domain, extending at the boundary of the taiga subzone, where stands show lower density and growth. The climate of the area is typically boreal, characterized by cold winters and moist summers. Temperature changes according to latitude and altitude with the northern and most elevated sites being the coldest in winter and least warm in summer. Mean temperatures range between  $-4.2$  and  $0.9^{\circ}\text{C}$ , with an annual precipitation that decreases with latitude. The sites experience between 206 and 235 frost days per year, increasing with latitude and altitude. During the growing season (May–September), mean temperature ranges between  $11.1$  and  $14.6^{\circ}\text{C}$ , with a total precipitation of 411–675 mm. Climatic data at the origin sites for the period 1979–2013 were extracted using CHELSA Bioclim (Karger et al., 2017) at a spatial resolution of 30 arcsec.

## 2.2 | Seed collection and common garden

Seeds were collected in June 2012, from 8 to 11 mother trees randomly selected in each site (Table 1). According to the availability and accessibility of the canopy, a minimum of 10 cones were

collected per trees from each stand. Seeds from each tree were kept separated to characterize the variability among half-sib families within populations. After seed extraction, the seedlings were grown under controlled conditions until reaching a suitable size to be planted in the field. In July 2014, a total of 422 seedlings were planted in a 0.5 ha forest gap in SIM, the southernmost site (Table 1). Seedlings were planted according to a random design at a distance of  $2\text{ m} \times 2\text{ m}$ . Two rows of non-experimental black spruce seedlings were planted on each side of the plantation to avoid edge effects.

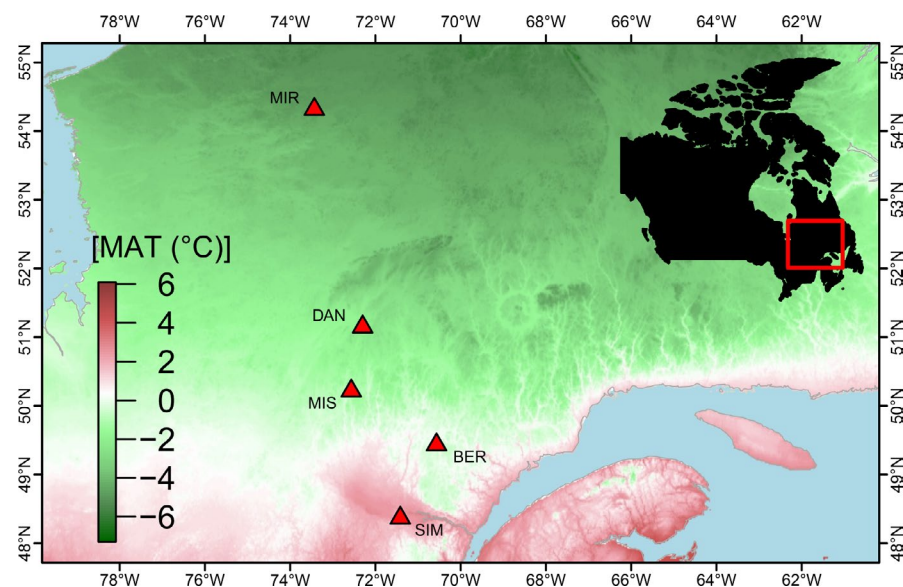
## 2.3 | Phenology

Bud phenology was recorded weekly between May and October in 2015, 2017–2019. The apical buds were used to discriminate the different phenological phases of bud burst and bud set (Dhont et al., 2010). Six bud burst phases were defined (a) open bud, with a pale spot at the tip; (b) elongated bud, with lengthening brown scales; (c) swollen bud, with smooth and pale-coloured scales; (d) translucent bud, with needles visible through the scales; (e) split bud, with needles still clustered; and (f) exposed shoot, with needles spreading outwards. Five phases of bud set were defined: (a) white bud; (b) beige bud, with beige scales; (c) brownish bud, with a substantial increase in bud size; (d) brown bud, with needles

**TABLE 1** Location of the five black spruce sites from the boreal forest of Quebec, Canada

ID	Site	Latitude ( $^{\circ}\text{N}$ )	Longitude ( $^{\circ}\text{W}$ )	Altitude (m a.s.l.)	Number of mother trees	Number of seedlings
SIM	Simoncouche	$48^{\circ}13'$	$71^{\circ}15'$	338	10	89
BER	Bernatchez	$48^{\circ}51'$	$70^{\circ}20'$	611	11	90
MIS	Mistassibi	$49^{\circ}43'$	$71^{\circ}56'$	342	9	84
DAN	Camp Daniel	$50^{\circ}41'$	$72^{\circ}11'$	487	8	84
MIR	Mirage	$53^{\circ}47'$	$72^{\circ}52'$	384	11	75

**FIGURE 1** Location of the five black spruce sites from the boreal forest of Quebec, Canada



spreading outwards; and (e) spread needles, with the needles completely spread outwards.

## 2.4 | Data analysis

A bioclimatic analysis was performed based on gridded BIOCLIM 1.4 raster maps in 2.5 arcminutes resolution (Hijmans et al., 2005) and using the seven thermal variables considered as limiting factors of geographical distribution of species used also in studies with provenance trials (Dyderski et al., 2018; Klisz et al., 2019). We included the seven variables in a principal component analysis by extracting the principal components with broken stick functions (Jackson, 1993) using PAST 4.0 (Hammer et al., 2001). The contribution of each bioclimatic variable to the total variance explained by the principal components was determined with *FactoMineR* 1.41 in R (R Core Team, 2020).

The phenological response to climate shift was calculated using linear regressions in the function of the climate transfer difference, that is, the difference in mean annual temperature between the provenance and planting sites. We used ordinal logistic models to quantify the relationship between mean annual temperature at the provenance origin and the sequential timings of each phenological phase, representing the response variable in day of the year.

We estimated the contribution of the factors provenance, half-sib family and year to the variance in timings of bud burst and bud set, where the family was nested in the provenance. Heritability was calculated by the proportion of phenotypic variance due to genetic factors (Frankham, 1996), according to the following formula:

$$h^2 = \frac{4\sigma_F^2}{\sigma_P^2 + \sigma_F^2 + \sigma_E^2},$$

where  $h^2$  is the heritability,  $\sigma_P^2$ ,  $\sigma_F^2$  and  $\sigma_E^2$  indicate the variance components of provenance, half-sib family and residual error respectively. Statistical analyses were performed in SAS 9.4 (SAS Institute Inc.).

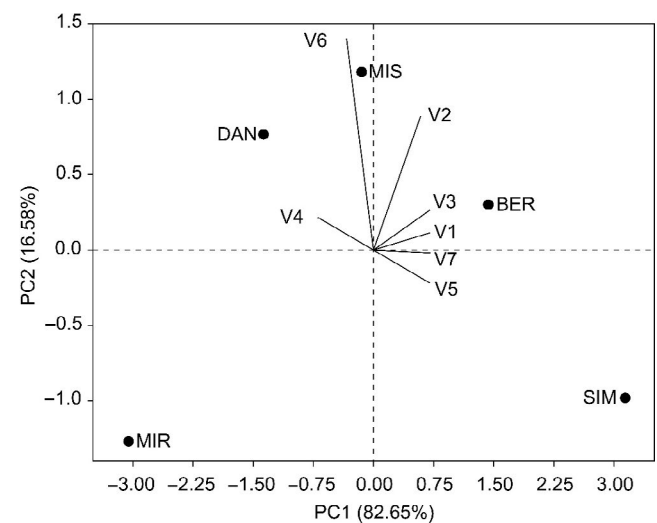
## 3 | RESULTS

### 3.1 | Bioclimatic analysis

The climatic characteristics represented by the seven thermal variables ordered the provenance sites according to the two main principal components (PCs), named PC1 and PC2 (Table 2). PC1 was statistically significant ( $p < 0.05$ ), explained 82.65% of the variance, and was strongly and positively correlated with all variables with the exception of temperature seasonality and temperature annual range that had a negative relationship. PC1 described the thermal variation well along the latitudinal gradient, with SIM and MIR being located at the opposite sides of the PC (Figure 2). PC2 explained 16.58% of the variance and was not significant ( $p > 0.05$ ). The correlation coefficients between PC2 and the thermal variables were generally weak and lower than 0.56, except for temperature annual range ( $r = 0.88$ ; Table 2).

**TABLE 2** Pearson's correlation coefficient and contribution of bioclimatic variables to the two principal components (PC1 and PC2)

Bioclimatic variables	Correlation		Contribution (%)	
	PC1	PC2	PC1	PC2
Annual mean temperature	0.99	0.07	16.92	0.45
Mean diurnal range	0.83	0.56	11.81	26.92
Isothermality	0.99	0.17	16.80	2.38
Temperature seasonality	-0.98	0.14	16.48	1.61
Minimum temperature of coldest month	0.99	-0.14	16.92	1.64
Temperature annual range	-0.47	0.88	3.84	67.00
Mean temperature of coldest quarter	1.00	-0.01	17.23	0.01



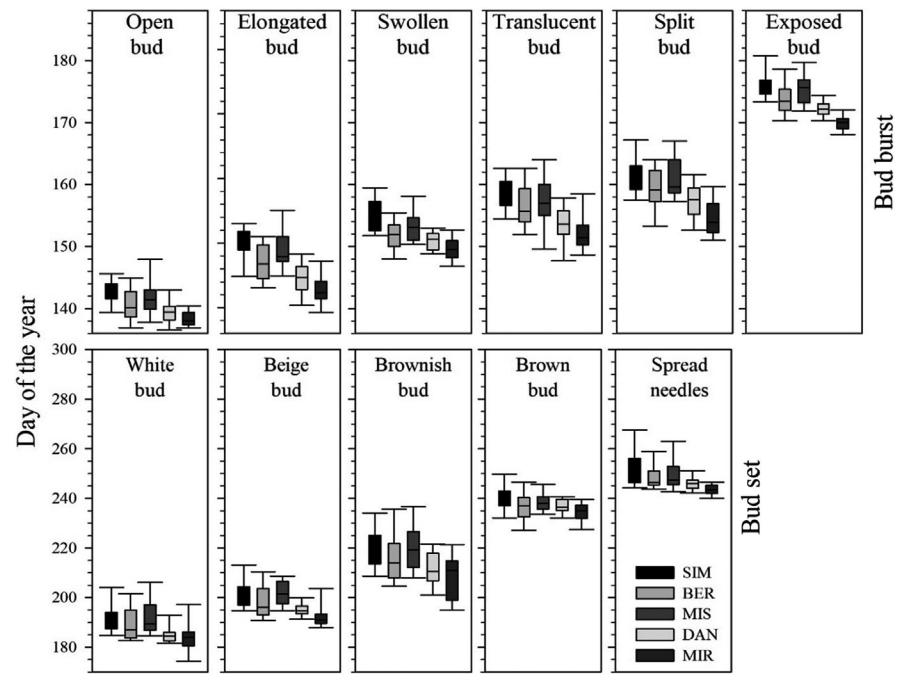
**FIGURE 2** Principal component analysis of climatic variability between the provenances and the experimental site of the common garden (SIM). The scores represent the provenances. Loadings are the bioclimatic variables annual mean temperature (V1), mean diurnal range (V2), isothermality (V3), temperature seasonality (V4), minimum temperature of coldest month (V5), temperature annual range (V6), mean temperature of coldest quarter (V7)

According to PC2, MIR and SIM showed lower temperature annual range and mean diurnal range than DAN, MIS and BER (Figure 2).

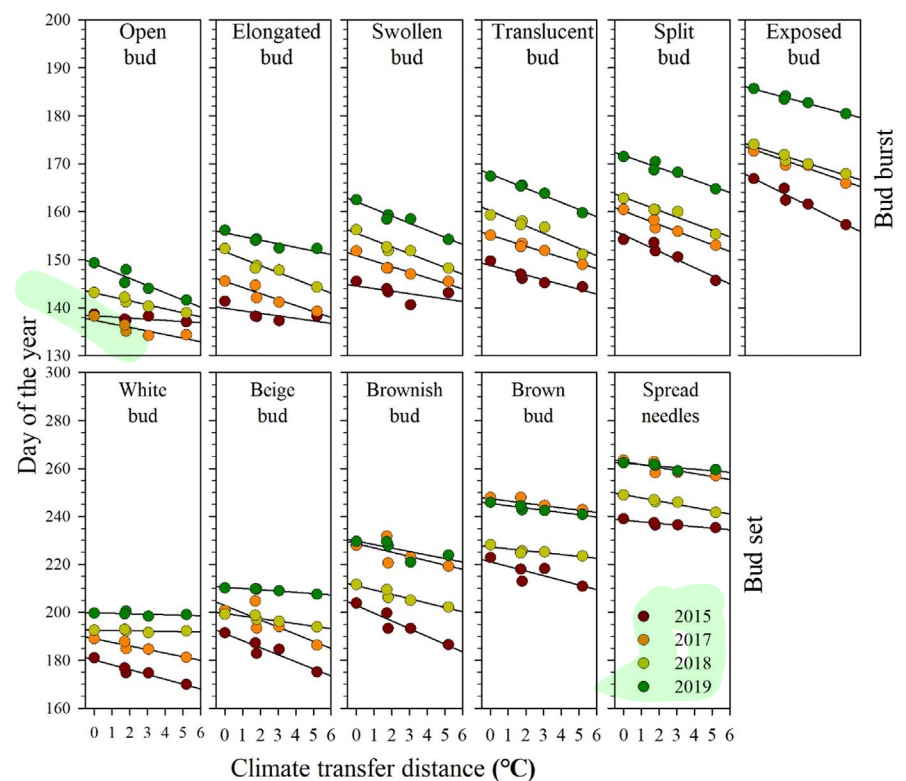
### 3.2 | Bud phenology

We observed a latitudinal gradient in the timings of bud burst among provenances. On average, bud burst started in mid-May (Figure 3). Open bud was observed first on saplings from MIR on day of the year (DOY)  $138 \pm 5$ , while SIM was the last provenance reactivating the buds, 1 week later (DOY  $143 \pm 7$ ). The phases of bud burst occurred in sequential order, with differences among provenances. Earlier timings of bud burst were observed in MIR, while SIM was the

**FIGURE 3** Timings of bud burst and bud set of five black spruce provenances growing in a common garden in the southern part of the boreal forest of Quebec, Canada, in the 2015 and 2017–2019 seasons. Boxes represent upper and lower quartiles, whiskers achieve the 10th and 90th percentiles, and the median is drawn as a horizontal solid line



**FIGURE 4** Timings of bud burst and bud set according to the climate transfer distance of five black spruce provenances (south to north gradient: SIM, BER, MIS, DAN and MIR) growing in a common garden in the southern part of the boreal forest of Quebec, Canada. Climate transfer distance represents the difference in mean annual temperature between provenance and planting site



latest provenance reactivating growth. The entire bud burst process lasted between 16 and 43 days, finishing in mid-June (exposed bud), from  $\text{DOY } 170 \pm 11$  in MIR to  $\text{DOY } 176 \pm 11$  in SIM (Figure 3). The other provenances followed a similar pattern, with BER and DAN being earlier than MIS.

The first phase of bud set (white bud) occurred in mid-July, shortly after the end of bud burst (Figure 3). The white bud phase was observed first in MIR ( $\text{DOY } 183 \pm 13$ ) and last in SIM ( $\text{DOY } 189 \pm 11$ ). Overall, the process of bud set lasted between 43 and 98 days,

with the different phases partially overlapping among sites. Spread needles were observed at the end of September, first in MIR ( $\text{DOY } 243 \pm 18$ ), and last in SIM ( $\text{DOY } 249 \pm 16$ ).

### 3.3 | Climate transfer distance

All transfer distances were positive, indicating that the individuals were planted in a warmer site than that of their origin (Figure 4).



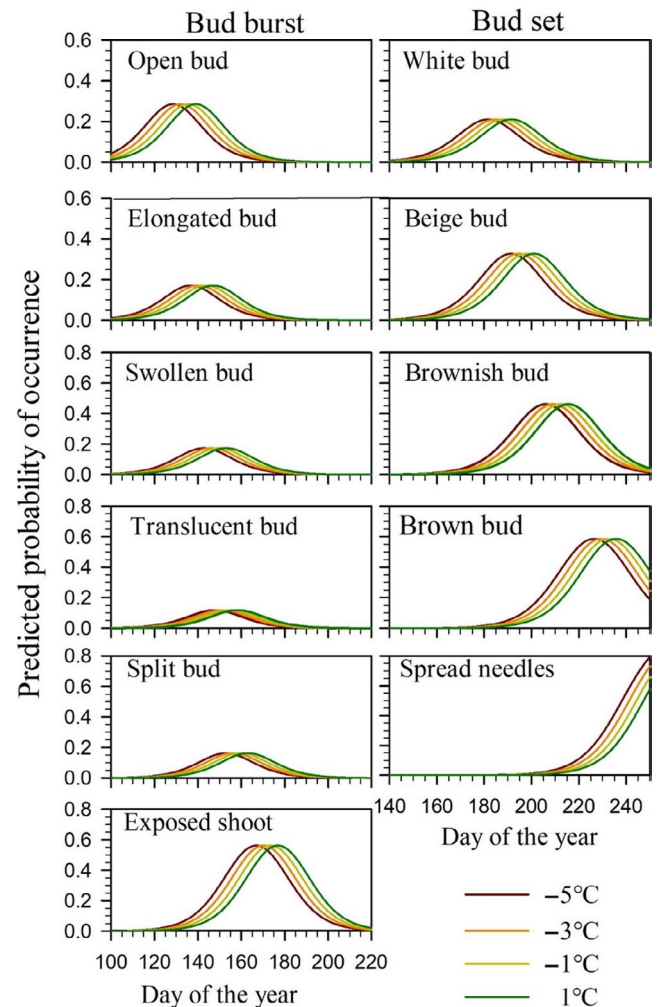
The local population (SIM) had a transfer distance of zero because the individuals grew at their origin site. All regressions had a negative slope, which suggests that the earlier bud burst occurred in the colder provenances, that is, those experiencing the larger temperature differences between plantation and origin site. On average, bud burst advanced by 1.1 days/°C of transfer distance. All bud burst phases occurred earlier in 2015, and later in 2019, except for open bud, which was earlier in 2017. Overall, the sequential phases followed a constant progression. Accordingly, a later reactivation of phase 1 resulted in a later reactivation in the successive phases.

The slopes of the regressions for bud set were negative, with slopes steeper than bud burst (Figure 4). The slopes ranged from  $-2.0$  for brownish bud to  $-0.9$  for white bud. On average, bud set occurred 1.4 days for every degree Celsius in mean annual temperature at the origin site. In MIR, the coldest provenance, the first bud set phase (white bud) occurred 5 days earlier under a temperature difference of  $5.2^{\circ}\text{C}$ , compared to saplings from SIM, the local provenance. For all bud set phases, the regressions were steeper in 2015 than in the other years, except for the last phase of spread needles. Differences between years ranged from 24 to 31 days, for white bud and brownish bud respectively. The earliest and latest occurrences of bud set were observed in 2015 and 2019, respectively, following the same pattern as for bud burst.

The ordinal logit model had a likelihood ratio with  $\chi^2 = 60,837.78$  ( $p < 0.0001$ ), indicating that the hypothesis that all regression parameters are zero was rejected. Also, the efficient score, testing for joint significance of the explanatory variables, was highly significant ( $\chi^2 = 28,383.71$ ,  $p < 0.0001$ ). The model described the whole sequential progression of bud burst and bud set by simulating the phenology of four theoretical provenances from sites with mean annual temperatures ranging from  $-5$  to  $1^{\circ}\text{C}$  (Figure 5). Estimates of association of predicted probabilities contain measures of association for assessing the predictive ability of a model. The number of pairs of observations concordant (92.4%), discordant (7.2%) and tied (0.4) indicated that the model was reliable. The probability of observing phase 1 (open bud) increased earlier in saplings originating from the colder provenances, culminating in mid-May. The model predicted a gap in bud reactivation of 11 days between provenances from sites with a  $6^{\circ}\text{C}$  difference in temperature. The same pattern was observed for the successive phases of bud burst and bud set, but with differences gradually increasing until 19 days for brown bud. Accordingly, the provenances from the colder sites were earlier and faster in completing bud development than provenances from warmer sites. On average, bud set was complete at the beginning of September, when the probability of the last phenological phase, spread needles, reached 0.5.

### 3.4 | Variance components and heritability

The factors year, provenance and sib family affected the phases of bud burst and bud set (Table 3) and were significant at  $p < 0.001$ .



**FIGURE 5** Predicted occurrence of the sequential phases of bud burst and bud set simulated by ordinal logistic model for four theoretical provenances from sites with mean annual temperatures ranging from  $-5$  to  $1^{\circ}\text{C}$

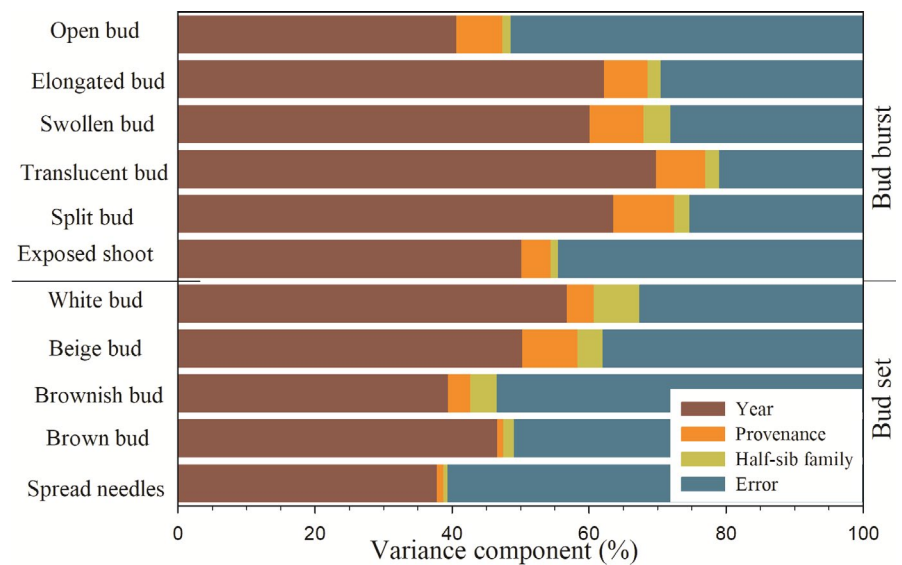
There was a huge inter-annual variation for all phenological phases, with the factor year contributing between 40.6% (open bud phase) and 69.7% (translucent bud phase) to the total variance for bud burst, and between 37.7% (spread needles) and 56.7% (white bud) for bud set (Figure 6). The percentage of variance in bud burst explained by the provenance ranged from 4.2% (exposed bud) to 8.8% (split bud). The half-sib family accounted for 1.0% (exposed bud) to 3.9% (swollen bud) of the variance in bud burst. The contribution of provenance was higher for white bud (3.9%), beige bud (8.1%) and brownish bud (3.2%), than for the two last phases of bud set (0.8%–0.9%). The effect of half-sib family ranged from 1.5% to 6.6% for the first four phases of bud set and dropped to 0.5% for spread needles.

On average, heritability estimates were similar between bud burst and bud set, with averages of 0.21 and 0.26 respectively. Variations were observed among the phases in spring and summer. Heritability estimates  $< 0.08$  occurred at the beginning and ending of bud phenology, and for phase 6 (exposed bud; Table 4).

**TABLE 3** Effect of year, provenance and half-sib family on the phenological phases of bud burst and bud set of black spruce provenances growing in a common garden in the southern part of the boreal forest of Quebec, Canada. \* and \*\* indicate  $p < 0.05$  and  $p < 0.001$  respectively

		Year	Provenance	Half-sib family
Bud burst	Open bud	289.48**	30.89**	1.62**
	Elongated bud	485.92**	33.77**	2.29**
	Swollen bud	645.26**	52.83**	4.43**
	Translucent bud	762.48**	39.87**	2.65**
	Split bud	854.48**	47.53**	3.09**
	Exposed bud	1,317.97**	78.15**	2.98**
Bud set	White bud	727.05**	30.84**	4.95**
	Beige bud	784.67**	56.89**	4.66**
	Brownish bud	621.21**	32.99**	5.72**
	Brown bud	1,060.83**	14.37**	4.64**
	Spread needles	849.82**	16.02**	2.17**

**FIGURE 6** Components of the variance in bud burst and bud set of black spruce provenances growing in a common garden in the southernmost part of the boreal forest of Quebec, Canada



**TABLE 4** Heritability estimates for the timings of bud burst and bud set of black spruce provenances growing in a common garden in the southern part of the boreal forest of Quebec, Canada

	Phenological phase	Heritability
Bud burst	Open bud	0.08
	Elongated bud	0.21
	Swollen bud	0.40
	Translucent bud	0.27
	Split bud	0.25
	Exposed bud	0.08
Bud set	White bud	0.61
	Beige bud	0.29
	Brownish bud	0.25
	Brown bud	0.12
	Spread needles	0.04

## 4 | DISCUSSION

### 4.1 | Variance of spring and autumn phenology

The experimental design of our common-garden experiment relies on five provenances originating from stands located along the latitudinal distribution of the boreal zone of Quebec, Canada. The stands show a clear thermal gradient, with the northernmost and southernmost sites representing the coldest and warmest conditions at the boundary of the closed boreal forest. When moved south towards warmer conditions, bud phenology of black spruce showed a significant negative relationship with mean annual temperature of the original sites, specifically, provenances from colder origins exhibited earlier bud burst and bud set. For bud burst, the factors related to temperature, such as requirements in winter chilling and spring forcing, are crucial for explaining the timings of growth reactivation. Previous studies have demonstrated that provenances from colder

origins or experiencing colder springs require lower temperatures to reactivate growth, thus advancing the process of seasonal transition between dormancy and growth (Wang et al., 2011; Zhang et al., 2016). This early reactivation of growth may allow plants to benefit from the lengthening day and higher solar radiation, thus to maximize carbon accumulation.

However, a late bud set in autumn, when day length and solar incident angle are decreasing, increases unnecessarily the risk of early frosts, with severe consequences for plant growth in the successive year without enhancing photosynthesis (Chuine & Beaubien, 2001). In order to avoid the prolonged bud set triggered by warming events in autumn, plants rely mostly on photoperiod, a stable signal over the years (Gallinat et al., 2015). The daylength threshold triggering bud set was longer for trees in colder sites, which induced earlier bud sets in populations from colder sites compared to those from warmer sites and growing under the same conditions (Liang, 2015). Thus, bud burst may represent a plastic response to the changing environment, while bud set could be more conservative, due to the link with the stable signal of photoperiod. The higher value of heredity and lower variance composition to the inter-annual environment variance in bud set also demonstrated the diverging strategies of growth mechanisms in spring and autumn phenology.

Compared to the Northern black spruce populations, provenances from warmer sites exhibit late bud burst because of higher forcing requirement to reactivate meristems. At the period of bud burst, populations in their native sites are submitted to different day lengths. As a consequence, a warming occurring in late winter or early spring could produce asynchronous impacts on the phenology of black spruce along its latitudinal distribution, because of the different requirements in degree-days accumulations and accumulations in photoperiod. Global warming, and the corresponding spring advancement in bud burst, are associated with higher risks of frost occurrence (Liu et al., 2018), which increases the possibility of frost damage to the developing buds and young needles. Frost damage to plant tissues also depends on frost tolerance, that is, the ability of the tissue to resist frost events, which decreases according to a latitudinal cline from North to South (Repo et al., 2000). The combination of these factors could make some populations more prone to frost damage in a context of climate changes.

Previous studies have demonstrated that the sequential phases of bud growth and development are highly correlated. When compared in common gardens, populations with earlier bud bursts also show earlier bud sets (Keenan & Richardson, 2015). Similar results were also reported for cambial activity and secondary growth in black spruce provenances, where earlier onset of xylem cell production corresponded to earlier ending of xylem maturation (Perrin et al., 2017). This demonstrates that primary and secondary growth follow similar rules and could be connected to the same endogenous processes or environmental signals. Autumn phenology is considered to be affected by the accumulated carbon derived from photosynthesis. Fulfilling of specific critical thresholds of sugars or reserves could trigger dormancy of the meristems at the end of

summer. Thus, earlier bud bursts could speed up carbon accumulation and lead to earlier bud sets (Zani et al., 2020). Models predict that global warming will advance bud burst and delay bud set in species of cold climates, thus possibly lengthening the growing season (Linderholm, 2006; Peñuelas & Filella, 2001; Reyes-Fox et al., 2014). However, our results raise questions about such a prediction. Also if the evergreen black spruce could benefit from a longer period for photosynthesis under the warmer conditions occurring during spring and autumn, the period spent for developing its buds could remain unchanged. Mechanisms and drivers of bud set have been dramatically neglected by phenological studies, and still remain largely unknown or misunderstood. Our study highlights the importance of studying autumn phenology using a more comprehensive understanding of the effects of spring phenology, carbon accumulation and the dependence on environmental factors (Gallinat et al., 2015), thus better simulating the future timings of bud set under global changes.

Epigenetic mechanisms could also play an important role in regulating bud phenology. They involve differential expressions of genes during embryogenesis (Bossdorf et al., 2008), which affect the subsequent plant development. Although little has been done in this field, the effect of epigenetic memory on bud phenology has already been reported for several tree species (Carneros et al., 2017; Yakovlev et al., 2010). For example, the different environmental conditions during seed development in Norway spruce are expected to influence the phenology of the offspring (Johnsen et al., 2009). The seasonal cycle of meristem activity is the result of an interaction between climatic and physiological signals triggering the sequence of phenological events. Under such a complex system, the role of endogenous factors other than weather and originating from epigenetic mechanisms during embryogenesis could have been overlooked or underestimated and need to be tested and quantified in the future.

## 4.2 | Variation in bud phenology among and within populations

Our study observed a higher variance in bud phenology among provenances (5.3%) than within provenances (2.6%). To increase fitness under specific conditions, individuals of the same species can develop different genetic pools, the ecotypes (Hufford & Mazer, 2003). The occurrence of ecotypes is related to the spatial heterogeneity in the growing conditions (Kawecki & Ebert, 2004). The provenances analysed in our common garden covered a wide latitudinal gradient, representing a difference in mean annual temperature of 5.2°C, corresponding to a wide range in length of the growing season, lasting from 80 to 130 days (Girard et al., 2015) or 90 to 160 days (Antonucci et al., 2017), according to the definition of a growing season. During stand development, the ecotypes with the highest fitness, that is, better matching between phenological requirements and period available for growth, may be gradually selected and have higher probabilities to be transferred to the successive generations. Thus, as a result of directional selection and



natural thinning of a growing stand, the variability in genotypes reduces (Kawecki & Ebert, 2004), mirroring adaptation of the population to the local climate. The variance in bud phenology among provenances revealed the heterogeneous climatic conditions experienced by the populations across the natural distribution of the species. Ecotypic differentiation for bud phenology has also been reported widely in other tree species, including Pedunculate oak (*Quercus robur* L.; Puchałka et al., 2017), white pine (*Pinus strobus* L.; Li et al., 1997), European beech (*Fagus sylvatica* L.; Gömöry & Paule, 2011) and sugar maple (*Acer saccharum* Marshall; Guo, Khare, et al., 2020).

A variability in bud phenology within provenances increases the chance of adaptation under changing conditions. The large heterogeneity in phenology represented by the residual variance may suggest a high diversity among individuals, in addition to the effect of microsite conditions and sampling errors. Individuals or phenotypes with lower degree-days requirements may benefit from an earlier growth reactivation in spring, a longer growing season, but also a higher risk of late frost damage. Nevertheless, those genotypes that better match the local dynamics of climate could achieve higher competitive ability and fitness. Therefore, the presence of various and divergent phenotypes within populations ensures that some of them can match the environmental conditions, thus allowing local persistence of the species. For conifer species, a long dispersal distance of pollen and continuous distribution of the species was proved to be an efficient way to increase gene diversity both within and among populations (Savolainen et al., 2007).

### 4.3 | Inter-annual variation of bud phenology

Our study found that the inter-annual environmental variation explained most variance in bud phenology, that is, the variability in bud phenology among years largely exceeded that from provenances. Specifically, the bud burst of black spruce in 2015 started earlier than other years, irrespective of the provenance. April–May temperature was 10.5°C in 2015, between 1.1 and 3.4°C higher than in the other studied years (data not shown). Thus, the quicker warming occurring in 2015 explains the advancement of bud phenology. This ability to adjust bud phenology under changing weather conditions, referred to as inter-annual phenotypic plasticity, helps plants to gain a longer period for carbon fixation and gives high competitive abilities. This is crucial for trees, which have a long life span, slow and intermittent regeneration periods (masting years), and high sensitivity to the changing environment (Fox et al., 2019). Under global warming, the rapid and unexpected weather changes may mismatch plant phenology from the optimal growing period, and thus endanger the persistence of some local populations. Our study may demonstrate that to some extent, this inter-annual phenotypic plasticity may help to cope with the environmental changes over temporal scales, thus achieving sufficient fitness and a successful establishment of extended populations (Van Kleunen & Fischer, 2005).

## 5 | CONCLUSIONS

The boreal forest could be submitted to both warming and cooling events produced by the ongoing climate changes that affect air mass movements at the higher latitudes. These rapid and unexpected changes may challenge the ability of trees to adjust its phenology to the new weather conditions and affect the persistence of some local populations. Because of their long life span and slow reproductive rates, trees are the most sensitive species of these ecosystems. In this study, bud phenology of five black spruce populations originating from the latitudinal range of the closed boreal forest was monitored in a common garden to investigate the local adaptation process. Our study observed a higher variance of bud phenology among populations than within populations and demonstrated a relationship between bud phenology and mean annual temperature at the origin sites. The diverging timing of bud burst and bud set detected among populations may be the result of the plastic response of bud burst to changing temperature and long-term adaptation of bud set to the photoperiod at the origin sites respectively. Besides, populations with earlier bud burst also complete bud set earlier, indicating a close correlation between the phenological phases occurring in spring and autumn. As a result, the buds of Northern and Southern populations exhibited similar periods of development. Our study suggested that the occurrence of ecotypes and the relationship between spring and autumn phenology should be considered in phenological modelling, which could help to precisely predict the fate of boreal species under climate change.

## ACKNOWLEDGEMENTS

This work was funded by the Direction général du secteur nord-est, région du Saguenay–Lac-St-Jean, Ministère des Forêts, de la Faune et des Parcs du Québec, Forêt d'Enseignement et de Recherche Simoncouche, the Fondation de l'Université du Québec à Chicoutimi, National Natural Science Foundation of China (41861124001), Natural Science Foundation of Guangdong Province (2019B121202007) and the International Collaborative Key Project of the CAS (GJHZ1752). X. Guo received a State Scholarship Fund (201904910365) provided by the China Scholarship Council to conduct this research. This paper was developed under a scholarship granted by the Scholarship Fund of the Forest Research Institute, pursuant to the decision of the Head of the Institute dated 17 October 2018 (based on an Agreement concluded on 18 October 2018). The authors thank L. Balducci, I. Allie, V. Néron, I. Froment and T. Ziegler for technical support, and A. Garside for editing the English text.

## AUTHORS' CONTRIBUTIONS

S.R. was involved in experimental design and project coordination; E.B. and R.S. were involved in data collection; S.R. and R.P. were involved in data analysis; X.G. and M.K. were involved in first draft. All authors were involved in comments and final version.

## PEER REVIEW

The peer review history for this article is available at <https://publons.com/publon/10.1111/1365-2745.13582>.

## DATA AVAILABILITY STATEMENT

Data are available from the Dryad Digital Repository <https://doi.org/10.5061/dryad.866t1g1pr> (Guo, Klisz, et al., 2021).

## ORCID

Xiali Guo  <https://orcid.org/0000-0002-8497-1837>

Radosław Puchałka  <https://orcid.org/0000-0002-4764-0705>

Roberto Silvestro  <https://orcid.org/0000-0003-2739-1534>

Patrick Faubert <http://orcid.org/0000-0003-0237-3188>

Jianguo Huang  <https://orcid.org/0000-0003-3830-0415>

Sergio Rossi  <https://orcid.org/0000-0002-9919-0494>

## REFERENCES

- Allevato, E., Saulino, L., Cesarano, G., Chirico, G. B., D'Urso, G., Bolognesi, S. F., Rita, A., Rossi, S., Saracino, A., & Bonanomi, G. (2019). Canopy damage by spring frost in European beech along the Apennines: Effect of latitude, altitude and aspect. *Remote Sensing of Environment*, 225, 431–440. <https://doi.org/10.1016/j.rse.2019.03.023>
- Anderson, J. T., Inouye, D. W., McKinney, A. M., Colautti, R. I., & Mitchell-Olds, T. (2012). Phenotypic plasticity and adaptive evolution contribute to advancing flowering phenology in response to climate change. *Proceedings of the Royal Society B: Biological Sciences*, 279, 3843–3852. <https://doi.org/10.1098/rspb.2012.1051>
- Antonucci, S., Rossi, S., Deslauriers, A., Morin, H., Lombardi, F., Marchetti, M., & Tognetti, R. (2017). Large-scale estimation of xylem phenology in black spruce through remote sensing. *Agricultural and Forest Meteorology*, 233, 92–100. <https://doi.org/10.1016/j.agrfo.2016.11.011>
- Bosssdorf, O., Richards, C. L., & Pigliucci, M. (2008). Epigenetics for ecologists. *Ecology Letters*, 11, 106–115.
- Bronson, D. R., Gower, S. T., Tanner, M., & Van Herk, I. (2009). Effect of ecosystem warming on boreal black spruce bud burst and shoot growth. *Global Change Biology*, 15, 1534–1543. <https://doi.org/10.1111/j.1365-2486.2009.01845.x>
- Byington, T. S., Gottschalk, K. W., & McGraw, J. B. (1994). Within-population variation in response of red oak seedlings to herbivory by gypsy moth larvae. *American Midland Naturalist*, 328–339. <https://doi.org/10.2307/2426589>
- Carneros, E., Yakovlev, I., Viejo, M., Olsen, J. E., & Fossdal, C. G. (2017). The epigenetic memory of temperature during embryogenesis modifies the expression of bud burst-related genes in Norway spruce ecotypes. *Planta*, 246, 553–566. <https://doi.org/10.1007/s00425-017-2713-9>
- Chmura, H. E., Kharouba, H. M., Ashander, J., Ehlman, S. M., Rivest, E. B., & Yang, L. H. (2019). The mechanisms of phenology: The patterns and processes of phenological shifts. *Ecological Monographs*, 89, e01337. <https://doi.org/10.1002/ecm.1337>
- Chuine, I., & Beaubien, E. G. (2001). Phenology is a major determinant of tree species range. *Ecology Letters*, 4, 500–510. <https://doi.org/10.1046/j.1461-0248.2001.00261.x>
- Dhont, C., Sylvestre, P., Gros-Louis, M., & Isabel, N. (2010). *Field guide for identifying apical bud break and bud formation stages in white spruce* (pp. 9–11). Natural Resources Canada.
- Dyderski, M. K., Paź, S., Frelich, L. E., & Jagodziński, A. M. (2018). How much does climate change threaten European forest tree species distributions? *Global Change Biology*, 24, 1150–1163. <https://doi.org/10.1111/gcb.13925>
- Fox, R. J., Donelson, J. M., Schunter, C., Ravasi, T., & Gaitan-Espitia, J. D. (2019). Beyond buying time: The role of plasticity in phenotypic adaptation to rapid environmental change. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, 374, 20180174. <https://doi.org/10.1098/rstb.2018.0174>
- Frankham, R. (1996). *Introduction to quantitative genetics* (4th ed., D. S. Falconer & T. F. C. Mackay Longman, Eds., pp. xv–464). Elsevier Current Trends.
- Gallinat, A. S., Primack, R. B., & Wagner, D. L. (2015). Autumn, the neglected season in climate change research. *Trends in Ecology & Evolution*, 30, 169–176. <https://doi.org/10.1016/j.tree.2015.01.004>
- Gauthier, S., Bernier, P., Kuuluvainen, T., Shvidenko, A., & Schepaschenko, D. (2015). Boreal forest health and global change. *Science*, 349, 819–822. <https://doi.org/10.1126/science.aaa9092>
- Girard, M.-J., Rossi, S., & Morin, H. (2015). Mapping events: Cambium phenology across the latitudinal distribution of black spruce. *IAWA Journal*, 36, 270–285. <https://doi.org/10.1163/22941932-20150091>
- Gömöry, D., & Paule, L. (2011). Trade-off between height growth and spring flushing in common beech (*Fagus sylvatica* L.). *Annals of Forest Science*, 68, 975–984. <https://doi.org/10.1007/s13595-011-0103-1>
- Guo, X., Khare, S., Silvestro, R., Huang, J., Sylvain, J.-D., Delagrangé, S., & Rossi, S. (2020). Minimum spring temperatures at the provenance origin drive leaf phenology in sugar maple populations. *Tree Physiology*, 40, 1639–1647. <https://doi.org/10.1093/treephys/tpaa096>
- Guo, X., Klisz, M., Puchałka, R., Silvestro, R., Faubert, P., Belien, E., Huang, J. G., & Rossi, S. (2021). Data from: Common-garden experiment reveals clinal trends of bud phenology in black spruce populations from a latitudinal gradient in the boreal forest. *Dryad Digital Repository*, <https://doi.org/10.5061/dryad.866t1g1pr>
- Hammer, Ø., Harper, D. A., & Ryan, P. D. (2001). PAST: Paleontological statistics software package for education and data analysis. *Palaeontologia Electronica*, 4, 9.
- Hijmans, R. J., Cameron, S. E., Parra, J. L., Jones, P. G., & Jarvis, A. (2005). Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology: A Journal of the Royal Meteorological Society*, 25, 1965–1978. <https://doi.org/10.1002/joc.1276>
- Hufford, K. M., & Mazer, S. J. (2003). Plant ecotypes: Genetic differentiation in the age of ecological restoration. *Trends in Ecology & Evolution*, 18, 147–155. [https://doi.org/10.1016/S0169-5347\(03\)00002-8](https://doi.org/10.1016/S0169-5347(03)00002-8)
- Jackson, D. A. (1993). Stopping rules in principal components analysis: A comparison of heuristic and statistical approaches. *Ecology*, 74, 2204–2214. <https://doi.org/10.2307/1939574>
- Johnsen, Ø., Kvaalen, H., Yakovlev, I. A., Dæhlen, O. G., Fossdal, C. G., & Skrøppa, T. (2009). An epigenetic memory from time of embryo development affects climatic adaptation in Norway spruce. In L. Gusta, M. Wisniewski, & K. Tanino (Eds.), *Plant cold hardiness. From the laboratory to the field* (pp. 99–107). CABI.
- Jump, A. S., Marchant, R., & Penuelas, J. (2009). Environmental change and the option value of genetic diversity. *Trends in Plant Science*, 14, 51–58. <https://doi.org/10.1016/j.tplants.2008.10.002>
- Karger, D. N., Conrad, O., Böhrner, J., Kawohl, T., Kreft, H., Soria-Auza, R. W., Zimmermann, N. E., Linder, H. P., & Kessler, M. (2017). Climatologies at high resolution for the earth's land surface areas. *Scientific data*, 4(170122). <https://doi.org/10.1038/sdata.2017.122>
- Kawecki, T. J., & Ebert, D. (2004). Conceptual issues in local adaptation. *Ecology Letters*, 7, 1225–1241. <https://doi.org/10.1111/j.1461-0248.2004.00684.x>
- Keenan, T. F., & Richardson, A. D. (2015). The timing of autumn senescence is affected by the timing of spring phenology: Implications for predictive models. *Global Change Biology*, 21, 2634–2641. <https://doi.org/10.1111/gcb.12890>
- Kim, E., & Donohue, K. (2013). Local adaptation and plasticity of *Erysimum capitatum* to altitude: Its implications for responses to climate change. *Journal of Ecology*, 101, 796–805.
- Klisz, M., Ukalska, J., Koprowski, M., Tereba, A., Puchałka, R., Przybylski, P., Jastrzębowski, S., & Nabais, C. (2019). Effect of provenance and

- climate on intra-annual density fluctuations of Norway spruce. *Agricultural and Forest Meteorology*, 269, 145–156.
- Kremer, A., Ronce, O., Robledo-Arnuncio, J. J., Guillaume, F., Bohrer, G., Nathan, R., Bridle, J. R., Gomulkiewicz, R., Klein, E. K., Ritland, K., Kuparinen, A., Gerber, S., & Schueler, S. (2012). Long-distance gene flow and adaptation of forest trees to rapid climate change. *Ecology Letters*, 15, 378–392. <https://doi.org/10.1111/j.1461-0248.2012.01746.x>
- Li, P., Beaulieu, J., Daooust, G., & Plourde, A. (1997). Patterns of adaptive genetic variation in eastern white pine (*Pinus strobus*) from Quebec. *Canadian Journal of Forest Research*, 27, 199–206.
- Liang, L. (2015). Geographic variations in spring and autumn phenology of white ash in a common garden. *Physical Geography*, 36, 489–509. <https://doi.org/10.1080/02723646.2015.1123538>
- Linderholm, H. W. (2006). Growing season changes in the last century. *Agricultural and Forest Meteorology*, 137, 1–14. <https://doi.org/10.1016/j.agrformet.2006.03.006>
- Liu, Q., Piao, S., Janssens, I. A., Fu, Y., Peng, S., Lian, X., Ciais, P., Myneni, R. B., Penuelas, J., & Wang, T. (2018). Extension of the growing season increases vegetation exposure to frost. *Nature Communications*, 9. <https://doi.org/10.1038/s41467-017-02690-y>
- Peñuelas, J., & Filella, I. (2001). Responses to a warming world. *Science*, 294, 793–795.
- Perrin, M., Rossi, S., & Isabel, N. (2017). Synchronisms between bud and cambium phenology in black spruce: Early-flushing provenances exhibit early xylem formation. *Tree Physiology*, 37, 593–603. <https://doi.org/10.1093/treephys/tpx019>
- Puchałka, R., Koprowski, M., Gričar, J., & Przybylak, R. (2017). Does tree-ring formation follow leaf phenology in Pedunculate oak (*Quercus robur* L.)? *European Journal of Forest Research*, 136, 259–268. <https://doi.org/10.1007/s10342-017-1026-7>
- R Core Team. (2020). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. <https://www.r-project.org/>
- Ren, P., Néron, V., Rossi, S., Liang, E., Bouchard, M., & Deslauriers, A. (2020). Warming counteracts defoliation-induced mismatch by increasing herbivore–plant phenological synchrony. *Global Change Biology*, 26, 2072–2080. <https://doi.org/10.1111/gcb.14991>
- Repo, T., Zhang, G., Ryyppo, A., Rikala, R., & Vuorinen, M. (2000). The relation between growth cessation and frost hardening in Scots pines of different origins. *Trees-Structure and Function*, 14, 456–464. <https://doi.org/10.1007/s004680000059>
- Reyes-Fox, M., Steltzer, H., Trlica, M., McMaster, G. S., Andales, A. A., LeCain, D. R., & Morgan, J. A. (2014). Elevated CO<sub>2</sub> further lengthens growing season under warming conditions. *Nature*, 510, 259–262. <https://doi.org/10.1038/nature13207>
- Richardson, A. D., Keenan, T. F., Migliavacca, M., Ryu, Y., Sonnentag, O., & Toomey, M. (2013). Climate change, phenology, and phenological control of vegetation feedbacks to the climate system. *Agricultural and Forest Meteorology*, 169, 156–173. <https://doi.org/10.1016/j.agrformet.2012.09.012>
- Rossi, S. (2015). Local adaptations and climate change: Converging sensitivity of bud break in black spruce provenances. *International Journal of Biometeorology*, 59, 827–835. <https://doi.org/10.1007/s00484-014-0900-y>
- Salmela, M. J., Cavers, S., Cottrell, J. E., Iason, G. R., & Ennos, R. A. (2013). Spring phenology shows genetic variation among and within populations in seedlings of Scots pine (*Pinus sylvestris* L.) in the Scottish Highlands. *Plant Ecology & Diversity*, 6, 523–536.
- Savolainen, O., Bokma, F., García-Gil, R., Komulainen, P., & Repo, T. (2004). Genetic variation in cessation of growth and frost hardiness and consequences for adaptation of *Pinus sylvestris* to climatic changes. *Forest Ecology and Management*, 197, 79–89. <https://doi.org/10.1016/j.foreco.2004.05.006>
- Savolainen, O., Pyhäjärvi, T., & Knürr, T. (2007). Gene flow and local adaptation in trees. *Annual Review of Ecology Evolution and Systematics*, 38, 595–619.
- Van Kleunen, M., & Fischer, M. (2005). Constraints on the evolution of adaptive phenotypic plasticity in plants. *New Phytologist*, 166, 49–60. <https://doi.org/10.1111/j.1469-8137.2004.01296.x>
- Wang, X., Piao, S., Ciais, P., Li, J., Friedlingstein, P., Koven, C., & Chen, A. (2011). Spring temperature change and its implication in the change of vegetation growth in North America from 1982 to 2006. *Proceedings of the National Academy of Sciences United States of America*, 108, 1240–1245. <https://doi.org/10.1073/pnas.1014425108>
- Wilczek, A., Burghardt, L., Cobb, A., Cooper, M., Welch, S., & Schmitt, J. (2010). Genetic and physiological bases for phenological responses to current and predicted climates. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 365, 3129–3147. <https://doi.org/10.1098/rstb.2010.0128>
- Yakovlev, I. A., Fossdal, C. G., & Johnsen, Ø. (2010). MicroRNAs, the epigenetic memory and climatic adaptation in Norway spruce. *New Phytologist*, 187, 1154–1169. <https://doi.org/10.1111/j.1469-8137.2010.03341.x>
- Yamahira, K., & Takeshi, K. (2008). Variation in juvenile growth rates among and within latitudinal populations of the medaka. *Population Ecology*, 50, 3–8. <https://doi.org/10.1007/s10144-007-0055-3>
- Zani, D., Crowther, T. W., Mo, L., Renner, S. S., & Zohner, C. M. (2020). Increased growing-season productivity drives earlier autumn leaf senescence in temperate trees. *Science*, 370, 1066–1071. <https://doi.org/10.1126/science.abd8911>
- Zhang, J., Tian, W., Chipperfield, M. P., Xie, F., & Huang, J. (2016). Persistent shift of the Arctic polar vortex towards the Eurasian continent in recent decades. *Nature Climate Change*, 6, 1094–1099. <https://doi.org/10.1038/nclimate3136>

**How to cite this article:** Guo X, Klisz M, Puchałka R, et al. Common-garden experiment reveals clinal trends of bud phenology in black spruce populations from a latitudinal gradient in the boreal forest. *J Ecol.* 2021;00:1–11. <https://doi.org/10.1111/1365-2745.13582>