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Ecological genomics of variation in bud-break phenology and mechanisms of response to climate warming in *Populus trichocarpa*

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Summary

- Spring bud-break phenology is a critical adaptive feature common to temperate perennial woody plants. Understanding the molecular underpinnings of variation in bud-break is important for elucidating adaptive evolution and predicting outcomes relating to climate change.
- Field and controlled growth chamber tests were used to assess population-wide patterns in bud-break from wild-sourced black cottonwood (*Populus trichocarpa*) genotypes. We conducted a genome-wide association study (GWAS) with single nucleotide polymorphisms (SNPs) derived from whole genome sequencing to test for loci underlying variation in bud-break.
- Bud-break had a quadratic relationship with latitude, where southern- and northern-most provenances generally broke bud earlier than those from central parts of the species' range. Reduced winter chilling increased population-wide variation in bud-break, whereas greater chilling decreased variation. GWAS uncovered 16 loci associated with bud-break. Phenotypic changes connected with allelic variation were replicated in an independent set of *P. trichocarpa* trees. Despite phenotypic similarities, genetic profiles between southern- and northern-most genotypes were dissimilar based on our GWAS-identified SNPs.
- We propose that the GWAS-identified loci underpin the geographical pattern in *P. trichocarpa* and that variation in bud-break reflects different selection for winter chilling and heat sum accumulation, both of which can be affected by climate warming.

Introduction

In perennial plants, phenological adaptation to unfavourable seasonal conditions often involves a pause, or dormant state, before resuming growth and activities for carbon assimilation. In temperate and boreal environments, the most evident occurrence of cyclical dormancy in deciduous trees and shrubs is the overwintering of buds followed by spring bud-break, canopy flush and new growth (Cooke *et al.*, 2012). This induction and subsequent release of bud dormancy is adaptive, with strong effects on plant survivorship to climate and significant impacts on overall biomass gain (reviewed in Saxe *et al.*, 2001; Cooke *et al.*, 2012; Brunner *et al.*, 2017). The timing of bud-break is presumed to balance the need to maximize growing season length while minimizing risks of spring frost damage and/or dehydration following premature bud-break (Lechowicz, 1984; Cannell & Smith, 1986; Heide, 1993; Farmer, 1996; Saxe *et al.*, 2001; Körner *et al.*, 2016). Scaled up, these perennial cycles of growth and overwintering dormancy in forests have substantial impacts through nutrient and carbon cycling, and energy balance (Chuine *et al.*, 2000). Consequently, the impacts of climate warming on bud-break phenology have garnered particular attention due to the effects of advancing spring on individual species, communities and landscapes (Cannell & Smith, 1986; Murray *et al.*, 1989; Heide, 1993; Kramer, 1995; Menzel & Fabian, 1999; Beaubien & Freeland, 2000; Saxe *et al.*, 2001; Wolkovich *et al.*, 2012; Guy, 2014; Kollas *et al.*, 2014).

Although the term *dormancy* implies inactivity, this state is dynamic in buds and not static. Before entering dormancy, trees and shrubs (with indeterminate growth) will respond to photoperiodic and/or temperature cues towards the end of the growing season to initiate 'bud set' (Wareing, 1948; Rohde *et al.*, 2011; Cooke *et al.*, 2012). In this phase, terminal meristems respond by slowing growth and often developing protective bud scales around preformed leaves. Once the meristem becomes inactive and does not resume growth, even if favourable conditions exist, it is considered dormant (reviewed in Arora *et al.*, 2003; Cooke *et al.*, 2012). A dormant bud must be released by a specific signal, or set of signals, and this state is often termed *endodormancy* as it is imposed physiologically (Lang *et al.*, 1987). During this time,

the bud also acclimatizes to cold, becoming hardened to survive overwintering and freezing temperatures. The dynamic nature of bud dormancy is reflected in the concept of 'depth of dormancy' (cf Brunner *et al.*, 2017) as dormancy-releasing signals, such as temperature, can act in a cumulative manner to modify the duration of bud dormancy. The period following bud set and lasting through winter is monitored by plants and often interpreted in quantitative terms of accumulated 'chilling units' (i.e. the duration and depth of cold experienced over time). Increasing chilling units decreases the depth of bud dormancy and when adequate chilling has been accumulated to pass an inherent threshold, the bud is released from its endodormant state. At this point, the bud enters a quiescent period, or *ecodormancy* (Lang *et al.*, 1987). During this phase, the meristem can respond to signals in anticipation of resuming growth. The warmer temperatures encountered during the late winter and spring period also are monitored by plants and interpreted as accumulated 'thermal units' or 'heat sum' (i.e. the duration and extent of warmth experienced over time). As the threshold for particular cues is reached, such as sufficient heat sum and/or appropriate photoperiod, buds will swell and 'burst' or 'break' as the preformed leaves expand and the meristem resumes growth.

The progression of meristems through different phases of dormancy is key to surviving temperate and boreal climates, and often affects the survivability of buds along with other mechanisms, such as frost hardiness and cold tolerance (Arora *et al.*, 2003). Accordingly, mathematical models predicting progression through dormancy and timing of bud-break in different woody taxa have long been of interest (e.g. Hänninen, 1990, 1995; Hunter & Lechowicz, 1992; Hannerz, 1999; Pop *et al.*, 2000; Hänninen *et al.*, 2007; Caffara *et al.*, 2011; Ferguson *et al.*, 2013; Chuine *et al.*, 2016). These models generally integrate different aspects of chilling units, thermal units/heat sum, temperature and/or photoperiod to provide estimates of the timing of bud-break. However, the genetics of the physiological mechanisms underlying these intrinsic factors, such as chilling and heat sum requirements, and response to environmental cues have yet to be fully integrated (Kramer *et al.*, 2015; Chuine *et al.*, 2016). Particular genes implicated in bud-break and dormancy of woody plants include phytochromes and hormone signalling genes (reviewed in Cooke *et al.*, 2012; Howe *et al.*, 2015; Brunner *et al.*, 2017). A set of dormancy-associated MADS-box transcription factors (DAM genes) was identified regulating endodormancy induction and release (Bielenberg *et al.*, 2008; Horvath *et al.*, 2010; Jiménez *et al.*, 2010; Ríos *et al.,* 2014). In aspens, other genes connected with bud-break include *EARLY BUD-BREAK 1* (*EBB1*) (Yordanov *et al.*, 2014) and *DEMETER-LIKE 10* DNA demethylase (Conde *et al.*, 2017). Nevertheless, the particular genes and molecular mechanisms that support ecological variation in bud-break have remained elusive (Arora *et al.*, 2003; Cooke *et al.*, 2012; Singh *et al.*, 2017).

The phenotype of spring phenology is complex and disentangling the genetics contributing to endodormancy and ecodormancy is complicated (Howe *et al.*, 2015). It incorporates variation in distinct physiological processes, including release of endodormancy through sufficient winter chilling and release of ecodormancy through accumulation of spring heat sums. Progression through these phases depends on the actual immediate season encountered, and also can require appropriate photoperiods for some species (Wareing, 1948; Caffara *et al.*, 2011; Junttila & Hänninen, 2012). The chilling necessary to transition from endodormancy to ecodormancy is highly heritable in most species, indicating a strong genetic component. Experiments with diverse woody species (e.g. *Picea*, *Vitis*, *Quercus*, *Populus*, *Salix*, *Betula*) have shown that the timing of bud-break also is strongly influenced by spring temperatures, as a certain heat sum is needed to trigger growth (Heide, 1993; Saxe *et al.*, 2001; Caffarra & Donnelly, 2011). For woody plants in temperate and boreal environments, it is critical to understand how modified temperatures stemming from climate change are expected to affect both overwintering and spring flush-out processes (Arora *et al.*, 2003; Nanninga *et al.*, 2017). Although plasticity has broadly been observed through warmer temperatures advancing the spring date of bud-break (Menzel & Fabian, 1999; Wolkovich *et al.*, 2012), the ability of different species (or populations) to respond to fluctuating conditions may be determined by the complexity of the genetic architecture underlying spring bud-break.

In order to provide insights into these questions, we investigated the ecological genomics of bud-break in black cottonwood (*Populus trichocarpa*), a species used extensively to investigate heritable adaptive traits in woody plants (Bradshaw & Stettler, 1995; Howe *et al.*, 1999; Frewen *et al.*, 2000; Evans *et al.*, 2014; McKown *et al.*, 2014b). Poplars (*Populus* spp.) are good models to explore adaptive patterns, as the genus occupies a wide range of habitats and latitudes across Europe, North America and Asia (Farmer, 1996). Provenances often show substantial phenotypic variation and strong local adaptation reflecting selection for heterogeneous environments (e.g. Luquez *et al.*, 2008; Keller *et al.*, 2011; Olson *et al.*, 2013; McKown *et al.*, 2014a; Evans *et al.*, 2016). In the present study, we tested the variability of spring bud-break in wild-sourced *P. trichocarpa* (originating throughout the species' range) using field studies and growth chamber tests to understand geographical patterns in bud-break, and the effects of temperature and chilling duration. We used a genome-wide association study with single nucleotide polymorphisms derived from whole genome sequencing to determine the underlying genetic variation in the timing of bud-break. We hypothesized that modifying the duration of winter chilling and spring temperatures may have different implications for the timing of bud-break, and importantly suggest that trait variation under future climate change may depend largely on the particular genetic background of the population.

Materials and Methods

Plant materials

Extensive collections of wild-sourced *Populus trichocarpa* Torr & Gray genotypes were used for field and growth chamber tests, association studies, and subsequent validation tests for bud-break (Fig. 1a; Supporting Information Table S1). Field data for all genotypes were obtained from individuals grown in common

gardens (with replicated clonal ramets) located within the natural range of the species. The 'UBC' collection was made by the British Columbia Ministry of Forests, Lands, Natural Resource Operations & Rural Development across the northern two-thirds of the species' range (44.0–59.6°N, 121.2–137.9°W). From this source, genotypes were planted in 2008 at Totem Field, Vancouver, BC (49.25°N, 123.10°W; McKown *et al.*, 2013) and in 2009–2010 at Agassiz, BC (49.25°N, 121.95°W; McKown *et al.*, 2017). The 'GW/BESC' collection originating from Oregon–Washington (44.5–48.9°N, 121.7–123.7°W) in the southern-central portion of the species' range was obtained from GreenWood Resources, Portland, OR, and the BioEnergy Science Center, Department of Energy, Oak Ridge, TN. Stem cuttings from the GW/BESC collection were planted at Agassiz in 2009–2010 alongside the UBC genotypes (McKown *et al.*, 2017). From these collections, we assessed 475 UBC and 822 GW/BESC genotypes.

Field assessments of bud-break

Spring bud-break in the field was identified as the emergence of leaf tips from the terminal bud of individual trees during early spring months and recorded using Julian number days (JD). At Totem Field, bud-break was assessed in 2010 and 2011 for 462 genotypes (with clonal ramet replication = 2047 trees in total; data from McKown *et al.*, 2014a). At Agassiz, bud-break was assessed in 2012 and 2013 for 1027 genotypes (with clonal ramet replication = 1795 trees in total; data from McKown *et al.*, 2017). Bud-break dates were tested for correlation across years within and across common gardens using Pearson product moment correlations in the standard R statistical package (R Core Team; http://www.R-project.org).

Daily minimum, maximum and mean temperatures (°C) were extracted for Totem Field (2009–2011) and Agassiz (2011–2013) using publicly available historical weather records (Table S2) from the Government of Canada (http://climate.weather.gc.ca/) and Weather Underground (www.wunderground.com). For each winter–spring season at each site, we determined the number of winter days with daily mean temperatures < 5°C (cf Landsberg, 1974; Cannell & Smith, 1983, 1986) from 1 November to the median bud-break date as a general weather metric. We also calculated the spring warming period as the number of days between the last episode of spring frost (i.e. a sustained period of minimum 5 d with a daily mean temperature < 2°C) and the median bud-break date for each year (following Hänninen, 1990). We determined the mean spring forcing temperature as the average of the daily maximum temperatures reported during the spring warming period.

We used a model to estimate winter chilling units for each season considering the different contributions of temperatures to overall chilling (following Hänninen, 1990, 1995). Using daily mean temperatures ($T(t)$) from 1 November to the last episode of spring frost, we applied the following calculations from Hänninen (1990) to determine chilling units:

$$0 \text{ CU h}^{-1}, \quad T(t) \leq -3.4^{\circ}\text{C or } T(t) > 10.4^{\circ}\text{C} \qquad \text{Eqn 1}$$

$$a_1 \times T(t) + a_2, \quad -3.4^{\circ}\text{C} < T(t) \leq 3.5^{\circ}\text{C} \qquad \text{Eqn 2}$$

$$a_3 \times T(t) + a_4, \quad 3.5^{\circ}\text{C} < T(t) \leq 10.4^{\circ}\text{C} \qquad \text{Eqn 3}$$

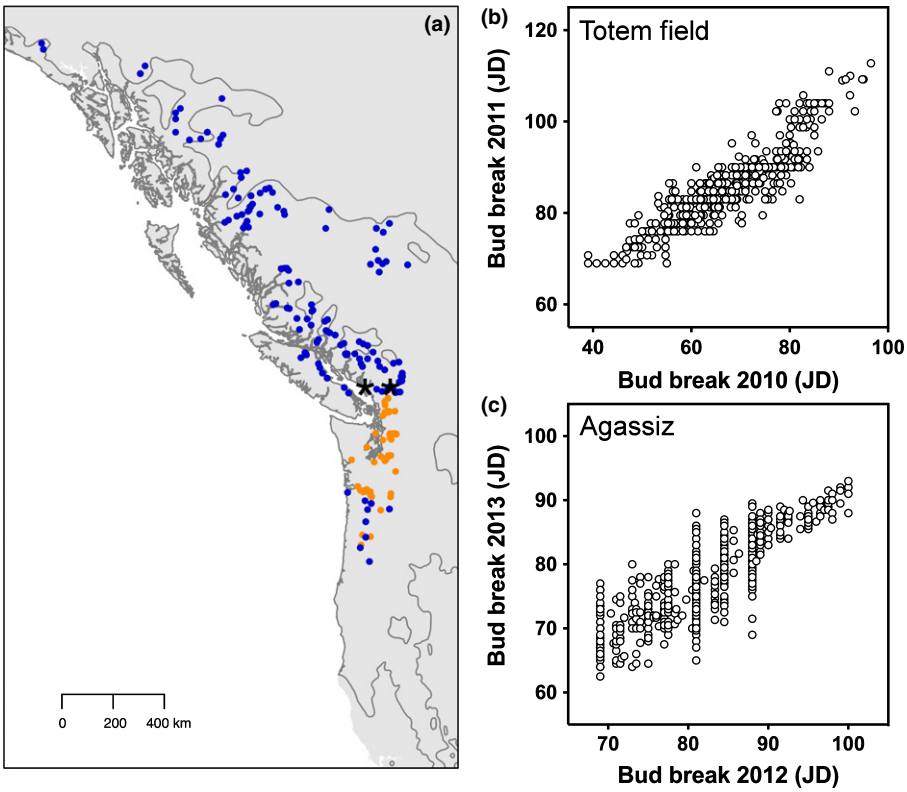

Fig. 1 Origin of *Populus trichocarpa* used for bud-break studies at Totem Field and Agassiz, BC, Canada. (a) Distribution map of *P. trichocarpa* provenances collected by British Columbia Ministry of Forests, Lands and Natural Resource Operations & Rural Development planted at Totem Field and Agassiz ('UBC', blue) and GreenWood/BioEnergy Science Center ('GW/BESC', orange) planted at Agassiz. Species' range (grey lines) obtained from USGS data (https://esp.cr.usgs.gov/data/little/). Asterisks indicate the locations of common gardens in the central region of the species distribution where trees were grown and monitored. (b) Scatterplot illustrating correspondence of spring bud-break (reported as Julian number days, JD) across two consecutive years at Totem Field (2010–2011) (c) Scatterplot illustrating correspondence of spring bud-break (JD) across two consecutive years at Agassiz (2012–2013). In both plots, circles represent individual genotypes. Correspondence of spring bud-break across genotypes planted at both common gardens is illustrated in Supporting Information Fig. S1.

where $a_1 = 0.159$ CU h^{-1} °C^{-1} (CU = arbitrary chilling unit), $a_2 = 0.506$ CU h^{-1}, $a_3 = -0.159$ CU h^{-1} °C^{-1} and $a_4 = 1.621$ CU h^{-1}. These estimates yielded a dimensionless value (on a per-day basis) and were summed to give total chilling units for each winter season. We also summed daily chilling units from 1 November to the date of bud-break for each genotype. This overestimates the critical chilling required by each genotype (which is unknown) but provides a reasonable comparison as the depth and duration of chilling modifies heat sum requirements (reviewed in Cooke *et al.*, 2012).

Bud-break under controlled conditions

A total of 446 UBC genotypes originating throughout the species' range were selected to test the timing of bud-break under controlled conditions within growth chambers. Cuttings with a dormant terminal vegetative bud were collected in early December 2012 from coppiced trees at Totem Field (McKown *et al.*, 2016). These were stored on ice within a cold room maintained at 4°C (full details in Methods S1). We split genotypes into two groups to test two chilling duration scenarios ensuring geographical coverage within each group. Paired plantings of genotypes were used within these treatments to test effects of forcing temperature. For all chamber trials, cuttings were planted in soil within small pots. Humidity averaged 72% and the photosynthetic photon flux density (PPFD) was *c.* 450 µmol m^{-2} s^{-1} for 24 h each day to prevent any differential photoperiodic response.

We tested the following scenarios: (1) a shortened chilling period (30 d at 4°C), followed by two spring forcing temperatures (10°C vs 20°C); or (2) a longer chilling period (98 d at 4°C), followed by these same forcing temperatures (10°C vs 20°C). Temperatures selected followed normal spring temperatures observed at Totem Field (*c.* 10°C, see Table 1), and a warm spring temperature (20°C). We included two additional, smaller trials: (3) testing 207 genotypes with a shortened chilling period (30 d at 4°C) and the lower forcing temperature (10°C) under a highly reduced photoperiod (6 h light daily), and (4) testing 182 genotypes with an extended period of prolonged chilling (135 d at 4°C) followed by the warmer forcing temperature (20°C).

Geographical patterns in bud-break

Population-wide relationships between the geography of tree origin (latitude, elevation) and bud-break from field observations were tested using mixed-effects models with LME4 (Bates *et al.*, 2015) and LMERTEST (Kuznetsova *et al.*, 2017) in R. No significant interaction between latitude and elevation was found, and models report these factors independently. We tested null, linear and quadratic models with 'genotype' as a random effects term to account for multiple clonal ramets within a site. For Agassiz, we also included 'planting year' as a random effects term to account for any differences in tree age (see McKown *et al.*, 2017). We retested bud-break data from Agassiz with accessions originating from 46 to 52°N to determine whether the southern- and northern-most accessions were driving the linear pattern observed. The best-fit models for each year and site were chosen according to the Akaike information criterion (AIC) with the 'model.sel' function in the MUMIN package (Bartoń, 2018). The variance explained (r^2) in each mixed-effects model was determined using MUMIN. We used the same strategy for data from controlled chamber experiments and compared linear and quadratic models against latitude of tree origin to assess the effects of chilling and forcing temperature on bud-break patterns.

Genome-wide association study (GWAS) for loci associated with bud-break

We filtered 2.2M single nucleotide polymorphisms (SNPs) from whole genome sequencing (WGS) (Suarez-Gonzalez *et al.*, 2016; full details in Methods S2) for a minor allele frequency of < 0.05 and a SNP call rate of > 0.9, resulting in 1357 029 bi-allelic SNPs. For our GWAS, we used 424 genotypes and field bud-break data measured from Totem Field in 2010 (described above). The association analysis was performed using the 'qtscore' function in GENABEL package (Aulchenko *et al.*, 2007):

$$y = \mu + X\beta + e \qquad \text{Eqn 4}$$

(y, vector of phenotypes; µ, intercept (overall population mean); X, vector of SNP genotypes; β, vector for SNP effects; e, vector of residual effects).

We evaluated population structure corrections in our GWAS using a principal components analysis (Price *et al.*, 2006), a parametric clustering-based inference ('Q matrix', see McKown *et al.*, 2014b) and a 'simple' regression without any additional correction (further details in Methods S3). We compared models with Bayesian information criterion (BIC) values and selected the model with the lowest BIC value indicating the best fit for the phenotype (Yu *et al.*, 2006). Owing to our population sample size and linkage disequilibrium (LD) between SNPs (i.e. nonindependence of each association test), we used a Bonferroni multiple testing correction as a strict cut-off for our GWAS results and considered SNP–trait associations significant at $\alpha = 0.05/1357029$ ($P < 3.68 \times 10^{-08}$). We then calculated composite pairwise LD for these SNPs based on genotypic correlations (Weir *et al.*, 2004).

Testing GWAS-identified SNPs for phenotypic outcomes

We determined the bud-break phenotypes associated with each allele using data from Totem Field (2010) and mixed-effects modelling with 'SNP' as a fixed effect and 'genotype' as a random effects term. Allelic-based phenotypic differences were calculated with Least Squares Means tests using the 'difflsmeans' function and confidence intervals with the 'lsmeansLT' function in the LMERTEST package. Based on P-values generated by LMERTEST, we assigned a relative bud-break phenotype to each allele ('early', 'mid-range' or 'late'). Using this scoring regime, each accession was given a general genotype–phenotype profile (assuming independent effects due to low LD; see the Results section).

We then tested differences in the timing of bud-break using SNP-based allelic groupings and field data from 156 UBC and

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Table 1 Seasonal temperatures, chilling units and median spring bud-break dates for wild-sourced *Populus trichocarpa* genotypes grown at Totem Field and Agassiz common gardens

Common garden	Period	Winter days (no. of days < 5°C)[a]	Winter chilling[b]	Spring period (no. of days)[c]	Mean forcing temperature (°C)[d]	Bud-break (JD)
Totem Field	2009–2010	33	29.9	68	10.0	7 March (66)
	2010–2011	67	69.1	26	9.4	26 March (85)
Agassiz	2011–2012	85	55.9	60	7.9	22 March (81)
	2012–2013	73	49.3	62	8.2	19 March (78)

The number of winter days, accumulated winter chilling, duration of the spring warming period, and mean spring forcing temperatures are determined from daily weather records for each common garden. JD, Julian number day.
[a]Number of days with daily mean temperatures < 5°C from 1 November to the median bud-break date.
[b]Accumulated chilling units from 1 November to the last episode of spring frost.
[c]Number of days between the last episode of spring frost (a sustained period of minimum 5 d with a daily mean temperature < 2°C) and the median bud-break date.
[d]Mean of daily maximum temperatures reported during the spring warming period. (Full weather data is available in Supporting Information Table S2).

420 GW/BESC genotypes grown at Agassiz. The GW/BESC trees (and associated phenotypic data) were not included in our GWAS and were used as a proof-of-concept and validation for our GWAS-identified SNPs. To obtain SNPs for GW/BESC genotypes, we downloaded publicly available, independently sequenced SNPs (annotated to v.3.0 of the genome) from Phytozome (http://phytozome.jgi.doe.gov/pz/portal.html). We tested bud-break data with mixed-effects modelling including 'SNP' as a fixed effect, 'genotype' as a random effects term to account for multiple clonal ramets, and 'planting year' due to differing tree ages at Agassiz. Using linear modelling, we also tested standardized bud-break data from our controlled chamber experiments for significant interactions between fixed effects ('SNP', 'chilling duration' and 'forcing temperature').

Data accessibility

Raw whole-genome sequencing data and alignment information have been deposited on SRA (accession: PRJNA276056; ID 276056) available at this link: http://www-ncbi-nlm-nih-gov.ezproxy.library.ubc.ca/sra/?term=PRJNA276056.

Results

Field patterns in bud-break phenology

Field bud-break dates (JD) were recorded over consecutive years at common gardens planted at Totem Field, BC (49.3°N, 123.1°W; years = 2010, 2011) and Agassiz, BC (49.3°N, 122.0°W; years = 2012, 2013) for 1297 *P. trichocarpa* genotypes originating from a large portion of the species' natural range (44–60°N, 121–138°W; Fig. 1a; Table S1). At Totem Field, median bud-break in 2010 was 7 March (JD 66; Fig. 2; Table 1). Weather conditions in 2010 showed that the last persistent winter frost was followed by a long spring period, and median bud-break was not achieved until after a period of 68 d with sustained warming. In 2011, median bud-break occurred later (compared to 2010) after a season where trees experienced

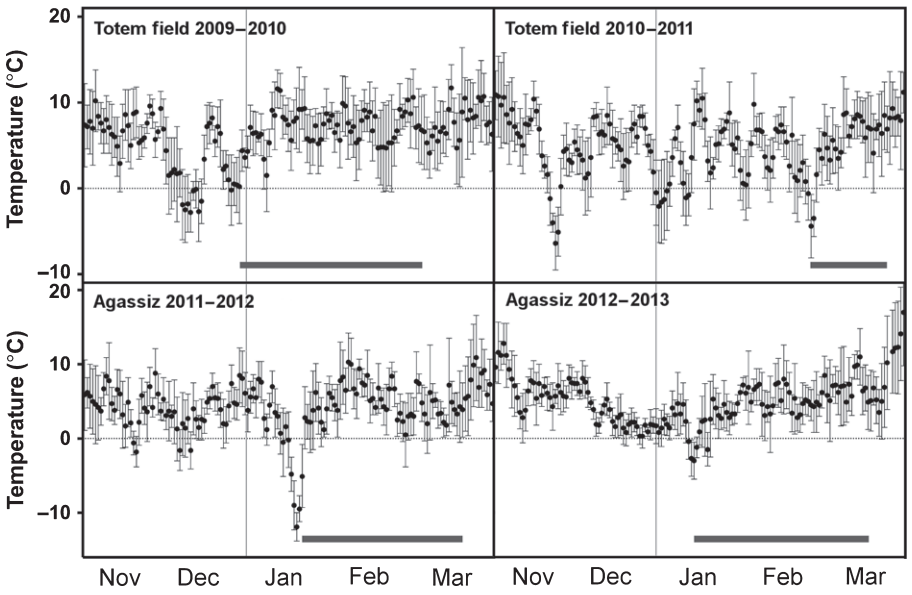

Fig. 2 Seasonal winter and spring temperature patterns from November to March at Totem Field, BC, Canada (2009–2011) and Agassiz, BC (2011–2013). Grey vertical lines indicate 1 January and grey horizontal bars indicate the spring warming period defined as the number of days between the last episode of winter chilling (a sustained cold period of minimum 3 d with a daily mean temperature ≤ 2°C), and the median bud-break date. Circles represent mean daily temperature and vertical bars indicate the daily maximum and minimum temperatures (full weather data available in Supporting Information Table S2).

much greater winter chilling overall and a substantially shorter spring warming period. At Agassiz, median bud-break was slightly earlier in 2013 compared to 2012. Overall winter chilling was lower in 2013, and was offset by a slightly longer spring period. Across all years, this same pattern was apparent where a lower amount of winter chilling resulted in a longer spring warming period (relative to the last period of winter frost). At each site, bud-break dates were significantly correlated across years (Totem Field $r^2 = 0.80$, $P < 0.0001$; Agassiz $r^2 = 0.73$, $P < 0.0001$) indicating consistent genotypic rankings (Fig. 1b,c). Bud-break also was highly correlated across common gardens comparing 185 genotypes planted at both sites (average $r^2 = 0.72$, $P < 0.0001$; Fig. S1).

Population-wide latitudinal patterns in bud-break showed the same trends within each common garden assessed either as total chilling units or JD (Figs 3, S2). We found a significant linear pattern between bud-break and latitude of tree origin at Agassiz (Fig. 3; Table 2). Genotypes from southernmost provenances generally had earlier bud-break dates than their counterparts from the central part of the species' range (50–54°N). By contrast, bud-break data from Totem Field (spanning the greatest geographical range of tree origin) had a quadratic relationship with latitude. At Totem Field, the earliest bud-break dates generally occurred in genotypes from the southern- and northern-most provenances. Notably, we found no corresponding relationship between bud-break and the elevation of tree origin (Figs 4, S3). Accessions at Totem Field had the greatest accumulation of chilling units over the 2011 season, whereas year-to-year differences were less pronounced at Agassiz, corresponding with our other weather metrics (Fig. 2; Table 1). Within each common garden, patterns between bud-break and latitude were similar across years, regardless of timing within the spring season, suggesting a low impact of photoperiod in determining genotypic rankings and trait variation (Figs 3, S2). By contrast, the yearly differences in total accumulation of chilling units likely had a greater influence on bud-break timing. It also was clear that the lower extent of chilling in 2010 (and subsequently longer spring warming period) strongly increased population-wide trait variation compared to the following year with greater chilling.

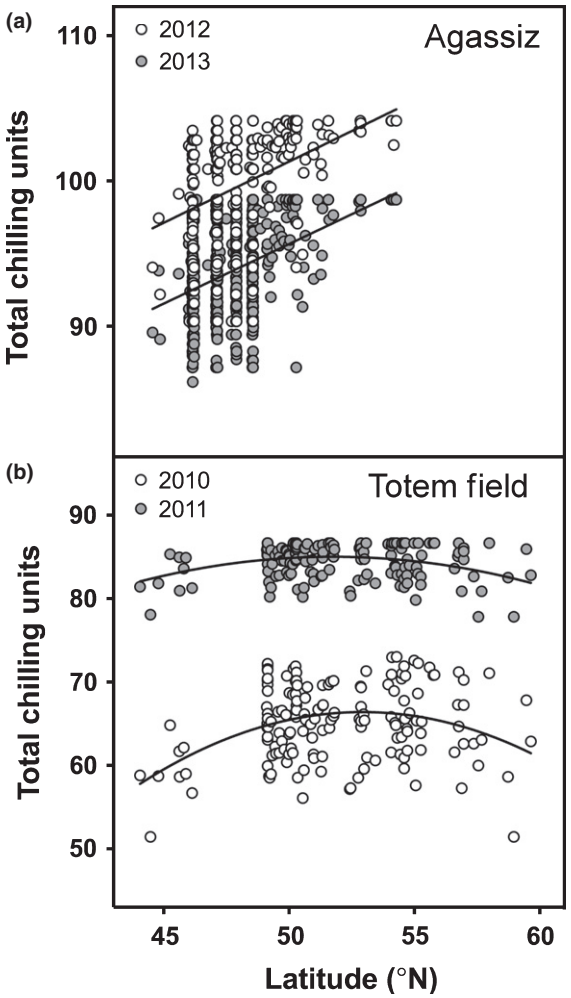

Fig. 3 Relationships between spring bud-break in provenances of *Populus trichocarpa* reported as total chilling units (accumulated throughout the winter–spring season) and latitude of tree origin for trees grown at Agassiz and Totem Field. (a) A linear relationship is observed at Agassiz (statistics and models available in Table 2). Further tests using accessions from 46 to 52°N confirm that the pattern at Agassiz is not driven by genotypes from lower or higher latitudes (details in Table 2). (b) A significant quadratic relationship is observed at Totem Field where the genotypes originate from a much broader portion of the species' range (statistics and models available in Table 2). Circles represent provenances in both graphs.

Phenotypic variation in bud-break under controlled warming

We tested the timing of bud-break relating to the length of winter cold (chilling duration) and spring warmth (forcing temperature) using 446 genotypes grown under controlled conditions within growth chambers. Genotypes were evaluated under shortened chilling (30 d at 4°C, *c.* 29.5 total chilling units) followed by two different forcing temperatures (10°C vs 20°C), or a longer chilling period (98 d at 4°C, *c.* 96.5 total chilling units) followed by the same forcing temperatures. Results from these different scenarios showed that chilling duration and forcing temperature both had strong effects on the timing of bud-break (Fig. 5) while maintaining relatively consistent genotypic rankings (Figs S1, S4). The first scenario (less accumulated chilling) resulted in a median bud-break of 28 d (after planting) at 10°C and 12 d at 20°C. The second scenario (more accumulated chilling) resulted in a median bud-break of 11 and 5 d at 10°C and 20°C, respectively (Fig. 5). On a population-wide basis, three-fold more chilling (+ 68 d at 4°C) advanced bud-break by 61% across both forcing temperatures, whereas two-fold higher forcing temperatures (+ 10°C) advanced bud-break by 54% within both chilling scenarios. Notably, the treatment combination with more chilling and the lower forcing temperature (10°C) had relatively similar results to the combination with less chilling but a warmer forcing temperature (20°C).

The quadratic relationship between days to bud-break and latitude of tree origin was apparent only at the lower forcing temperature for both chilling scenarios (Fig. 5; Table 2). By contrast, the

higher forcing temperature effectively reduced population-wide variation and 'flattened' the pattern in bud-break. This further supports the geographical trend not necessarily being driven by particular environmental effects, such as changing photoperiod (which was held constant in the growth chamber). The 'flattening' effects of higher forcing temperature were confirmed with our additional experiment of extended chilling (Fig. S5). The trial testing bud-break under 6 h of light at the lower forcing temperature (10°C) resulted in 201 out of 207 (97%) genotypes breaking, confirming that a longer and/or changing photoperiod was unnecessary to induce bud-break (data not shown).

Association genetics of bud-break

We used GWAS with 424 *P. trichocarpa* individuals from the UBC collection and 1.4 million filtered, bi-allelic SNPs to uncover genes associated with the variation in bud-break (using data from Totem Field in 2010). The simple model (with no population structure correction) provided the best fit for the phenotypic data (BIC = 3286.4) compared to the principal components-based structure correction (BIC = 3292.3) or clustering-based Q matrix (BIC = 3295.4). Using results assessed with the simple model, GWAS uncovered 31 SNPs within 16 genes annotated to the *P. trichocarpa* reference genome (v.3.0) under the threshold for the Bonferroni multiple testing correction at $\alpha = 0.05$ ($P < 3.68 \times 10^{-8}$; Fig. 6; Tables 3, S3).

GWAS identified SNPs in genes putatively responsible for meristem growth and bud activation (Potri.005G209100: *FAF2*, *FANTASTIC FOUR 2*), cell proliferation and expansion (Potri.016G022700: *MAPR4*, *MEMBRANE-ASSOCIATED PROGESTERONE BINDING PROTEIN 4*; Potri.017G081000: *TUA6*, *TUBULIN ALPHA-6*), carbohydrate binding (Potri.015G118800: *PP2-A9*, *PHLOEM PROTEIN 2-A9*; Potri.015G120100: *PP2-A10*, *PHLOEM PROTEIN 2-A10*), cold acclimation (Potri.007G032000: cold acclimation protein WCOR413 family) and heat response (Potri.001G148500: electron carrier/iron ion-binding protein, Potri.016G019800: heat shock protein 70 (HSP70) family protein). Among the GWAS-identified loci, the greatest numbers of SNPs were retrieved within Potri.014G102700 or *CYP78A6* (*CYTOCHROME P450, FAMILY 78, SUBFAMILY A* monooxygenase). LD across all 16 GWAS-identified loci was low (average $r^2 = 0.02$), whereas LD within loci was high ($r^2 > 0.7$; Table S4). Based on LD values, we found that *CYP78A6* had two distinct linkage regions (Fig. 7). One region upstream of the gene had two SNPs in high LD ($r^2 = 0.78$) that showed little relationship to other SNPs associated with *CYP78A6*. The second region in high LD (average $r^2 = 0.80$) included SNPs from the *CYP78A6* coding sequence and the downstream intergenic region.

Individuals with major homozygous alleles had significantly different phenotypes compared to those with minor homozygous alleles (Tables 4, S5). Some heterozygous individuals had 'mid-range' bud-break phenotypes compared to the major and minor homozygotes, whereas others were phenotypically similar to minor allelic homozygotes, suggesting partial or incomplete dominance of the major allele. Across the GWAS results, the difference between an 'early' vs 'late' phenotype was up to 21 d. Strikingly, the phenotypic effects associated with SNPs from the two unlinked genetic regions of *CYP78A6* were reversed. SNPs

Table 2 Models testing bud-break in *Populus trichocarpa* against latitude of tree origin using phenotypic data assessed from two common gardens (Totem Field, Agassiz) and controlled growth chamber studies

Dataset[a]	Latitudinal range (°N)	AIC null model	AIC linear model	AIC quadratic model	Final model	Equation	r^{2b}
Agassiz (2012) – CU	44.5–54.25	9699.8	**9561.6**	9562.7	Linear	$0.920x + 56.11$	0.11 (0.75)
Agassiz (2012 subset) – CU	46.0–51.7	9464.2	**9344.3**	9348.2	Linear	$0.925x + 55.89$	0.10 (0.74)
Agassiz (2013) – CU	44.5–54.25	8354.8	**8148.7**	8153	Linear	$0.886x + 51.89$	0.17 (0.86)
Agassiz (2013 subset) – CU	46.0–51.7	8155.9	**7974.1**	7979.1	Linear	$0.900x + 51.23$	0.16 (0.86)
Totem Field (2010) – CU	44.0–59.6	10373.4	10368.1	**10351.2**	Quadratic	$-0.0904x^2 + 9.622x - 190.4$	0.068 (0.85)
Totem Field (2011) – CU	44.0–59.6	7876.5	7882.5	**7867.4**	Quadratic	$-0.0440x^2 + 4.582x - 34.6$	0.048 (0.81)
Agassiz (2012) – JD	44.5–54.25	11456.1	**11210.6**	11216.5	Linear	$2.085x - 16.84$	0.19 (0.82)
Agassiz (2012 subset) – JD	46.0–51.7	11179.1	**10967.5**	10973.1	Linear	$2.084x - 16.82$	0.17 (0.81)
Agassiz (2013) – JD	44.5–54.25	10707.8	**10467.8**	10473.6	Linear	$1.847x - 10.35$	0.20 (0.87)
Agassiz (2013 subset) – JD	46.0–51.7	10440.2	**10238.2**	10242.5	Linear	$1.825x - 9.28$	0.18 (0.86)
Totem Field (2010) – JD	44.0–59.6	13540.8	13532.5	**13517.3**	Quadratic	$-0.1863x^2 + 19.89x - 462.0$	0.065 (0.86)
Totem Field (2011) – JD	44.0–59.6	12437.1	12439.1	**12431.6**	Quadratic	$-0.1169x^2 + 12.29x - 236.5$	0.033 (0.85)
Growth chamber – Less chilling, 10°C	44.0–59.6	1413.7	1414.4	**1410.5**	Quadratic	$-0.0812x^2 + 8.034x - 169.6$	0.032
Growth chamber – Less chilling, 20°C	44.0–59.6	**1090.6**	1092.5	1094.4	Null	1	0
Growth chamber – More chilling, 10°C	44.0–59.6	1157.6	1159	**1151.4**	Quadratic	$-0.05475x^2 + 5.682x - 136.3$	0.045
Growth chamber – More chilling, 20°C	44.0–59.6	**929.7**	930.9	936.3	Null	1	0

Field data were tested with mixed-effects modelling and growth chamber data were tested using linear modelling. Final models were selected using Akaike information criterion (AIC) values and are highlighted in bold. The final equation and r^2 for the best model is shown for each dataset. CU, total seasonal chilling units; JD, Julian number day.

[a]A subset of trees from Agassiz were tested to determine that accessions from the furthermost provenances were not driving the linear trends.

[b]r^2 values for mixed-effects models determined using MuMIn package. Values are estimated for fixed effects and values in brackets are for fixed and random effects (total model).

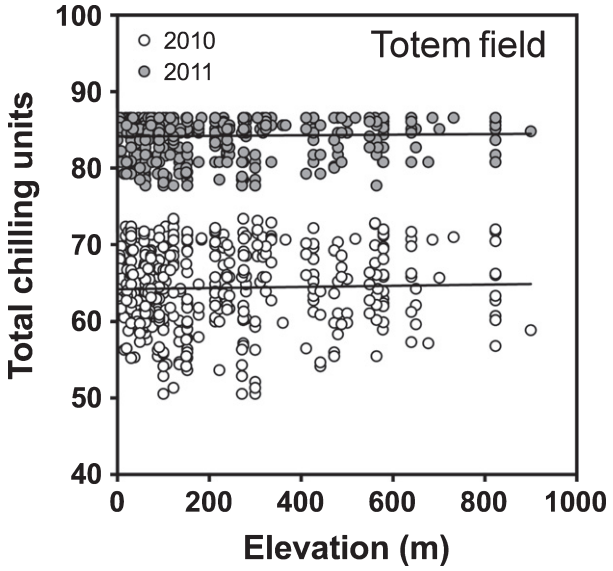

Fig. 4 Relationship between spring bud-break in *Populus trichocarpa* reported as total chilling units (accumulated throughout the winter–spring season) and the elevation of tree origin for genotypes cultivated at Totem Field. No significant pattern is observed between elevation and bud-break. Circles represent individual genotypes.

from the upstream region showed that major homozygous allelic variants had later bud-break dates, whereas SNPs from the coding sequence/downstream region showed that major homozygous allelic variants had earlier bud-break dates (boxplots in Fig. 7; Table 4). As a consequence of low LD and contrasting phenotypic effects, we considered both regions around *CYP78A6* separately in subsequent analyses (i.e. 17 genetic regions were assessed in subsequent phenotypic validation tests).

Phenotypic validation of GWAS-identified loci

We tested bud-break data from UBC genotypes at Totem Field and Agassiz for consistency of phenotypes among allelic groups (Tables 5, S5). At Totem Field, significance and the same directionality of phenotypic change were maintained in 2011 over a longer chilling period and shorter spring warming period (compared to 2010). At Agassiz, significant phenotypic differences for 14 of the GWAS-identified genetic regions were found using bud-break data for 156 UBC genotypes with WGS. In this set of individuals, only SNPs on chromosome 15 were not significant. We then tested bud-break data for 420 GW/BESC genotypes (originating from the southern-central part of the species' range, 44.5–48.9°N) with available WGS data from Phytozome (see the Materials and Methods section) and planted at Agassiz. Using this independent collection, 12 of the GWAS-identified genetic regions showed significant differences in the timing of bud-break across allelic groups (Tables 4, S5). Only SNPs from *FAF2* and *FZL* on chromosome 5 did not show significant differences among allelic groups (in either year of phenotypic observations), whereas SNPs on chromosome 15 were invariant in the GW/BESC population.

Using data from our controlled chamber study, we standardized bud-break dates (within each of our four trials) and used linear modelling to test for significant interactive effects between GWAS-identified genetic regions, chilling duration and forcing temperature (Table S6). All three fixed effects were significant; however, we identified only four instances of significant interactions between loci and chilling duration, including the poplar homologues of *FZL*, HSP70, *MAPR4* and *TUA6*. Other interactive terms involving loci as a fixed effect were not significant for any test.

Genotypic vs phenotypic patterns in bud-break

Data from field and growth chamber studies indicated that the southern- and northern-most *P. trichocarpa* provenances shared comparable 'early' bud-break phenotypes. To assess whether provenances from these disparate regions of the species' range also had similar genetic profiles, we assigned 'early', 'mid-range' or 'late' bud-break phenotypes to each of the GWAS-identified loci. Using this scoring regime, we generated genotype–phenotype profiles (assuming independent effects of the loci due to low LD) for all sequenced UBC accessions. Genotype–phenotype profiles sorted by latitude of tree origin highlighted that genotypes from the southern- and northern-most portions of the species' range did not share similar overall genetic profiles

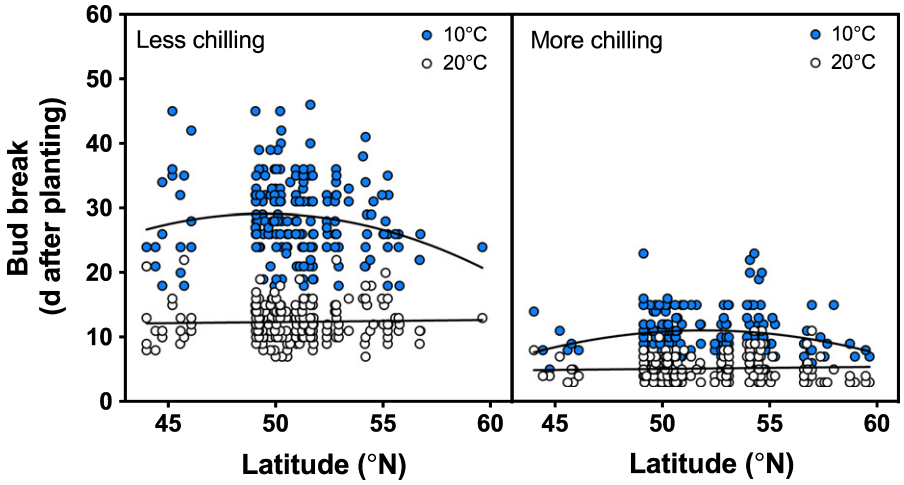

Fig. 5 Timing of bud-break in *Populus trichocarpa* genotypes tested under different climate scenarios altering the length of chilling and forcing temperature. The increased chilling represents a longer period of cold exposure (+ 68 d). Genotypes (individual circles) are paired across the two forcing temperatures (10°C vs 20°C) within each chilling scenario (correspondence between genotypes within chilling treatments available in Supporting Information Fig. S4). A significant quadratic relationship is observed between bud-break and latitude of tree origin with the cooler forcing temperature (10°C), whereas at the higher forcing temperature (20°C), no relationship is apparent (statistics and regressions available in Table 2).

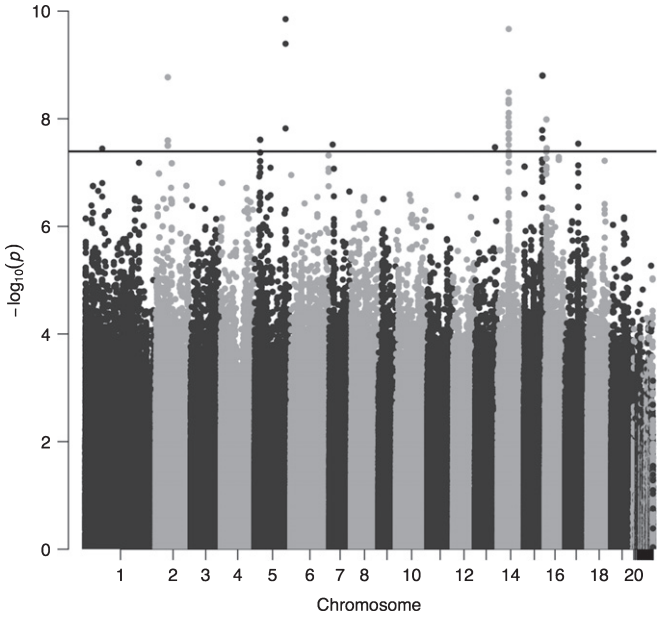

Fig. 6 Manhattan plot of single nucleotide polymorphism (SNP) markers uncovered by a genome-wide association study (GWAS) for spring bud-break in *Populus trichocarpa*. Genotypes from the UBC collection and phenotypic data from Totem Field (2010) were used for GWAS. A Bonferroni multiple testing correction cut-off (horizontal line) was used to determine significant SNP–trait associations at $\alpha = 0.05/1\,358\,312$ ($P < 3.68 \times 10^{-08}$).

(Fig. 8). Notably, genotypes from the northern regions of the species' range had a much higher proportion of 'late' bud-break alleles and a lower proportion of 'early' bud-break alleles than genotypes from more southerly regions.

When we assessed individual loci, we found different trends apparent with latitude. In some loci, northern provenances possessed higher frequencies of major homozygous individuals compared to mid-range and southern provenances, typified by the cold acclimation protein (Potri.007G032000), *FAF2* and *QQT1* (Figs 9a–c, S6). This trend also was observed for *CYP78A6* (upstream region) and an electron carrier/iron-binding protein (Potri.001G148500; Fig. S6). The opposite pattern was found for other loci, such as *CYP78A6* (coding sequence and downstream region), *FZL* and *TUA6* (Figs 9d–f, S6), and a chloroplast-localized protein (Potri.014G102400; Fig. S6). In a third trend, southern and northern provenances had similar SNP frequencies, apparently following the quadratic geographical trend. This was observed in the ATPase, F1 complex, OSCP/delta subunit protein (Potri.014G102100), HSP70, and *MAPR4* (Figs 9g–i, S6). This pattern also was apparent in the cellular apoptosis susceptibility protein/importin-alpha re-exporter (Potri.014G100000; Fig. S6).

Discussion

An interesting pattern from our study was the general quadratic relationship apparent between the timing of bud-break and latitude of tree origin. This pattern in spring phenology contrasts the strongly clinal, negative trend with latitude consistently observed in the autumn phenology of *Populus trichocarpa* (Evans *et al.*, 2014; McKown *et al.*, 2014a). Although linear trends were observed at Agassiz, this is likely due to a lack of genotypes from northernmost regions (provenances originated from < 54.3°N), indicating that the accessions selected for Agassiz (and planted in other common gardens, see Evans *et al.*, 2014; Guerra *et al.*, 2016) capture only a portion of the geographical trend in bud-break across the species. Prior investigations in poplar have shown weak linear patterns between bud-break and latitude (e.g. Farmer & Reinholt, 1986; Olson *et al.*, 2013; Guerra *et al.*, 2016) or no trends (e.g. Hall *et al.*, 2007; Luquez *et al.*, 2008; Soolanayakanahally *et al.*, 2015; Evans *et al.*, 2016). Nevertheless, there are reported results that follow a nonlinear pattern. Soolanayakanahally *et al.* (2013) observed earlier bud-break in northern and southern accessions of *P. balsamifera* grown within a common garden setting (Fig. S7). Another common garden study reported a clinal trend where bud-break occurred earlier in northern provenances of *P. tremuloides* compared to mid-latitudinal provenances (Li *et al.*, 2010). Earlier bud-break also was observed in southern *P. tremuloides* provenances but was reported as a 'reversed' cline.

In our study, the quadratic geographical pattern observed at Totem Field was also detected in the growth chamber trials, with the loss of the trend occurring only under very high forcing temperatures. Farmer & Reinholt (1986) surmised that earlier bud-break in trees might relate to earlier bud set (i.e. lengthening the overwintering dormancy period), as this would act to modify the depth of bud dormancy by increasing the time for accumulating chilling units. This explanation is plausible for northern *P. trichocarpa*, but not for earlier bud-break observed in the southern genotypes, which set bud much later in the autumn than northern genotypes at Totem Field (McKown *et al.*, 2013). In addition, the accessions planted at Agassiz also were planted further south in multiple common gardens (see Evans *et al.*, 2014; Guerra *et al.*, 2016), and bud-break results from these trials (specifically in genotypes originating from 50 to 54°N) failed to exhibit this effect.

Genes underlying ecological variation in bud-break

Using a genome-wide association study (GWAS) with 1.4 million single nucleotide polymorphisms (SNPs) retrieved only a small number of loci associated with variation in the timing of bud-break in *P. trichocarpa*. Notably, SNPs from the poplar homologue of *CYP78A6* were previously identified with associations to bud-break and leaf flush (McKown *et al.*, 2014b) and retrieved in F_{ST} analyses indicating diversifying selection (Evans *et al.*, 2014; Geraldes *et al.*, 2014; Table S3). We found that loci had repeatable phenotypes, reflecting conserved genotypic rankings (Fig. 1b,c) and high trait heritability estimates previously determined for bud-break in *P. trichocarpa* ($H^2 > 0.9$; Bradshaw & Stettler, 1995; McKown *et al.*, 2014a; Guerra *et al.*, 2016). Although GWAS-identified loci implicated a number of different effects on plant growth, response to heat and/or signalling, most showed little interaction with modified chilling duration or forcing temperature (under our controlled conditions). This suggests that many of these genes may contribute to predictable trait

Table 3 Genes identified by a genome-wide association study (GWAS) underlying variation in spring bud-break in *Populus trichocarpa*

Potri. gene (v.3.0)	SNPs	*Arabidopsis* homolog	Annotation	Physiological function
001G148500	1	AT1G32730	Electron carrier/iron ion-binding protein	Interacts with histone deacetylase regulating heat response (Buszewicz *et al.*, 2016)
002G118100	4	AT5G22370	*QQT1* (*QUATRE-QUART 1*)	Interacts with microtubules during cell division (Lahmy *et al.*, 2007)
005G057900	1	AT3G18670	Ankyrin repeat family protein	Transmembrane protein (Becerra *et al.*, 2004)
005G209100	1	AT1G03170	*FAF2* (*FANTASTIC FOUR 2*)	Regulates shoot meristem size and activity (Wahl *et al.*, 2010); Involved in cytokinin-mediated bud activation (Müller *et al.*, 2015)
005G209200	2	AT1G03160	*FZL* (*FZO-LIKE*)	Determinant of thylakoid and chloroplast morphology (Gao *et al.*, 2006)
007G032000	1	AT4G37220	Cold acclimation protein WCOR413 family	Induced by abscisic acid (Hoth *et al.*, 2002); Responds to sugar (Gonzali *et al.*, 2006)
014G100000	1	AT2G46520	Cellular apoptosis susceptibility protein/importin-alpha re-exporter	Root to shoot phloem-mobile transcript (Thieme *et al.*, 2015)
014G102100	2	AT4G00895	ATPase, F1 complex, OSCP/delta subunit protein	Shoot-to-root phloem-mobile transcript (Thieme *et al.*, 2015)
014G102400	1	AT3G61870	Chloroplast-localized protein	Chloroplast inner-membrane protein (Ferro *et al.*, 2002)
014G102700	11	AT2G46660	*CYP78A6* (*CYTOCHROME P450, FAMILY 78, SUBFAMILY A*)	Responds to diurnal thermocycles (Pan *et al.*, 2009); Promotes cell proliferation and cell expansion (Wang *et al.*, 2008; Fang *et al.*, 2012)
015G118800	1	AT1G31200	*PP2-A9* (*PHLOEM PROTEIN 2-A9*)	Involved in carbohydrate binding and intercellular signalling (Dinant *et al.*, 2003)
015G119700	1	AT5G04690	Ankyrin repeat family protein	Transmembrane protein (Becerra *et al.*, 2004)
015G120100	1	AT1G10155	*PP2-A10* (*PHLOEM PROTEIN 2-A10*)	Involved in carbohydrate binding and intercellular signalling (Dinant *et al.*, 2003)
016G019800	1	AT4G16660	Heat shock protein 70 (HSP70) family protein	Upregulated under chilling stress (Ma *et al.*, 2016)
016G022700	1	AT4G14965	*MAPR4* (*MEMBRANE-ASSOCIATED PROGESTERONE BINDING PROTEIN 4*)	Cytochrome b5-like heme/steroid-binding domain, upregulated in response to hormones, affects cell expansion (Yang *et al.*, 2005)
017G081000	1	AT4G14960	*TUA6* (*TUBULIN ALPHA-6*)	Affects cell proliferation and expansion (Xiong *et al.*, 2013); Target of DAM transcription factors (Kumar *et al.*, 2016)

Genes are annotated to the *P. trichocarpa* reference genome v.3.0 and include number of single nucleotide polymorphisms (SNPs), *Arabidopsis* homologue, and putative physiological function (full details in Supporting Information Table S3).

outcomes. Most of the GWAS-identified loci showed the same bud-break phenotypes (using SNP-based groupings) in our GW/BESC test population from the southern-central portion of the species' range. Although markers on chromosome 5 were not significant, they were variable in this population and showed the same direction of phenotypic change. Individuals with minor alleles were fewer in number within this population, suggesting insufficient power to detect statistically significant variation. By contrast, the markers on chromosome 15 were invariant within the second test population. Although chromosome 15 can show signatures of adaptive introgression with *P. balsamifera* in the central portion of the *P. trichocarpa* range (Suarez-Gonzalez *et al.*, 2016), SNPs on chromosome 15 from our study common to mid-range genotypes (52–53°N) also were found in more southerly accessions (44–46°N) (Fig. S6).

Some of the genes identified by GWAS present promising candidates for future studies. In *Arabidopsis*, some of these loci have characterized effects in temperature sensing and/or heat responses. The cytochrome P450 monooxygenase *CYP78A6* responds to temperature and daily thermocycles (Pan *et al.*, 2009). Other loci involved with temperature include an electron carrier/iron ion-binding protein that interacts with histone deacetylase regulating heat response (Buszewicz *et al.*, 2016), a cold acclimation protein that responds to both abscisic acid and sugar sensing (Hoth *et al.*, 2002; Gonzali *et al.*, 2006), and a heat shock protein (HSP70) that is upregulated under chilling stress (Ma *et al.*, 2016). In poplar, these loci may have direct roles in determining the chilling and/or heat sum required for the activity–dormancy cycle of the meristem. Other GWAS-identified loci implicate meristem regulation, cell growth and cell divisions, and cell-to-cell signalling. *FAF* genes are involved in meristem growth and size, acting to arrest meristem activity in *Arabidopsis* (Wahl *et al.*, 2010). They also have roles in cytokinin-mediated bud activation and outgrowth (Müller *et al.*, 2015). *QQT1* interacts with microtubules during cell division (Lahmy *et al.*, 2007) and plays a critical role in the structure of the meristem (Kwak *et al.*, 2017). *CYP78A6*, *TUA6* and *MAPR4* also have noted effects on cell proliferation and expansion in *Arabidopsis* (Yang *et al.*, 2005; Wang *et al.*, 2008; Fang *et al.*, 2012; Xiong *et al.*, 2013). Notably, *TUA6* is a target of DAM genes in *Malus* (Kumar *et al.*, 2016). Additional genes uncovered by the GWAS identify a role for phloem signalling. These include phloem proteins (*PP2-A9*, *PP2-A10*) involved in carbohydrate binding and intercellular signalling in *Arabidopsis* (Dinant *et al.*, 2003) and phloem-mobile messenger RNAs (cellular apoptosis susceptibility protein/importin-alpha re-exporter and an ATPase, F1 complex, OSCP/delta subunit protein; Thieme *et al.*, 2015). Cell-to-cell signalling has previously been connected with bud dormancy,

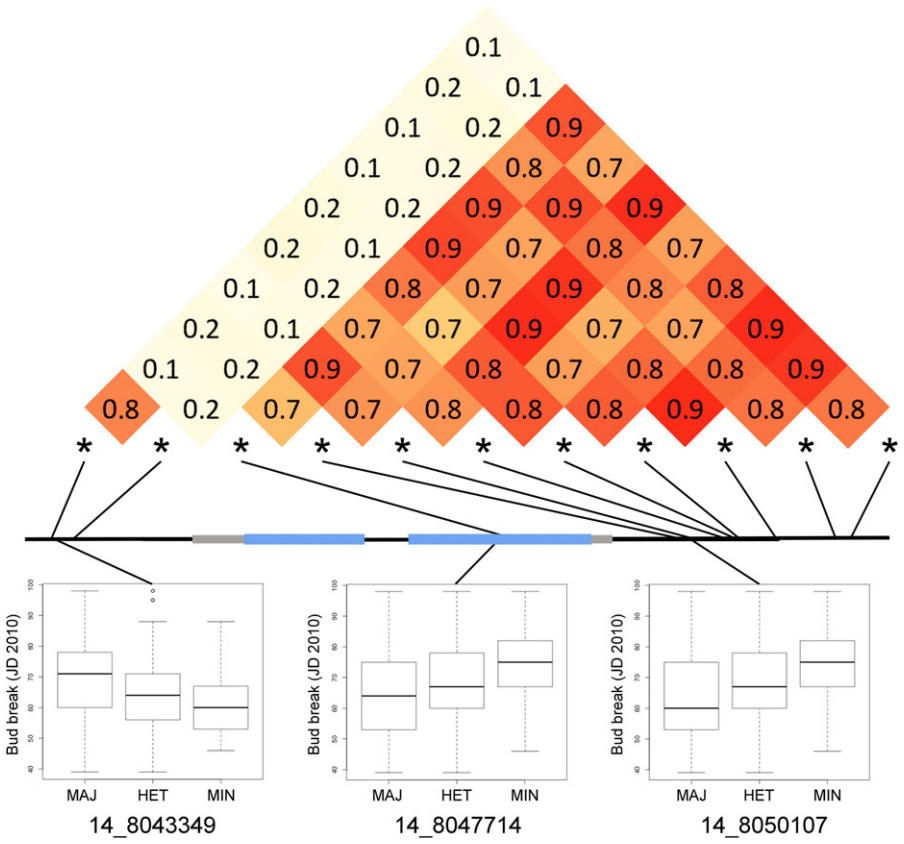

Fig. 7 Linkage disequilibrium (LD) between single nucleotide polymorhisms (SNPs) in Potri.014G102700 (homologue of *Arabidopsis CYTOCHROME P450, FAMILY 78, SUBFAMILY A; CYP78A6*) retrieved by genome-wide association study (GWAS) with spring bud-break in *Populus trichocarpa*. SNPs upstream of the *CYP78A6* locus suggest a region in high LD (highlighted in orange and red according to r^2 values), whereas SNPs within and downstream of the coding sequence suggest a second region in high LD. Different allelic patterns in bud-break phenotypes are associated with the upstream and gene/downstream regions. Box plots indicate the data distribution (median with quartile whiskers and maximum values indicated as circles) for bud-break at Totem Field (2010).

both through hormones and metabolites, such as carbohydrates (Cooke *et al.*, 2012).

Interestingly, genes previously implicated in bud-break in *Populus* were not identified (e.g. phytochromes, DAM genes, *CONSTANS*, *FLOWERING TIME*, *EBB1*) nor were those highlighted throughout dormancy (Howe *et al.*, 2015; Brunner *et al.*, 2017). Our study does not imply that these genes are not involved in dormancy and/or bud-break processes. GWAS is a

Table 4 Phenotypic differences between loci uncovered by a genome-wide association study (GWAS) in *Populus trichocarpa* using mixed-effects modelling testing

Gene	MAJ-HET	MAJ-MIN	HET-MIN	Bud-break range (d)
001G148500 (electron carrier/iron-binding)	***	***	*	11
002G118100 (*QQT1*)	***	**	ns	10
005G057900 (ankyrin repeat family protein)	***	***	ns	11
005G209100 (*FAF2*)	***	***	ns	10
005G209200 (*FZL*)	***	***	**	10
007G032000 (cold acclimation protein WCOR413)	***	***	*	15
014G100000 (cellular apoptosis susceptibility protein)	***	***	ns	12
014G102100 (ATPase, F1 complex)	***	***	ns	9
014G102400 (chloroplast-localized protein)	***	***	***	9
014G102700 (*CYP78A6* – upstream region)	***	***	ns	9
014G102700 (*CYP78A6* – gene/downstream region)	***	***	**	10
015G118800 (*PP2-A9*)	***	***	ns	17
015G119700 (ankyrin repeat family protein)	**	***	**	21
015G120100 (*PP2-A10*)	***	***	**	21
016G019800 (HSP70)	***	***	***	16
016G022700 (*MAPR4*)	***	***	***	17
017G081000 (*TUA6*)	*	***	***	8

The phenotypic range between mean bud-break associated with major and minor alleles for data from Totem Field (2010) are reported (full details in Supporting Information Table S5). MAJ, major homozygous; HET, heterozygous; MIN, minor homozygous.
***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; ns, not significant.

Table 5 Significance of phenotypic differences in spring bud break based on loci uncovered by a genome-wide association study (GWAS)

Tree collection	UBC		GW/BESC			
Site-year[a]	TF-2010	TF-2011	AG-2012	AG-2013	AG-2012	AG-2013
Genotypes (*n*)	*n* = 424	*n* = 424	*n* = 156	*n* = 156	*n* = 420	*n* = 420
Latitudinal range (°N)	44.0–59.6	44.0–59.6	49.1–54.3	49.1–54.3	44.5–48.9	44.5–48.9
001G148500 (electron carrier/iron-binding)	***	***	***	***	**	**
002G118100 (*QQT1*)	***	***	***	***	***	***
005G057900 (ankyrin repeat family protein)	***	***	**	**	**	***
005G209100 (*FAF2*)	***	***	*	*	ns	ns
005G209200 (*FZL*)	***	***	**	*	ns	ns
007G032000 (cold acclimation protein WCOR413)	***	***	**	***	***	***
014G100000 (cellular apoptosis susceptibility protein)	***	***	*	*	***	***
014G102100 (ATPase)	***	***	**	*	**	***
014G102400 (chloroplast-localized protein)	***	***	**	***	**	***
014G102700 (*CYP78A6* – upstream region)	***	***	***	***	***	***
014G102700 (*CYP78A6* – gene/downstream region)	***	***	*	**	***	***
015G118800 (*PP2-A9*)	***	***	ns	ns	na	na
015G119700 (ankyrin repeat family protein)	***	***	ns	ns	na	na
015G120100 (*PP2-A10*)	***	***	ns	ns	na	na
016G019800 (HSP70)	***	***	**	**	*	**
016G022700 (*MAPR4*)	***	***	***	***	**	***
017G081000 (*TUA6*)	***	***	***	***	ns	*

UBC genotypes at Totem Field (2011) and Agassiz (2012–13) were tested for repeatability of allelic effects on bud break phenotypes observed in UBC *Populus trichocarpa* genotypes and phenotypic data from 2010. GW/BESC genotypes at Agassiz were used to validate the phenotypic findings associated with GWAS-identified loci.

[a]Genotypes originating from wild sources (UBC and GW/BESC *P. trichocarpa* collections) were cultivated in two common gardens (AG, Agassiz; TF, Totem Field), and monitored for timing of bud-break over multiple years (2010–2013). For each dataset, phenotypic differences in spring bud-break are tested using mixed-effects modelling (full details in Supporting Information Table S5).

***, $P < 0.001$; **, $P < 0.01$; * $P < 0.05$, ns, not significant; na, not applicable (single nucleotide polymorphism is invariant).

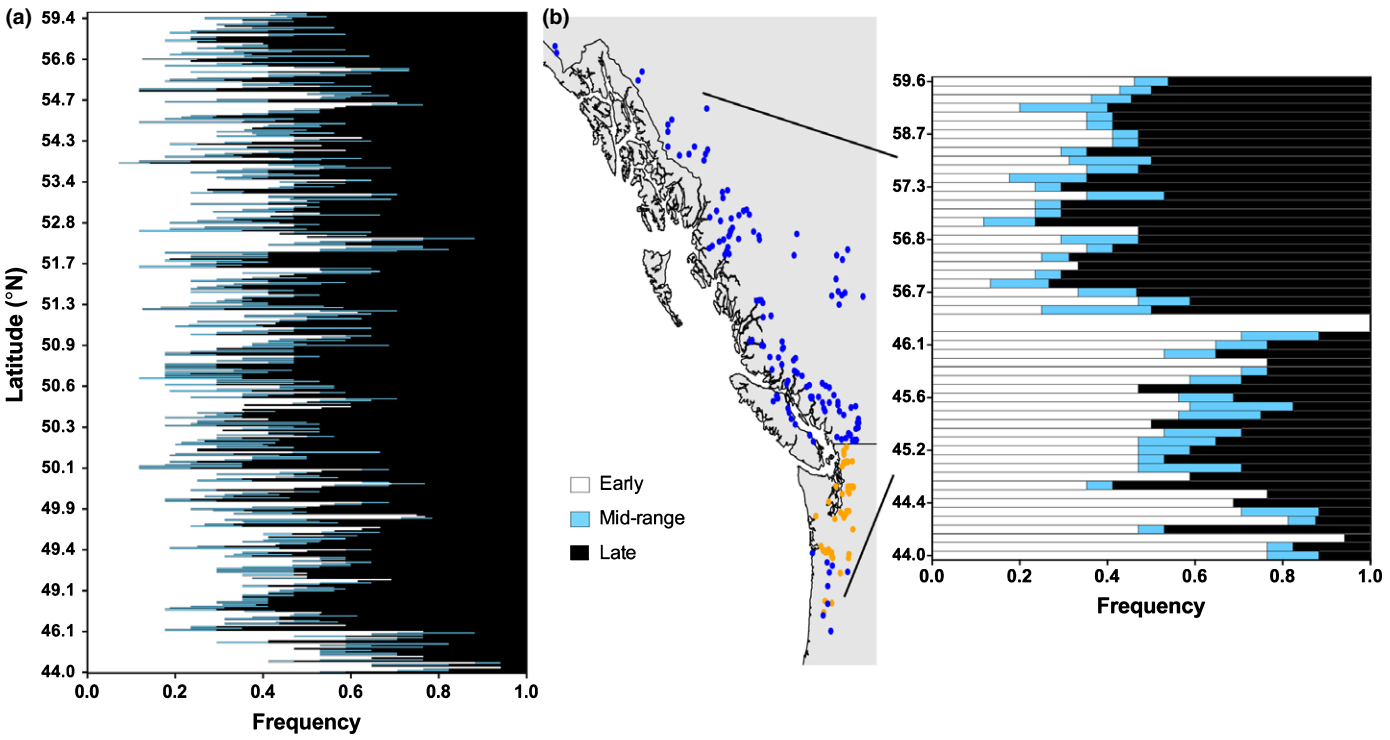

Fig. 8 Genotype–phenotype profiles of sequenced *Populus trichocarpa* individuals. (a) The total proportion of loci associated with 'early', 'mid-range' or 'late' bud-break phenotypic outcomes (see Tables 4, Supporting Information Table S5). (b) Individuals from southern- and northern-most *P. trichocarpa* provenances are highlighted for genetic differences despite apparent phenotypic similarities. Each bar presents an individual genotype.

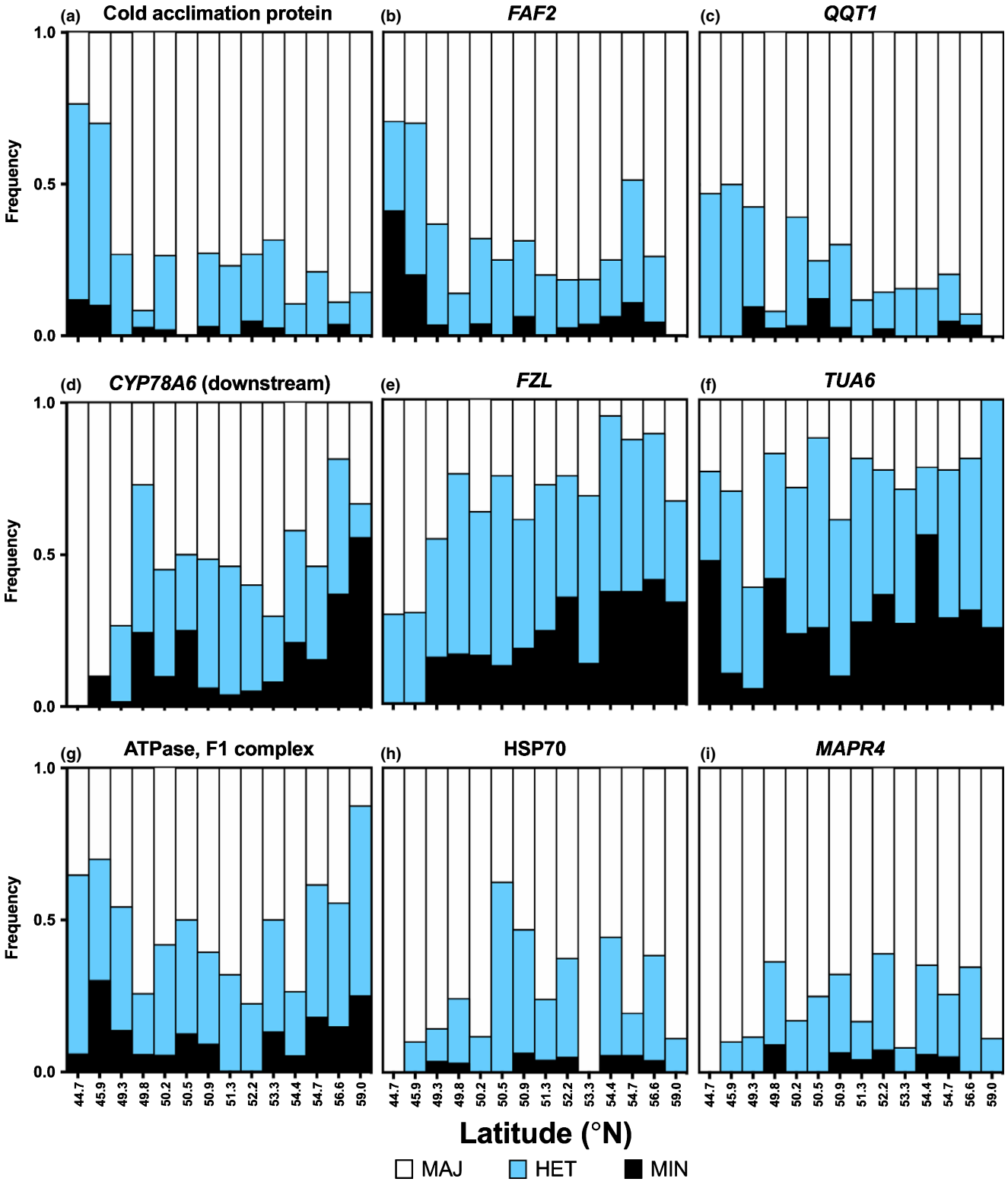

Fig. 9 Latitudinal patterns in the frequency of major, heterozygous, and minor allelic variants in provenances of *Populus trichocarpa*. Top graphs (a–c) show a general clinal pattern of increasing frequency in allelic variants associated with 'late' phenotypes following a south-to-north distributional pattern: (a) Cold acclimation protein (Potri.007G032000); (b) *FAF2* (Potri.005G209100); (c) *QQT1* (Potri.002G118100). Middle graphs (d–f) show this same pattern where heterozygous variants confer the 'mid-range' phenotype (between major and minor homozygous variants): (d) The locus/downstream region of *CYP78A6* (Potri.014G102700); (e) *FZL* (Potri.005G209200); (f) *TUA6* (Potri.017G081000). Bottom graphs (g–i) show higher frequencies of 'early' bud-break allelic variants in both southern- and northern-most provenances, and greater genetic heterogeneity in the central portion of the species' range. (g) ATPase, F1 complex, OSCP/delta subunit protein (Potri.014G102100). (h) HSP70 (Potri.016G019800). (i) *MAPR4* (Potri.016G022700). Bars represent frequencies of alleles in provenances grouped by genetic similarity within topographical 'drainages' (Geraldes *et al.*, 2014).

tool to search for loci with associations to trait *variation* within a population, and previously characterized genes may have less species-wide genetic variability and/or involvement in determining phenotypic diversity. The genes identified in our study are expressed throughout the phases of endodormancy and ecodormancy in *P. trichocarpa* (data from Howe *et al.*, 2015; Table S7) but are implicated in our study in the differential timing of bud-break and may have diverse effects within a landscape genomics context. Individuals with major homozygous alleles had significantly different phenotypes compared to those with minor homozygous alleles, suggesting dominant effects (Tables 4, S5). Further functional investigations are necessary to determine gene actions and interactions.

Genetic differences underlying similar phenotypic outcomes

The discrepancy in overall genetic profiles between northern and southern individuals, despite comparable phenotypic outcomes, suggests that there are different physiological mechanisms determining bud-break depending on the genetic background of the population (Figs 8, 9). Many GWAS-identified SNPs display frequencies in general clinal, south-to-north directional patterns (Fig. 9a–f). These may underpin latitudinal clines in bud chilling requirements during endodormancy. Lower total chilling requirements are common in northern tree populations (Farmer & Reinholt, 1986; Myking & Heide, 1995; Junttila & Hänninen, 2012). A lower threshold for accumulated chilling to release bud endodormancy can predominate if sustained cold temperatures throughout the winter easily achieve the necessary chilling. The converse applies as trees face milder winters. In these environments, extended endodormancy, particularly in more southerly regions, is necessary to prevent premature bud-break, which could expose young tissues to unfavourable growing conditions and/or shorten the growing period through the early onset of drought (Kalcsits *et al.*, 2009; Rohde *et al.*, 2011). Here, the potential ecological role of the poplar homologue for *CYP78A6* may be sensitivity to daily temperature throughout the winter and spring seasons and responding to daily thermocycles (cf Pan *et al.*, 2009).

In other loci, SNP frequencies follow the general geographical quadratic trend (Fig. 9g–i). These loci might contribute to differential response to heat sums acquired during ecodormancy. In *P. trichocarpa*, mid-range populations are more likely to encounter long periods of spring temperature fluctuations and damaging frosts. Selection for greater heat sums could counteract this uncertainty by delaying bud-break to mitigate tissue damage from freezing spring temperatures. By comparison, relaxing selection for high heat sum requirements (or modifying the rate of heat sum accumulation) at more northern and southern parts of the species' range could be advantageous where spring freezing is less likely and/or rapidly resuming aboveground growth is necessary as soon as conditions are favourable.

Another significant component in bud-break phenology is spring forcing temperature. Reduced winter chilling during the endodormancy period resulted in extended spring forcing periods, both in our field and growth chamber trials. Nevertheless, high forcing temperatures shifted bud-break forward substantially in the chamber trials, apparently overriding the effects of reduced chilling (Fig. 5). These results are not completely unexpected (e.g. Nanninga *et al.*, 2017) and poplars tend to have strong responses to warm temperatures, acting as 'opportunistic' species (Caffarra & Donnelly, 2011). Greater forcing temperature also strongly reduced genotypic differences in timing of bud-break due to the steep trajectory of heat sum accumulation (Fashler & El-Kassaby, 1987). Interestingly, loci that had significant interactions with chilling duration (Table S6) follow two geographical patterns: SNP frequencies in *FZL* and *TUA6* show a north–south trend, whereas HSP70 and *MAPR4* have similar SNP frequencies in northern and southern provenances (Fig. 9). These loci might play roles in sensing total chilling during endodormancy, acting to adjust total heat sums required during ecodormancy depending on the amount of chilling experienced. This type of mechanism could be particularly significant for genotypes from the central portion of the species range where temperatures throughout the winter may be more heterogeneous, varying winter chilling from year to year. Future effects on bud-break across the species' range will likely depend on when the climate warming occurs during the winter and spring seasons. Yet, the response also will be underpinned by the genetic background of the populations, and alleles putatively modifying bud-break response depending on chilling duration may become more ecologically important under future climate warming.

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Author contributions

R.D.G, Y.A.E-K. and S.D.M. designed the field studies; A.D.M. collected data, designed the controlled chamber tests and performed the experiments; A.D.M. and J.K. analysed the data; and A.D.M. wrote the manuscript with input from other co-authors.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article:

Fig. S1 Correspondence in bud-break between *P. trichocarpa* genotypes from the UBC collection used for different field trials and a controlled growth chamber study.

Fig. S2 Relationships between bud-break in provenances of *P. trichocarpa* reported as JD and latitude of tree origin for trees grown at Agassiz and Totem Field.

Fig. S3 Relationship between the elevation of tree origin for *P. trichocarpa* genotypes cultivated at Totem Field and bud-break reported as JD.

Fig. S4 Correspondence in bud-break for *P. trichocarpa* genotypes from the UBC collection assessed under controlled conditions with 30 d of chilling ('less') or 68 d of chilling ('more') and forced to flush under 10°C vs 20°C.

Fig. S5 Timing of bud-break in *P. trichocarpa* genotypes under a controlled climate scenario of an extended period of chilling followed by one forcing temperature (20°C).

Fig. S6 Latitudinal distribution of alleles associated with 'early', 'mid-range' or 'late' bud-break phenotypic outcomes in *P. trichocarpa*.

Fig. S7 Relationship between bud-break in *P. balsamifera* reported as JD and latitude of tree origin for trees grown at Indian Head, SK.

Table S1 List of *P. trichocarpa* accessions, geographical origin, collection and common gardens where genotypes were grown

Table S2 Daily temperatures (maximum, minimum, mean) for Totem Field and Agassiz sites in British Columbia

Table S3 Full details of SNP–trait associations using a GWAS

Table S4 Pairwise LD r^2 values between GWAS-identified SNP markers

Table S5 Phenotypic relationships between GWAS-identified SNPs and bud-break from two common gardens assessed using mixed-effects modelling

Table S6 Effects of chilling duration and forcing temperature on spring bud-break using linear modelling testing loci uncovered by a GWAS

Table S7 Expression data of loci uncovered by a GWAS in *P. trichocarpa* (this study) from Howe *et al.* (2015)

Methods S1 Testing bud-break under controlled growth chamber conditions.

Methods S2 Whole genome sequencing details and methodology.

Methods S3 Testing population structure correction in GWAS.

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