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Research

# Geographical and environmental gradients shape phenotypic trait variation and genetic structure in *Populus trichocarpa*

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## Summary

• *Populus trichocarpa* is widespread across western North America spanning extensive variation in photoperiod, growing season and climate. We investigated trait variation in *P. trichocarpa* using over 2000 trees from a common garden at Vancouver, Canada, representing replicate plantings of 461 genotypes originating from 136 provenance localities.

• We measured 40 traits encompassing phenological events, biomass accumulation, growth rates, and leaf, isotope and gas exchange-based ecophysiology traits. With replicated plantings and 29 354 single nucleotide polymorphisms (SNPs) from 3518 genes, we estimated both broad-sense trait heritability ($H^2$) and overall population genetic structure from principal component analysis.

• *Populus trichocarpa* had high phenotypic variation and moderate/high $H^2$ for many traits. $H^2$ ranged from 0.3 to 0.9 in phenology, 0.3 to 0.8 in biomass and 0.1 to 0.8 in ecophysiology traits. Most traits correlated strongly with latitude, maximum daylength and temperature of tree origin, but not necessarily with elevation, precipitation or heat : moisture indices. Trait $H^2$ values reflected trait correlation strength with geoclimate variables. The population genetic structure had one significant principal component (PC1) which correlated with daylength and showed enrichment for genes relating to circadian rhythm and photoperiod.

• Robust relationships between traits, population structure and geoclimate in *P. trichocarpa* reflect patterns which suggest that range-wide geographical and environment gradients have shaped its genotypic and phenotypic variability.

## Introduction

Tree species offer interesting cases for studying adaptive evolution, as many have extensive geographical distributions across large spatial areas, landscapes and/or environmental conditions (Eckert & Dyer, 2012). Within the range of a widespread tree species, individuals are likely to occur in a diversity of habitats along pronounced environmental gradients (Savolainen *et al.*, 2007; De Frenne *et al.*, 2013; Lasky *et al.*, 2013). The maintenance of a large range generally presents challenges in balancing adaptive evolution and maintaining species persistence and integrity (Savolainen *et al.*, 2007; Lexer *et al.*, 2013). Where an overlying geographical or environmental gradient exerts the strongest adaptive selection, clinally related variation both in genetic structure and phenotypic traits of a species is predicted, but will depend on the relative strength of selection, demographic history, and levels of dispersal and/or gene flow among populations (Savolainen *et al.*, 2007). Differing selection pressures may include temperature, precipitation, soil nutrient availability, growing season length, photoperiod and biotic agents. Many of these factors are directly affected by geographic position or elevation, and are therefore interrelated. Among them, only the seasonal photoperiodic regime is essentially unchanging across years whereas other patterns, such as seasonal temperatures or precipitation, might fluctuate on a yearly basis.

Genetic and phenotypic variability are both fundamental in determining intrinsic factors that define species and species biogeography (Stapley *et al.*, 2010). Phenotypic trait variation across large populations often mirrors the co-ordination of traits with components of geography and climate, suggesting that the distribution of genetic and/or genomic variation may follow similar patterns reflecting selective factors (Fournier-Level *et al.*, 2011; Ingvarsson & Street, 2011; Eckert & Dyer, 2012; Lasky *et al.*, 2013). Recent advances in tree genomics are beginning to describe the 'landscape genetics' whereby functional traits and genetic variance are coordinated with ecological information to

understand adaptation (Eckert & Dyer, 2012). For instance, phenotypic trait variance within a population can be related to genetic variance as an estimation of heritability. Heritability estimates are not fixed but instead depend on both the genetic architecture of the trait and the population sample analysed. Highly heritable traits, where a greater proportion of phenotypic variation is attributable to genetic variance, may help predict evolutionary response to selection. In theory, high estimates might suggest that a trait is subject to spatially variable selection rather than past strong directional selection across the entire species range, as this would be expected to eliminate genetic variation in the trait (Mousseau & Roff, 1987). Conversely, low estimates may indicate that a trait is largely plastic in response to the immediate environment, highlighting the role of phenotypic plasticity as an additional variability reservoir, or that the trait has one or few genes with large, epistatic effect(s). Many traits relating to range-wide distribution in trees often have a polygenic or complex genetic architecture, such as cold tolerance, phenology or growth (Savolainen *et al.*, 2007; Holliday *et al.*, 2008; Ingvarsson *et al.*, 2008; Eckert *et al.*, 2009; Ibáñez *et al.*, 2010; Ma *et al.*, 2010; Rohde *et al.*, 2010; Olson *et al.*, 2012). Identifying the underlying genetic variability and architecture of traits is an important, growing area of interest in tree biology; however, detecting causal genetic variation in natural populations is often difficult. Methods such as association genetics tend to incorporate a bias in marker discovery using small numbers of candidate or *a priori*-selected genes (Eckert & Dyer, 2012) and advances in genome-wide associations (GWAS) may not improve the issue where population structure confounds results (Ingvarsson & Street, 2011). For traits of ecological or evolutionary interest, studies must also address the extent to which population structure, trait variation and genetic architecture co-vary along ecological gradients.

Black cottonwood (*Populus trichocarpa*) is an ecologically important and widespread tree species ranging from Alaska to northern California (Farrar, 1995), with extensive genetic resources available (Cronk, 2005; Tuskan *et al.*, 2006; Jansson & Douglas, 2007). A number of traits in *Populus* species, including *P. trichocarpa*, have broad-sense trait heritability ($H^2$) estimates reported from common garden studies with clonal replication. While these estimates vary as an attribute of each population studied, phenology, biomass and growth, features of leaves, cold tolerance, wood biochemistry and lignin content tend to have high $H^2$ values within *Populus* (Yanchuk *et al.*, 1984; Bradshaw & Stettler, 1995; Riemenschneider *et al.*, 1996; Howe *et al.*, 2000; Rae *et al.*, 2004; Pliura *et al.*, 2007; Chamaillard *et al.*, 2011; Rohde *et al.*, 2011; Fabbrini *et al.*, 2012; Guerra *et al.*, 2013). In *P. trichocarpa*, studies show that natural phenotypic variation is often related to geographic origin (Farmer, 1996; Gornall & Guy, 2007; Xie *et al.*, 2009), which is also observed in the closely related species *P. balsamifera* (Soolanayakanahally *et al.*, 2009; Keller *et al.*, 2011, 2012; Olson *et al.*, 2012). *Populus balsamifera* experiences a range in climatic factors throughout its distribution across the northern interior of North America, which combines north–south and east–west trends in climate and dryness (Farrar, 1995; Farmer, 1996). Consequently, ecophysiological traits, such as photosynthetic gas exchange, carbon isotopes, leaf chlorophyll and leaf mass per area (LMA), have strong correlative relationships with environmental 'dryness' (i.e. continentality or summer dryness) and number of frost-free days (FFD) (Soolanayakanahally *et al.*, 2009). By comparison, the distribution of *P. trichocarpa* is west of the Rocky Mountains and essentially along a highly mountainous north–south axis. It is therefore likely that environmental gradients along this north–south axis exert stronger selection in *P. trichocarpa*.

In this study, we investigated the relationships between geography, trait variability and broad-sense heritability ($H^2$), and genome-wide population genetic structure using accessions from wild populations of *P. trichocarpa*. We assayed 40 biomass, ecophysiology and phenology traits in a common garden across multiple years and genotyped accessions with an Illumina iSelect Infinium 34K *Populus trichocarpa* single nucleotide polymorphism (SNP) array (Geraldes *et al.*, 2013). We used this genome-wide genetic variation to assess the $H^2$ of phenotypic traits in our population and to estimate population structure patterns through multivariate analysis. We related trait variability, population structure and trait $H^2$ to gradients in the geography and environment of tree origin (i.e. geoclimate variables) and predicted that (1) the north–south axis (and related variables) would correlate most strongly with these factors in *P. trichocarpa*. We also predicted that (2) in a species with a wide natural range, traits with the highest $H^2$ would show the strongest relationships to these gradients reflecting a theoretical optimum in fitness for that location if the selection pressure is clinal. We identified the photoperiodic gradient as the strongest correlation with PC1 (which best describes the genetic landscape) and with this understanding, we tested the hypothesis that (3) genes relating to light are enriched in PC1. Based on overall patterns from our study, we suggest that particular environmental gradients across the latitudinal range of *P. trichocarpa* have played a strong role in shaping species-wide phenotypic trait variation and genetic structure.

## Materials and Methods

### Tree field materials and geoclimate of tree origin

Branch cuttings from native-tree genotypes of *Populus trichocarpa* Torr. & A. Gray (black cottonwood) used in this study were collected from 136 provenances spanning the northern two-thirds of the species' range (44–60°N, 121–138°W) by the British Columbia Ministry of Forests, Lands and Natural Resource Operations (MFLNRO) (Fig. 1; Xie *et al.*, 2009; McKown *et al.*, 2013). In 2000, cuttings from wild trees were rooted and out-planted in common gardens at Surrey and Terrace, BC. Samples were taken from these sites in spring 2008 for use at Totem Field, University of British Columbia (UBC) (McKown *et al.*, 2013). We used cuttings from 461 accessions with 4–20 clonal replicates of each accession similar in age and condition, grew these as stecklings under glasshouse conditions, and out-planted young trees in June 2008. Within the Totem Field common garden, trees were planted in a random block design for the replicate clones at 1.5 × 1.5 m spacing in an area 40 × 54 m in size (total

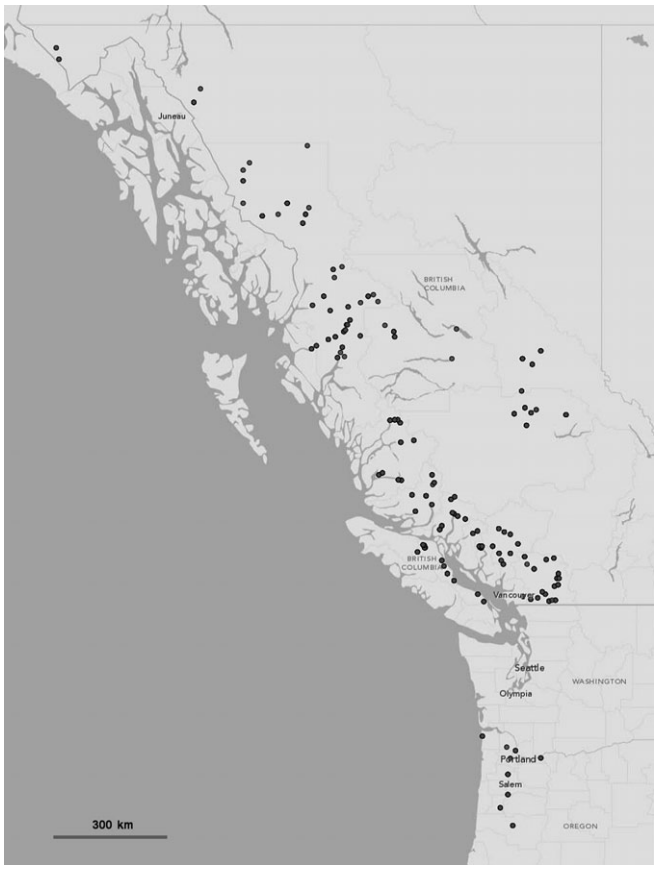


**Fig. 1** Distribution of collection sites for *Populus trichocarpa* (black cottonwood) accessions. Unrelated genotypes of *P. trichocarpa* (461 total) were collected from 136 provenances across the northern two-thirds of the species' range as it occurs west of the Rocky Mountains. Trees were replanted in the Totem Field common garden at the University of British Columbia, Vancouver, BC, Canada (map developed using ArcGIS Explorer; http://www.arcgis.com/explorer/).

area = 2160 $m^2$). Trees were watered daily throughout 2008–2009 from rainfall or using a drip irrigation system and the field was mowed to limit weed overgrowth. No fertilizers or other soil supplements were provided at any time during the study.

We compiled 12 'geoclimate' variables to describe each accession collection site. Positional information (latitude, longitude, elevation) was obtained from MFLNRO records (Table 1 and Supporting Information Table S1). We calculated maximum length of day (DAY; h) as a proxy for photoperiodic regime at each location based on Forsythe *et al.* (1995). We also estimated nine averaged climate variables for each site with ClimateWNA (Wang *et al.*, 2012a) based on 1971–2000 Canadian Climate Normals from nearby weather stations and climate maps. Variables derived in ClimateWNA included mean annual temperature (MAT; °C), mean warmest monthly temperature (MWMT; °C), mean coldest monthly temperature (MCMT; °C), mean annual precipitation (MAP; mm), mean summer precipitation from May to September (MSP; mm), annual heat moisture index (AHM; derived from (MAT + 10)/(MAP/1000)), summer heat moisture index (SHM; derived from (MWMT)/(MSP/1000)), and number of frost-free days (FFD).

**Table 1** Geography and climate data for *Populus trichocarpa* accessions

| Variable | Mean value (± SD) | Range |
|---|---|---|
| LAT (deg N)[1] | 51.55 ± 2.9 | 44–59.62 |
| LONG (deg W)[1] | 125.21 ± 2.9 | 121.17–137.92 |
| ELEV (m)[1] | 216.9 ± 209 | 0–900 |
| DAY (h:min) | 16:42 ± 0:34 | 15:29–18:44 |
| MAT (°C)[2] | 6.9 ± 2.2 | −0.1 to 12.2 |
| MWMT (°C)[2] | 16.2 ± 1.4 | 12.4–19.8 |
| MCMT (°C)[2] | −2.4 ± 4.2 | −14.6 to 5.5 |
| MAP (mm)[2] | 1608 ± 746 | 14.9–3519 |
| MSP (mm)[2] | 380 ± 160 | 147–840 |
| AHM[2] | 12.9 ± 6.1 | 5–40.1 |
| SHM[2] | 50.4 ± 22.3 | 18.2–130.6 |
| FFD (d)[2] | 233 ± 46.4 | 130–329 |

AHM, annual heat : moisture index; DAY, longest yearly daylength (photoperiod); ELEV, elevation; FFD, number of frost free days; LAT, latitude; LONG, longitude; MAP, mean annual precipitation; MAT, mean annual temperature; MCMT, mean coldest month temperature; MSP, mean summer May–September precipitation; MWMT, mean warmest month temperature; SMH, summer heat : moisture index.
[1]From British Columbia Ministry of Forests, Lands and Natural Resource Operations.
[2]Variables derived from 1971–2002 weather data using ClimateWNA (Supporting Information Table S1; Wang *et al.*, 2012a).

## Biomass, ecophysiology and phenology traits

We assessed all 461 accessions (total surviving trees = 2088) in Totem Field for 40 traits, including phenology, biomass and growth, photosynthetic gas exchange, leaf traits and stable isotopes (Tables 2, S2).

We measured 12 traits relating to aboveground growth rates and biomass accumulation for all trees throughout 2008–2012. General growth was estimated at the end of each season by measuring tree height (H; cm) and basal diameter at 10 cm from the ground (D; cm). Height : diameter ratios for each year were calculated (H : D; cm/cm). Bole volume ($cm^3$) was calculated assuming a cone: $(1/3 \times \pi \times (D/2)^2 \times H)$. Both height and volume gains were determined for each individual year. The number of branches was tallied at the end of 2009. Following 4 yr of field growth, 800 trees were harvested before spring bud break and measured for whole tree and bole fresh mass (kg). Density ($kg\ m^{-3}$) was calculated as bole fresh mass divided by bole volume. Active growth rate ($cm\ d^{-1}$) was determined from yearly height gain divided by the growth period (see later). Log height growth rate (log $cm\ d^{-1}$) and log volume growth rate (log $cm^3\ d^{-1}$) were used to reflect peak growth and determined from repeated measurements taken throughout the 2009 growing season calculating the linear portion of the log slope of height/volume curves over time.

The majority of trees ($n$ = 2046) were sampled in the field for 15 ecophysiological traits. We took measurements during the seasons of 2009–2011 before bud set, unless otherwise indicated. Due to inherent physiological changes occurring after bud set (McKown *et al.*, 2013), data collected in the 'post-bud set' phase were considered separately and are listed as such. We assessed 11 traits using gas exchange, and subsequent tissue sampling, on

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**Table 2** Clonal trait data and broad-sense heritability estimates ($H^2$) for biomass, ecophysiology, and phenology traits measured in *Populus trichocarpa* accessions across multiple years

| Trait | Year | *n* | Mean value (± SD) | Data range | $H^2$ (± SE) |
|---|---|---|---|---|---|
| Phenology | | | | | |
| Bud break (d) | 2010 | 461 | 67 ± 11 | 39–97 | 0.867* (± 0.009) |
| Bud break (d) | 2011 | 461 | 85 ± 9 | 69–113 | 0.893* (± 0.008) |
| Bud set (d) | 2008 | 457 | 264 ± 22 | 213–346 | 0.661* (± 0.020) |
| Bud set (d) | 2009 | 461 | 244 ± 22 | 173–316 | 0.822* (± 0.012) |
| Bud set (d) | 2010 | 461 | 224 ± 33 | 136–281 | 0.734* (± 0.016) |
| Canopy duration (d) | 2009 | 455 | 278 ± 22 | 213–328 | 0.266 (± 0.026) |
| Canopy duration (d) | 2010 | 461 | 248 ± 19 | 172–284 | 0.854* (± 0.010) |
| Growth period (d) | 2009 | 455 | 198 ± 28 | 87–275 | 0.373 (± 0.026) |
| Growth period (d) | 2010 | 461 | 157 ± 36 | 65–240 | 0.801* (± 0.013) |
| Height growth cessation (d) | 2009 | 461 | 228 ± 22 | 145–271 | 0.772* (± 0.015) |
| Leaf drop (d) | 2008 | 455 | 332 ± 15 | 275–358 | 0.579* (± 0.022) |
| Leaf drop (d) | 2009 | 461 | 323 ± 14 | 283–351 | 0.593* (± 0.021) |
| Leaf drop (d) | 2010 | 461 | 315 ± 15 | 250–323 | 0.627* (± 0.020) |
| Leaf flush (d) | 2010 | 461 | 82 ± 10 | 46–104 | 0.829* (± 0.011) |
| Leaf flush (d) | 2011 | 461 | 104 ± 8 | 85–125 | 0.877* (± 0.009) |
| Leaf flush (d) | 2012 | 461 | 99 ± 4 | 81–108 | 0.841 (± 0.013) |
| Leaf lifespan (d) | 2010 | 461 | 190 ± 27 | 98–258 | 0.666* (± 0.019) |
| Post-bud set period (d) | 2009 | 455 | 80 ± 13 | 35–126 | 0.484* (± 0.025) |
| Post-bud set period (d) | 2010 | 461 | 91 ± 21 | 42–144 | 0.635* (± 0.020) |
| Yellowing, 25% (d) | 2010 | 461 | 223 ± 25 | 163–272 | 0.528* (± 0.023) |
| Yellowing, 50% (d) | 2010 | 461 | 250 ± 27 | 173–293 | 0.570* (± 0.022) |
| Yellowing, 75% (d) | 2010 | 461 | 272 ± 25 | 189–310 | 0.569* (± 0.022) |
| Yellowing, 100% (d) | 2010 | 458 | 294 ± 24 | 214–319 | 0.589* (± 0.022) |
| Biomass | | | | | |
| Active growth rate (cm $d^{-1}$) | 2009 | 455 | 0.75 ± 0.23 | 0.01–1.25 | 0.596* (± 0.022) |
| Active growth rate (cm $d^{-1}$) | 2010 | 461 | 0.98 ± 0.32 | 0.07–1.70 | 0.575* (± 0.022) |
| Bole density (kg $m^{-3}$) | 2012 | 374 | 784.2 ± 164.5 | 334.2–1516.4 | 0.404 (± 0.041) |
| Bole mass (kg) | 2012 | 374 | 6.57 ± 3.70 | 0.05–21.32 | 0.437 (± 0.041) |
| Branches (#) | 2009 | 461 | 10.9 ± 7.0 | 0–37.5 | 0.553* (± 0.022) |
| Height (cm) | 2008 | 455 | 59.4 ± 20.0 | 10.5–135 | 0.470* (± 0.025) |
| Height (cm) | 2009 | 461 | 205.5 ± 65.8 | 25.5–376.1 | 0.665* (± 0.019) |
| Height (cm) | 2010 | 461 | 366.8 ± 128.6 | 57.1–669.3 | 0.746* (± 0.016) |
| Height (cm) | 2011 | 461 | 563.5 ± 178.1 | 113.6–878.1 | 0.706* (± 0.018) |
| Height gain (cm) | 2009 | 455 | 150.2 ± 52.1 | 2.0–293 | 0.767* (± 0.015) |
| Height gain (cm) | 2010 | 461 | 161.8 ± 69.0 | 7.3–305.3 | 0.629* (± 0.020) |
| Height gain (cm) | 2011 | 461 | 197.2 ± 58.7 | 33–319.3 | 0.287* (± 0.025) |
| H : D (cm : cm) | 2009 | 461 | 90.6 ± 14.2 | 35.6–131.1 | 0.509* (± 0.023) |
| H : D (cm : cm) | 2010 | 461 | 86.8 ± 13.4 | 50.8–133.5 | 0.384* (± 0.025) |
| H : D (cm : cm) | 2011 | 461 | 100.9 ± 18.9 | 61.5–188.6 | 0.456* (± 0.025) |
| Log height growth (log cm $d^{-1}$) | 2009 | 460 | 0.00493 ± 0.0012 | 0.00244–0.0120 | 0.474* (± 0.025) |
| Log volume growth (log $cm^3$ $d^{-1}$) | 2009 | 460 | 0.01245 ± 0.0026 | 0.00386–0.0224 | 0.287* (± 0.025) |
| Volume ($cm^3$) | 2009 | 461 | 435.8 ± 346.7 | 3.3–1924 | 0.375*† (± 0.025) |
| Volume ($cm^3$) | 2010 | 461 | 2973.0 ± 2439.0 | 15.9–13129.6 | 0.467*† (± 0.024) |
| Volume ($cm^3$) | 2011 | 461 | 8175.9 ± 6293.5 | 48.8–29122.6 | 0.515*† (± 0.023) |
| Volume gain ($cm^3$) | 2010 | 461 | 2542.5 ± 2122.8 | 9.3–11 332 | 0.461*† (± 0.024) |
| Volume gain ($cm^3$) | 2011 | 461 | 5205.7 ± 3989.1 | 32.5–20 221 | 0.432*† (± 0.025) |
| Whole tree mass (kg) | 2012 | 374 | 10.82 ± 6.34 | 0.076–31.68 | 0.466*† (± 0.040) |
| Ecophysiology[1] | | | | | |
| $A_{max}$ (μmol $CO_2$ $m^{-2}$ $s^{-1}$) | 2009 | 455 | 19.9 ± 2.7 | 12.2–28.6 | 0.184 (± 0.027) |
| $A_{max/mass}$ (μmol $CO_2$ $g^{-1}$ $s^{-1}$) | 2009 | 455 | 0.246 ± 0.037 | 0.130–0.404 | 0.167 (± 0.028) |
| C : N (mg $mg^{-1}$) | 2009 | 454 | 20.9 ± 2.8 | 14.7–30.1 | 0.205 (± 0.028) |
| $Chl_{spring}$ (CCI) | 2009 | 205 | 25.9 ± 7.8 | 12.4–59.2 | 0.287 (± 0.119) |
| $Chl_{summer}$ (CCI) | 2009 | 414 | 37.9 ± 10.7 | 15.8–81.3 | 0.313 (± 0.029) |
| $Chl_{summer}$ (CCI) | 2011 | 369 | 21.8 ± 5.4 | 10.6–47.2 | 0.338 (± 0.028) |
| $Chl_{post\text{-}budset}$ (CCI) | 2009 | 189 | 48.9 ± 13.4 | 13.3–85.1 | 0.180 (± 0.055) |
| $Chl_{post\text{-}budset}$ (CCI) | 2011 | 95 | 26.0 ± 6.2 | 13.4–42.3 | 0.301 (± 0.057) |
| $\Delta_{leaf}$ (‰) | 2009 | 455 | 19.6 ± 1.1 | 16.6–23.2 | 0.509 (± 0.026) |
| $\delta^{13}C_{wood}$ (‰) | 2012 | 370 | −26.4 ± 0.8 | −29.0 to −24.1 | 0.487 (± 0.039) |
| $\delta^{15}N$ (‰) | 2009 | 455 | 1.66 ± 0.69 | −0.32 to 3.52 | 0.079 (± 0.025) |

**Table 2** (Continued)

| Trait | Year | *n* | Mean value (± SD) | Data range | $H^2$ (± SE) |
|---|---|---|---|---|---|
| $g_s$ (mol $H_2O$ $m^{-2}$ $s^{-1}$) | 2009 | 455 | 0.396 ± 0.084 | 0.159–0.679 | 0.446 (± 0.027) |
| Leaf shape (length : width) | 2009 | 461 | 2.6 ± 0.4 | 1.4–4.7 | 0.374 (± 0.026) |
| Leaves per bud (#) | 2011 | 461 | 4.6 ± 0.8 | 3.0–8.2 | 0.334 (± 0.033) |
| Leaves per bud (#) | 2012 | 446 | 5.5 ± 1.1 | 3.0–10.5 | 0.485 (± 0.029) |
| $LMA_{spring}$ (mg $mm^{-2}$) | 2010 | 461 | 0.0643 ± 0.0101 | 0.0437–0.1003 | 0.820* (± 0.012) |
| $LMA_{spring}$ (mg $mm^{-2}$) | 2011 | 461 | 0.0604 ± 0.0093 | 0.0384–0.0957 | 0.800* (± 0.013) |
| $LMA_{summer}$ (mg $mm^{-2}$) | 2009 | 455 | 0.0824 ± 0.0085 | 0.0634–0.1145 | 0.173 (± 0.027) |
| $LMA_{summer}$ (mg $mm^{-2}$) | 2010 | 369 | 0.0966 ± 0.0108 | 0.0676–0.1334 | 0.265* (± 0.027) |
| $LMA_{summer}$ (mg $mm^{-2}$) | 2011 | 369 | 0.0743 ± 0.0081 | 0.0359–0.1062 | 0.323* (± 0.028) |
| $LMA_{post\text{-}budset}$ (mg $mm^{-2}$) | 2010 | 95 | 0.1094 ± 0.0155 | 0.0746–0.1525 | 0.180 (± 0.054) |
| $LMA_{post\text{-}budset}$ (mg $mm^{-2}$) | 2011 | 95 | 0.0629 ± 0.0125 | 0.0299–0.1252 | 0.121 (± 0.052) |
| $N_{area}$ (mg $mm^{-2}$) | 2009 | 455 | 0.00191 ± 0.00025 | 0.00116–0.0027 | 0.211 (± 0.028) |
| $N_{mass}$ (mg $mg^{-1}$) | 2009 | 455 | 0.0235 ± 0.0031 | 0.0116–0.0338 | 0.206 (± 0.028) |
| NUE (μmol $CO_2$ $g^{-1}$ N $s^{-1}$) | 2009 | 455 | 10.6 ± 1.31 | 6.45–15.72 | 0.177 (± 0.028) |
| WUE (μmol $CO_2$ $mmol^{-1}$ $H_2O$) | 2009 | 455 | 3.89 ± 0.56 | 2.22–6.01 | 0.282 (± 0.028) |

$A_{max}$, maximum photosynthetic rate; $A_{max/mass}$, photosynthetic rate per unit dry mass; C : N, carbon : nitrogen; Chl, chlorophyll content; Δ, net discrimination; $\delta^{13}C$, stable carbon isotope ratio; $\delta^{15}N$, stable nitrogen isotope ratio; $g_s$, stomatal conductance; H : D, height : diameter; LMA, leaf mass per unit area; N, nitrogen; NUE, photosynthetic nitrogen-use efficiency; WUE, instantaneous water-use efficiency.
[1]Data for replicated gas exchange and isotope measurements with fewer than 230 accessions located in Supporting Information Tables S2, S5.
*Data corrected for spatial trends within Totem Field.
†Data log transformed for normality.

fully exposed, upper canopy leaves taking measurements once from each tree throughout May–August 2009–2010. All sampling was done on clear, sunny days between 08:00 h and 14:00 h using either a LI-COR 6400 or LI-COR 6400 XT portable infrared gas exchange system (LI-COR Biosciences, Lincoln, NE, USA). We measured three gas exchange traits directly including maximum photosynthetic rate ($A_{max}$; μmol $CO_2$ $m^{-2}$ $s^{-1}$), stomatal conductance ($g_s$; mol $H_2O$ $m^{-2}$ $s^{-1}$), and instantaneous water-use efficiency as determined by photosynthetic rate over transpiration under constant vapour pressure deficit (WUE; μmol $CO_2$ $mmol^{-1}$ $H_2O$). Following gas exchange sampling, two leaf tissue discs (61 $mm^2$) were taken using a standard, hand-held punch. Samples were oven dried at 50°C for 48 h and weighed to determine leaf mass per unit area (LMA; mg $mm^{-2}$) and to calculate photosynthetic rate per unit dry mass ($A_{max/mass}$; μmol $CO_2$ $g^{-1}$ $s^{-1}$). Between 2 and 2.5 mg of dried tissue was analysed for carbon (C) and nitrogen (N) content and stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$, respectively; ‰) at the UC Davis Stable Isotope Facility (Davis, CA, USA). From these data, we calculated C to N ratio (C : N; mg/mg), leaf N content per unit dry mass ($N_{mass}$; mg/mg) and per unit area ($N_{area}$; mg $mm^{-2}$), and photosynthetic N-use efficiency (NUE; μmol $CO_2$ $g^{-1}$ N $s^{-1}$). $\delta^{13}C$ values were used with correction for sampling date (McKown *et al.*, 2013) to obtain net discrimination ($\Delta_{leaf}$; ‰) as a proxy measure for time-integrated water-use efficiency. From harvested trees (see above), we also analysed 1 mg of dried, ground wood from a basal homogenized stem disk at the UBC Stable Isotope Facility to determine $\delta^{13}C_{wood}$. We measured trees for additional leaf traits, including number of emerging/preformed leaves per bud, leaf shape, seasonal LMA, and seasonal chlorophyll content. Between 8 and 20 buds from tree tips and high lateral branches were assessed for numbers of emerged leaves. Leaf shape was estimated by length : width ratio and binned into five shape categories (1, most orbicular; 5, most narrow). Leaf tissue samples for seasonal LMA were obtained using a hole punch and taken once from an upper canopy leaf on each tree during simultaneous sampling across the garden in spring and summer seasons. Chlorophyll absorbance measurements (Chl; unit-less) were taken on these same leaves for the chlorophyll content index (CCI) using a CCM-200 plus SPAD chlorophyll meter (Opti-Sciences Inc., Hudson, NH, USA).

All trees were monitored for 13 phenology traits. We recorded seasonal canopy events directly from observations of trees and calculated additional traits using phenological date information. The Julian dates of spring, summer and autumn phenology events were recorded for each tree throughout 2008–2011, including bud break, leaf flush, final bud set, canopy senescence (indicated by 25%, 50%, 75% or 100% yellowing) and leaf drop. Phenology events were marked using visual observations of the terminal bud on the main bole or canopy as a whole (Soolanayakanahally *et al.*, 2013). Repeated tree height measurements in 2009 were used to estimate height growth cessation dates. The full canopy duration was calculated as the time from bud break to leaf drop. Leaf lifespan, or green cover period, was calculated as canopy leaf flush to 75% canopy yellowing. Growth and post-bud set periods were calculated using days from bud break to final bud set, and from bud set to leaf drop, respectively.

## SNP genotyping and population structure analysis

We successfully genotyped 97% of our samples (448/461 individual *P. trichocarpa* accessions) with a 34K *Populus* SNP genotyping array (Geraldes *et al.*, 2013). Full details of SNP discovery/selection from 3518 candidate genes across the *P. trichocarpa* genome, array development, array performance

and data filtering criteria are given in Geraldes *et al.* (2011, 2013). Of 34 131 SNPs available in the array, we used 29 354 SNPs after filtering (following Geraldes *et al.*, 2013).

For population genetic structure, we conducted two principal component analyses (PCA) using SNP markers (Patterson *et al.*, 2006) with the 'prcomp' function implemented in the 'base' R package (R Core Development Team, 2011). We first performed PCA using 29 354 filtered SNP markers. We compared this to a smaller dataset of 8749 SNPs with no missing data, no-low linkage disequilibrium (LD) at $r^2 < 0.2$ (Wang *et al.*, 2009), and in Hardy-Weinberg Equilibrium (HWE) using the 'Chisq' function in R package 'HardyWeinberg' (Graffelman & Morales, 2008). Significant principal components (PC) in both 29K and 8K SNP analyses were identified with greater than predicted variance using simple broken-stick modelling (Jackson, 1993). Tree accessions were projected with eigenvector data to determine distributions and compare components from both analyses (Table S3). Top loading SNPs from the 29K PCA were identified and reported through assessing SNP eigenvalues (Table S4).

In order to test the hypothesis that PC1 is enriched for genes relating to light, we assigned gene ontology (GO) classifications to genes identified as the top 0.1–1% SNPs. The total numbers of genes categorized as circadian rhythm (GO:0007623), photoperiodism (GO:0009648), response to light stimulus (GO:0009416), response to blue light (GO:0009637) and response to red or far-red light (GO:0009639) were tested against total numbers on the array using Fisher's exact tests.

### Heritability estimations

We estimated broad-sense trait heritability ($H^2$) using data from individual ramets (clonal replication) of the 448 successfully genotyped *P. trichocarpa* accessions. Trait data were checked for normality using the regression model approach and logarithmic transformation was applied to six volume estimates and tree mass to improve residual distribution. We included effects of environment/spatial trends within Totem Field where the data collected included > 75% of the field and the spatial correction improved the model fit (Burnham & Anderson, 2002; Dutkowski *et al.*, 2006; Table S5). We used the linear mixed model implemented in the ASReml package (Gilmour *et al.*, 2002) to estimate $H^2$ variance components as follows:

$$\boldsymbol{y} = \mathbf{X}\beta + \mathbf{Z}\boldsymbol{u} + \boldsymbol{e}$$

($\boldsymbol{y}$, vector of measurements; $\beta$ and $\boldsymbol{u}$, vectors of fixed (population) and random (genetic) values; $\mathbf{X}$ and $\mathbf{Z}$, incidence matrices assigning fixed and random effects to each measurement in $\boldsymbol{y}$; $\boldsymbol{e}$, vector of residuals (effect of ramets within clone) following $E \sim N(0, \mathbf{I}\sigma_e^2)$ where $\sigma_e^2$ is residual (environmental) variance and $\mathbf{I}$ is the identity matrix). The vector of genotypic values follows $Var(u) = \mathbf{I}\sigma_g^2$ where $\sigma_g^2$ is total genetic variance containing both additive and nonadditive genetic components. The fixed effect of population structure was fitted by PC1 (see above). Variance components estimated in the models defined above were used to calculate $H^2$ as follows:

$$\widehat{H}^2 = \frac{\widehat{\sigma}_g^2}{\widehat{\sigma}_g^2 + \widehat{\sigma}_e^2}$$

### Correlation and regression analyses

All correlation and regression analyses were done using GraphPad Prism v6 (GraphPad Software Inc., La Jolla, CA, USA). We assessed the relationships among mean trait values, geoclimate variables and projected accession data from PCA using Pearson's Product-Moment Correlation ($r$) with Bonferroni correction for multiple correlations (Table S6). We further tested correlations between geoclimate variables with mean trait values, trait heritability and PC1 of population structure analyses (8K and 29K PCA; Table S7). Because sampling sizes were large and correlations reflected linear relationships, we focused on significant results where $r \geq 0.5$, thus predicting goodness of fit ($R^2$) of at least 0.25. To estimate the predictability of relationships between trait heritability ($H^2$) and geoclimate, we calculated linear regressions between average trait $H^2$ and average trait correlation coefficients ($r$) with key factors (latitude, elevation, DAY, FFD, MAT, MWMT, MAP, MSP; Table S8). Clear outliers were removed and regressions recalculated for comparison.

## Results

### Variation and heritability of biomass, ecophysiology, and phenology traits

Substantial variability was observed among accessions grown in the Totem Field common garden for all phenology, biomass and ecophysiology traits (Tables 2, S2). Traits tended to be consistent and correlated within accessions across years (Table S6). Many traits had strong intercorrelations within phenotype categories (biomass, ecophysiology, phenology), particularly biomass and phenology traits (Fig. 2, Table S6). Some traits also showed correlation to traits from other categories, such as inherently linked growth and phenology traits. Notably, some ecophysiology traits correlated strongly with phenology and/or biomass traits, including leaf mass per area ($LMA_{spring}$) with bud break/leaf flush, and photosynthesis ($A_{max}$), stomatal conductance ($g_s$), and leaves per bud with bud set-related traits and tree height/volume.

Broad-sense heritability estimates ($H^2$) were generally consistent among traits with some variability in year–year estimates (Tables 2, S5). $H^2$ values were highest in phenology (average $H^2 = 0.67$), compared to biomass (average $H^2 = 0.51$) or ecophysiology traits (average $H^2 = 0.32$). Key phenology events (bud break, leaf flush, height growth cessation, bud set) and the related canopy duration/growth period had the highest $H^2$ values (range = 0.66–0.89). Several biomass traits (height/height gain, volume/volume gain, H : D, branch number, active growth rate, log height growth rate, whole tree mass) had moderate/high $H^2$ values (range = 0.46–0.77). Among ecophysiology traits, only $g_s$, carbon isotope traits ($\delta^{13}C_{wood}$, $\Delta_{leaf}$), $LMA_{spring}$ and leaves per bud had moderate/high $H^2$ values (range = 0.45–0.82).

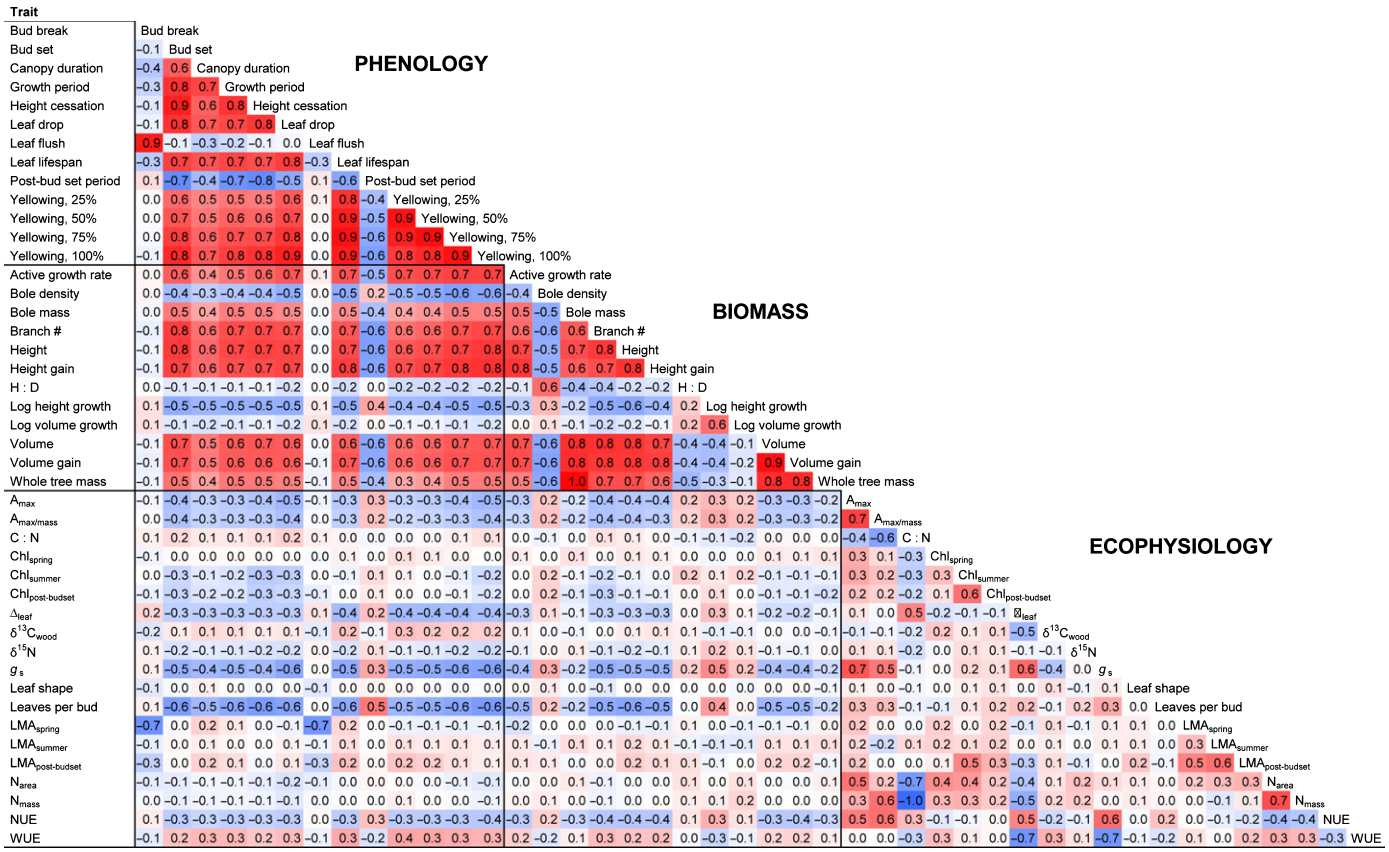


**Fig. 2** Phenotypic trait correlations for *Populus trichocarpa* accessions. Pearson's Product-Moment Correlation coefficients (*r*) indicated among all biomass, ecophysiology, and phenology traits listed in Table 2. Values are averaged across repeated years where appropriate (individual correlation coefficients in Supporting Information Table S6). Correlation coefficient of 1 is indicated by dark red and −1 by dark blue. Traits are listed in order of category as follows: Phenology traits; bud break, bud set, canopy duration, growth period, height growth cessation, leaf drop, leaf flush, leaf lifespan, post-bud set period, yellowing 25%, yellowing 50%, yellowing 75%, yellowing 100%. Biomass traits; active growth rate, bole density, bole mass, branch number, height, height gain, H : D, log height growth rate, log volume growth rate, volume, volume gain, whole tree mass. Ecophysiology traits; $A_{max}$, $A_{max/mass}$, C : N, $Chl_{spring}$, $Chl_{summer}$, $Chl_{post\text{-}budset}$, $\Delta_{leaf}$, $\delta^{13}C_{wood}$, $\delta^{15}N$, $g_s$, leaf shape, leaves per bud, $LMA_{spring}$, $LMA_{summer}$, $LMA_{post\text{-}budset}$, $N_{area}$, $N_{mass}$, NUE, WUE.

## Population genetic structure

In our multivariate PCA, only the first component (PC1) was significant with broken-stick modelling. The same result was obtained using either the reduced 8K SNP dataset or the full 29K SNP dataset suggesting an overarching, one-dimensional population stratification pattern across the range of *Populus trichocarpa* studied. Projected tree accessions using PC1 eigenvectors from either analysis were highly correlated, indicating that the overall population structure was equivalent in the 8K and 29K analyses ($R^2 = 0.99$, $P < 0.0001$; Table S3). This was also true of the orthogonal axis PC2 ($R^2 = 0.97$, $P < 0.0001$; Table S3). In the 29K SNP dataset, PC1 and PC2 explained only 2.1% and 1.2% of data variance, respectively. Other components explained < 1% of the variability and were disregarded. The degree to which SNPs contributed to a component (i.e. SNP eigenvalue loadings) varied and SNP loadings ranged from zero to |0.025|. The vast majority of SNPs contributing to either component had very low values (Fig. S1).

## Trait and population structure relationships to latitude and climate

Most geoclimate variables significantly co-varied (Tables 3, S7; see the Materials and Methods section). We focused on latitude, elevation and growing season-related climate variables as latitude and longitude were inherently intercorrelated due to the species' natural range and distribution ($r = 0.74$, $P < 0.001$), and mean coldest month temperature (MCMT) was strongly correlated to mean annual temperature (MAT) and frost-free days (FFD) (Table S7). Between geography and climate variables, latitude, daylength (DAY), FFD, MAT and mean warmest month temperature (MWMT) strongly co-varied (Tables 3, S7). Elevation showed strong correlations only with FFD and MAT. By comparison, mean annual precipitation (MAP) and mean summer precipitation (MSP) co-varied with each other and with annual and summer heat : moisture indices (AHM, SHM), but not strongly with geography or other climate variables.

The strength of co-variation between geoclimate variables and population structure or phenotypic traits differed considerably

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**Table 3** Coefficient of correlation (*r*) between selected geography and climate variables, phenotypic traits, and population structure principal components (PC) in *Populus trichocarpa* using Pearson's Product-Moment Correlations

| Variable[1,2] | LAT | ELEV | DAY | FFD | MAT | MWMT | MAP | MSP | AHM | SHM |
|---|---|---|---|---|---|---|---|---|---|---|
| ELEV | 0.16 | | | | | | | | | |
| DAY | **0.99** | 0.15 | | | | | | | | |
| FFD | **−0.69** | **−0.66** | **−0.67** | | | | | | | |
| MAT | **−0.83** | **−0.59** | **−0.81** | **0.95** | | | | | | |
| MWMT | **−0.75** | −0.32 | **−0.71** | **0.67** | **0.81** | | | | | |
| MAP | −0.28 | −0.45 | −0.31 | **0.57** | 0.47 | 0.16 | | | | |
| MSP | 0.04 | −0.25 | 0.01 | 0.31 | 0.16 | −0.08 | **0.89** | | | |
| AHM | 0.18 | 0.48 | 0.20 | −0.42 | −0.34 | −0.02 | **−0.86** | **−0.71** | | |
| SHM | −0.36 | 0.10 | −0.31 | 0.03 | 0.20 | 0.40 | **−0.65** | **−0.82** | **0.66** | |
| PC1(29K SNP) | **0.72** | 0.24 | **0.78*** | **−0.54** | **−0.62** | **−0.50** | −0.47 | −0.21 | 0.42 | 0.05 |
| PC2 (29K SNP) | **0.50** | −0.27 | 0.45 | −0.11 | −0.27 | −0.42 | 0.34 | 0.41 | −0.45 | **−0.59*** |
| Phenology | | | | | | | | | | |
| Bud break | 0.14 | 0.03 | 0.11 | −0.01 | −0.10 | −0.20 | 0.19 | 0.24 | −0.17 | −0.27* |
| Bud set | **−0.85** | −0.23 | **−0.86*** | **0.61** | **0.74** | **0.65** | 0.29 | 0.01 | −0.21 | 0.24 |
| Canopy duration | **−0.63** | −0.18 | **−0.64*** | 0.42 | **0.53** | 0.47 | 0.19 | −0.02 | −0.18 | 0.17 |
| Growth period | **−0.77*** | −0.18 | **−0.77*** | **0.52** | **0.65** | **0.60** | 0.20 | −0.05 | −0.15 | 0.25 |
| Height growth cessation | **−0.83** | −0.27 | **−0.85*** | **0.60** | **0.73** | **0.65** | 0.30 | 0.01 | −0.22 | 0.22 |
| Leaf drop | **−0.81** | −0.29 | **−0.83*** | **0.60** | **0.71** | **0.57** | 0.38 | 0.09 | −0.34 | 0.14 |
| Leaf flush | 0.11 | 0 | 0.07 | 0.03 | −0.05 | −0.17 | 0.22 | 0.27 | −0.20 | −0.30* |
| Leaf lifespan | **−0.72** | −0.19 | **−0.74*** | 0.45 | **0.58** | **0.52** | 0.19 | −0.07 | −0.15 | 0.24 |
| Post-bud set period | **0.69*** | 0.13 | **0.68** | −0.47 | **−0.58** | **−0.56** | −0.14 | 0.07 | 0.04 | −0.27 |
| Yellowing, 25% | **−0.54** | −0.14 | **−0.59*** | 0.33 | 0.41 | 0.33 | 0.18 | −0.01 | −0.15 | 0.11 |
| Yellowing, 50% | **−0.63** | −0.15 | **−0.67*** | 0.39 | 0.49 | 0.42 | 0.22 | −0.01 | −0.18 | 0.14 |
| Yellowing, 75% | **−0.72** | −0.21 | **−0.76*** | 0.49 | **0.60** | 0.49 | 0.28 | 0.02 | −0.24 | 0.14 |
| Yellowing, 100% | **−0.83** | −0.25 | **−0.86*** | **0.59** | **0.70** | **0.59** | 0.36 | 0.07 | −0.29 | 0.13 |
| Biomass | | | | | | | | | | |
| Active growth rate | **−0.58** | −0.18 | **−0.61*** | 0.42 | 0.49 | 0.38 | 0.30 | 0.12 | −0.21 | 0.04 |
| Bole density | 0.36 | 0.29 | 0.39* | −0.29 | −0.34 | −0.21 | −0.24 | −0.06 | 0.34 | −0.03 |
| Bole mass | −0.40 | −0.31 | −0.41 | 0.37 | 0.42* | 0.30 | 0.24 | 0.09 | −0.24 | 0.06 |
| Branch # | **−0.72*** | −0.26 | **−0.72*** | **0.56** | **0.65** | **0.55** | 0.23 | −0.05 | −0.22 | 0.24 |
| Height | **−0.71** | −0.23 | **−0.73*** | **0.55** | **0.63** | **0.51** | 0.34 | 0.11 | −0.25 | 0.10 |
| Height gain | **−0.69** | −0.22 | **−0.72*** | **0.50** | **0.59** | 0.49 | 0.30 | 0.06 | −0.21 | 0.14 |
| H : D | 0.12 | 0.15 | 0.09 | −0.16 | −0.16 | −0.10 | −0.21 | −0.11 | 0.30* | 0.08 |
| Log height growth | 0.49 | 0.16 | **0.51*** | −0.36 | −0.42 | −0.33 | −0.25 | −0.09 | 0.26 | −0.02 |
| Log volume growth | 0.16 | 0.07 | 0.13 | −0.18 | −0.17 | −0.13 | −0.14 | −0.09 | 0.22* | 0.05 |
| Volume | **−0.62*** | −0.25 | **−0.62*** | **0.51** | **0.58** | 0.48 | 0.32 | 0.10 | −0.27 | 0.08 |
| Volume gain | **−0.63*** | −0.25 | **−0.63*** | **0.50** | **0.58** | 0.47 | 0.32 | 0.09 | −0.27 | 0.10 |
| Whole tree mass | −0.45 | −0.32 | −0.46* | 0.39 | 0.45 | 0.33 | 0.21 | 0.04 | −0.22 | 0.12 |
| Ecophysiology | | | | | | | | | | |
| $A_{max}$ | 0.49* | 0.11 | 0.49* | −0.34 | −0.41 | −0.35 | −0.24 | −0.07 | 0.22 | −0.06 |
| $A_{max/mass}$ | 0.41 | 0.23 | 0.41 | −0.39 | −0.43* | −0.33 | −0.27 | −0.10 | 0.26 | −0.03 |
| C : N | −0.20 | −0.20 | −0.19 | 0.30* | 0.28 | 0.20 | 0.30* | 0.22 | −0.28 | −0.10 |
| $Chl_{spring}$ | 0.07 | −0.09 | 0.09 | −0.01 | −0.01 | −0.01 | −0.12 | −0.10 | 0.08 | 0.08 |
| $Chl_{summer}$ | 0.34 | 0.19 | 0.35 | −0.29 | −0.33 | −0.20 | −0.32 | −0.16 | 0.36* | 0.07 |
| $Chl_{post\text{-}budset}$ | 0.21 | 0.31 | 0.20 | −0.36* | −0.35 | −0.22 | −0.32 | −0.18 | 0.35 | 0.16 |
| $\Delta_{leaf}$ | 0.25 | 0.04 | 0.27* | −0.04 | −0.13 | −0.12 | 0.07 | 0.17 | 0 | −0.13 |
| $\delta^{13}C_{wood}$ | −0.03 | 0.09 | −0.04 | −0.07 | −0.03 | 0.03 | −0.11 | −0.08 | 0.14 | 0.05 |
| $\delta^{15}N$ | 0.22 | 0.09 | 0.23 | −0.18 | −0.23 | −0.25* | −0.11 | −0.05 | 0.05 | −0.03 |
| $g_s$ | **0.54** | 0.09 | **0.55*** | −0.29 | −0.39 | −0.30 | −0.10 | 0.11 | 0.15 | −0.17 |
| Leaf shape | 0 | 0.08 | −0.01 | −0.04 | −0.03 | 0.05 | 0.09 | 0.14 | −0.05 | −0.13 |
| Leaves per bud | **0.59** | 0.21 | **0.63*** | −0.45 | **−0.52** | −0.44 | −0.22 | −0.02 | 0.16 | −0.14 |
| $LMA_{spring}$ | 0.07 | −0.15 | 0.09 | −0.02 | −0.01 | 0.01 | −0.17 | −0.18* | 0.10 | 0.13 |
| $LMA_{summer}$ | 0.03 | −0.18 | 0.03 | 0.11 | 0.07 | −0.04 | 0.12 | 0.11 | −0.12 | −0.11 |
| $LMA_{post\text{-}budset}$ | −0.10 | −0.05 | −0.09 | 0.14 | 0.12 | 0.09 | 0.13 | 0.16 | 0.03 | −0.01 |
| $N_{area}$ | 0.20 | 0.06 | 0.20 | −0.20 | −0.21 | −0.17 | −0.22* | −0.17 | 0.21 | 0.07 |
| $N_{mass}$ | 0.15 | 0.20 | 0.15 | −0.28* | −0.25 | −0.16 | −0.27 | −0.21 | 0.26 | 0.11 |
| NUE | 0.32* | 0.06 | 0.32* | −0.17 | −0.24 | −0.21 | −0.04 | 0.09 | 0.03 | −0.14 |
| WUE | −0.22 | −0.01 | −0.23* | 0.02 | 0.08 | 0.01 | −0.10 | −0.21 | 0.01 | 0.16 |

Traits in bold indicate both correlative significance and $r \geq 0.5$. Asterisks indicate the highest correlation for each trait significant at $P < 0.001$ after Bonferroni correction.

[1]See Tables 1 and 2 for units and abbreviations.

[2]Values indicate mean *r* where repeated measurements were taken (see Supporting Information Table S7 for full results).

(Tables 3, S7). Population genetic structure (PC1) correlated most strongly with latitude, DAY and temperature metrics (FFD, MAT, MWMT), whereas the orthogonal PC2 correlated with latitude and SHM. The strength of relationships between phenotypic traits and geoclimate variables varied depending on the trait. Phenology traits showed the strongest relationships to geoclimate variables among all traits measured. Most phenology events (height growth cessation, bud set, leaf yellowing, leaf drop) and period metrics (growth period, canopy duration, leaf lifespan) had strong correlations to latitude, DAY, FFD, MAT and MWMT. Biomass traits showed similarly robust relationships with geoclimate, particularly with height, volume, branch numbers and growth rate traits. Among ecophysiology traits, only $g_s$ and number of leaves per bud strongly co-varied with latitude, DAY and MAT, whereas most ecophysiology traits had lower associations with geoclimate variables.

Considering all correlations with geoclimate variables across each PC or trait showed that DAY and latitude tended to have the strongest associations (asterisks, Table 3). The strongest significant relationship was DAY for PC1 and SHM for PC2. Both DAY and latitude were the strongest associations for the majority of phenology traits, most biomass traits and several ecophysiological traits. Notably, bud break and leaf flush did not align with any of these variables and showed greater correlation with SHM. Only a small number of biomass and ecophysiology traits co-varied more strongly with other geoclimate variables (temperature, precipitation), and no trait correlated strongly with elevation.

### Relationship of trait heritability and geography

Linear regressions between trait $H^2$ estimates and trait correlation coefficients ($r$) to eight geoclimate variables (latitude, elevation, DAY, FFD, MAT, MWMT, MAP, MSP) yielded four significant, predictive relationships (Fig. 3, Table S8). Trait $H^2$ was significantly related to trait correlations with latitude, DAY, MAT and MWMT ($P < 0.05$). By comparison, there was no significant relationship with elevation, FFD, MAP or MSP. The same patterns were observed testing $H^2$ against coefficients of determination ($R^2$) from trait–geoclimate correlations (data not shown). Among the four significant trends between trait heritability and correlation to geoclimate, the strongest predictive trends occurred between $H^2$ and DAY (slope = 0.58), latitude (slope = 0.56) and MWMT (slope = 0.42; Table S8). Outliers affected the strength and/or significance of the regression (Fig. 3, outliers with $H^2 > 0.8$; see the Materials and Methods section). Removing three clear, outlying traits (bud break, leaf flush, $LMA_{spring}$) made all regressions significant, and relationships between $H^2$ and latitude, DAY, MAT and MWMT gained in statistical strength ($P < 0.001$; Fig. 3, Table S8).

### Contributing SNPs and gene enrichment related to population structure

We identified the top 600 SNP markers (99th percentile) with high loading values (|0.015–0.025|) in the significant PC1 and orthogonal PC2. Markers implicated 375 genes spanning all 19 chromosomes: PC1 had 181 highly contributing genes while PC2 had 205 genes. Ten genes contributed to both components, including one gene involving response to light (POPTR_0012s13550 encoding homologues of Arabidopsis *FRS5* [*FAR1-related sequence 5*]). Many of the 375 genes had known gene function annotations, such as transcription factors, signalling genes, aquaporins, kinases, ion transporters, phytohormone-related genes, light-associated genes, disease and stress response genes, and cell wall metabolism genes (Table S4). Others were differentially expressed candidates and uncharacterized genes.

Maximum daylength (i.e. photoperiod) had the highest correlation with PC1 (Table 3). In PC1, genes annotated as light-associated genes included *ARR3* and *9* (*RESPONSE REGULATOR 3* and *9*), *CCA1* (*CIRCADIAN CLOCK ASSOCIATED 1*), *COL* (*CONSTANS-LIKE 14*), *FAR1* (*FAR-RED IMPAIRED RESPONSE 1*), *CYP78A9* (*CYTOCHROME P450 78A9*), *FHY3* (*FAR-RED ELONGATED HYPOCOTYLS 3*), *FRS5* and *11* (*FAR1-RELATED SEQUENCE 5* and *11*), *PCL1* (*PHYTOCLOCK 1*), *PIL6* (*PHYTOCHROME INTERACTING FACTOR 3-LIKE 6*), *PHOT1* (*PHOTOTROPIN 1*), *PRR7* (*PSEUDO-RESPONSE REGULATOR 7*) and *TOC1* (*TIMING OF CAB EXPRESSION 1*). SNPs in phenology-related genes implicated in other *Populus* species (*FRIGIDA*, *GIGANTEA*, *PHYTOCHROME A* and *B*; cf. Ingvarsson *et al.*, 2008; Olson *et al.*, 2012) were successfully genotyped but not highly contributing to either PC axes.

We tested for enrichment of genes within five light-associated GO categories (circadian rhythm, photoperiod, light response, blue light response, red/far-red light response) in PC1. All GO categories except blue light response showed at least twice greater numbers of light-associated genes compared to all genes within the top 0.1% (SNPs ≥ |0.0191|) contributing to the overall population stratification pattern in *P. trichocarpa* (Table 4). Enrichment within the top 0.1% SNPs from PC1 relative to the SNP array was significant in the circadian rhythm and photoperiod GO categories ($P < 0.05$). Within the 0.5–1% SNP categories, the proportions of genes in each GO category were higher relative to total numbers on the SNP array; however, no significant enrichment in any GO category was observed.

## Discussion

Our results demonstrate high variation in phenotypic traits among a large collection of *P. trichocarpa* accessions spanning most of the species' natural range. Extensive assessments of traits through spatial and temporal replications underscored the consistency observed in phenotypic traits from year to year and to our knowledge, present the most comprehensive assessment of traits in *Populus* to date, particularly as many of these are partitioned to reflect seasonal changes (cf. McKown *et al.*, 2013).

### Traits and population structure reflect clinal distribution

Many traits strongly co-varied with latitude, photoperiodic regime (daylength) and/or temperature. This was observed particularly among phenology traits which represented the largest

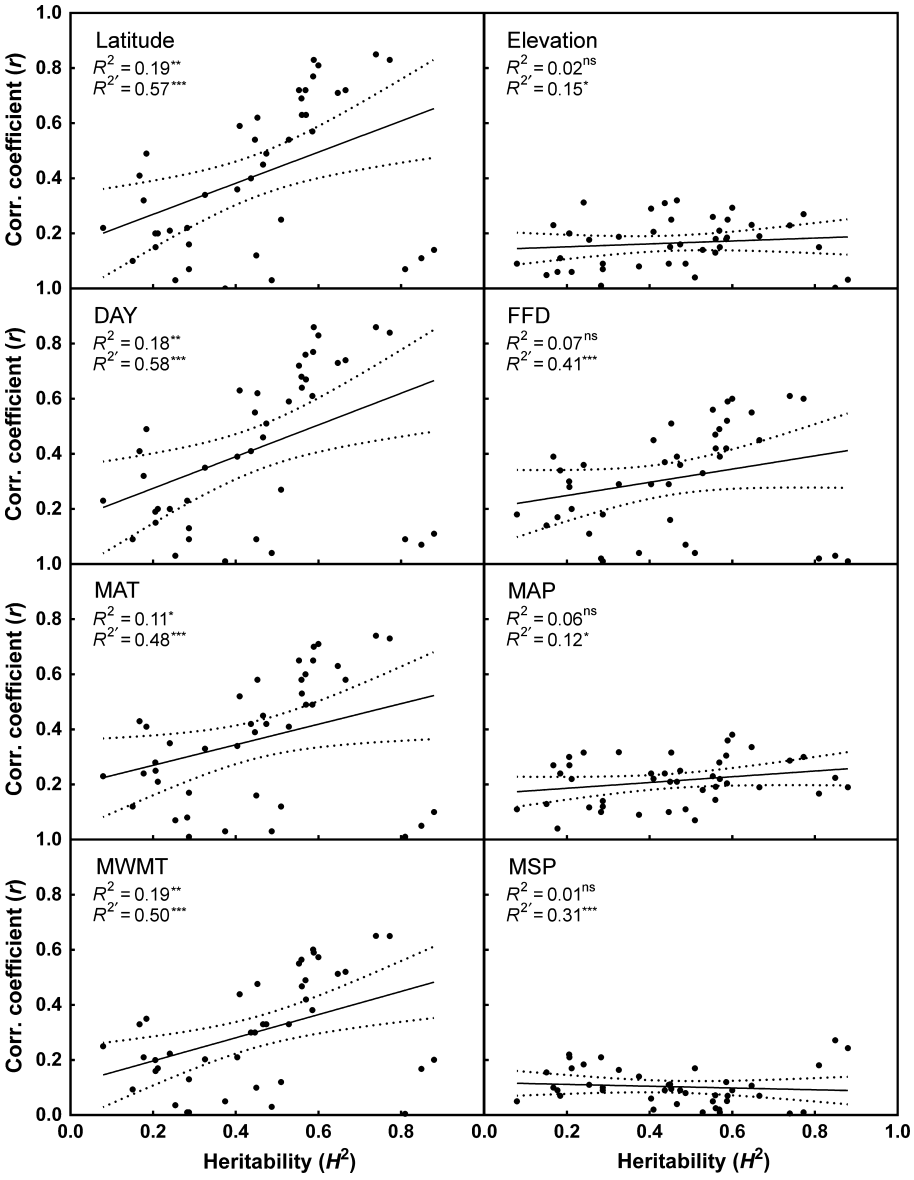


**Fig. 3** Relationships between phenotypic trait broad-sense heritability values ($H^2$) and trait correlation coefficients (|r|) with geography and climate variables in *Populus trichocarpa*. Regression significance and goodness of fit indicated between: $H^2$of all traits ($R^2$); and $H^2$ excluding bud break, leaf flush, and $LMA_{spring}$ ($R^{2\prime}$). Linear regressions of $H^2$ vs geoclimate *r* (considering all traits) with 95% confidence intervals shown. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, not significant. DAY, photoperiod/daylength; FFD, frost-free days; MAP, mean annual precipitation; MAT, mean annual temperature; MSP, mean summer precipitation; MWMT, mean warmest month temperature.

number of strong correlations (bold values, Table 3). Across geoclimate variables, the photoperiodic gradient was the strongest significant correlation for the majority of traits and only in a small number of cases were trait correlations higher with other geoclimate variables (asterisks, Table 3). In comparison, a previous study of a much smaller number of *P. trichocarpa* accessions found that ecophysiological traits ($A_{max}$, $g_s$, $N_{area}$) and height measured in a common garden were most strongly correlated with MWMT, and LMA co-varied with precipitation (Gornall & Guy, 2007). Our patterns also contrast those found in observations of *P. balsamifera* where growth and LMA co-varied highly with latitude while numerous gas-exchange based and isotope traits correlated more strongly with frost-free days, MAT and summer dryness index (Soolanayakanahally *et al.*, 2009). In addition, co-varying trait relationships observed in *P. balsamifera*, such as height/isotopes/LMA and gas-exchanged based traits/LMA (Soolanayakanahally *et al.*, 2009), were not present among our accessions of *P. trichocarpa*. Differences among these studies may relate to scale in terms of the numbers of genotypes and phenotypic traits assessed; however, accession sampling for this study and Soolanayakanahally *et al.* (2009) covered the majority of both species' extensive ranges and the results likely reflect some species divergence and adaptive differences between *P. trichocarpa* and *P. balsamifera*. This is not unexpected as traits affecting physiology are known to be dissimilar between both species (Farrar, 1995; Gornall & Guy, 2007).

Genome-wide genetic variation in *P. trichocarpa* co-varied with the same geoclimate variables as many phenotypic traits, and may reflect similar selective factors. Population structure among our accessions was best described by PC1, regardless of the number of SNPs in the analysis (8K vs 29K), confirming that results were representative of genome-wide variation and not influenced by SNPs in linkage, markers not in HWE, or missing data. The overall genomic structure from PC1 reflected the original location for our *P. trichocarpa* accessions but represented only 2% of the genomic variability across the species. The vast majority of

**Table 4** Enrichment of gene ontology (GO) gene categories implicated by high single nucleotide polymorphism (SNP) eigenvalue contributions (top 0.1–1.0% of SNP loadings) to population structure principal component 1 (PC1) in *Populus trichocarpa* compared to the 34K *Populus* SNP array using Fisher's exact tests

| Dataset[1] | SNP array | 0.1% PC1 | 0.5% PC1 | 1% PC1 |
|---|---|---|---|---|
| All genes | | | | |
| Number of genes | 3436 | 20 | 78 | 160 |
| Circadian rhythm | | | | |
| Number of genes | 99 | **3** | 4 | 4 |
| Proportion | 0.029 | **0.15** | 0.051 | 0.025 |
| *P*-value* | | **0.027** | 0.30 | 1 |
| Light response | | | | |
| Number of genes | 334 | 4 | 10 | 19 |
| Proportion | 0.097 | 0.2 | 0.13 | 0.12 |
| *P*-value* | | 0.16 | 0.45 | 0.42 |
| Photoperiod | | | | |
| Number of genes | 49 | **2** | 2 | 2 |
| Proportion | 0.014 | **0.10** | 0.026 | 0.013 |
| *P*-value* | | **0.040** | 0.3181 | 1 |
| Blue light | | | | |
| Number of genes | 30 | 0 | 0 | 1 |
| Proportion | 0.0087 | 0 | 0 | 0.0063 |
| *P*-value* | | – | – | 1 |
| Red and Far-red light | | | | |
| Number of genes | 133 | 3 | 5 | 10 |
| Proportion | 0.039 | 0.15 | 0.064 | 0.063 |
| *P*-value* | | 0.054 | 0.24 | 0.15 |

Significantly enriched gene GO categories are indicated in bold.
[1]Gene information from Geraldes *et al.* (2013).
**P*-values for one- and two-tailed Fisher exact tests equivalent.

SNPs had low contribution values while a limited number of SNPs were considered high (i.e. loadings were not identical among all 29K SNPs). Eckert & Dyer (2012) proposed that a large portion of adaptive genetic diversity lies within regulatory regions of the genome, and, supporting this idea, two-thirds of markers among the top 600 SNPs identified in our analysis were categorized as intergenic or intronic (Table S4).

Where geographical patterns accompany patterns in environmental factors causing selection pressure, the ability to detect genetic variants responsible for clinal adaptation depends on the level of selection intensity. Principal components in multivariate analyses based on genetic markers often show strong relationships with geographical distribution of a population sample covering extensive regions (Novembre *et al.*, 2008; Wang *et al.*, 2012b). Thus, a complicating issue is the ability to distinguish isolation-by-distance, or genetic drift, from selection processes (Neale & Ingvarsson, 2008). In *P. trichocarpa*, there is strong co-variation between population genetic structure (PC1), environmental gradients and numerous phenotypic traits. Genes associated with the PC1 axis should represent both a mixture of population history (i.e. drift, migration, introgression) and selection due to adaptation. For instance, PC1 co-varied along similar ecological gradients to many phenology traits, associating most with latitude, and photoperiod (Table 3), and was enriched for circadian rhythm and photoperiodic genes (Table 4). Many of the light-associated genes implicated by multivariate analysis are known to play important roles in determining circadian rhythms in Arabidopsis (Hudson *et al.*, 2003; Huang *et al.*, 2012) and the timing of phenological traits in *Populus* (Ruttink *et al.*, 2007; Ingvarsson *et al.*, 2008; Cooke *et al.*, 2012; Olson *et al.*, 2012). By comparison, the orthogonal axis PC2 was not significant in describing population structure. PC2 and the highly heritable traits bud break/leaf flush co-varied with SHM, and were less related to other environmental gradients. Thus, PC2 might provide 'orthogonal' genomic information about genes or traits and range-wide demographic history or selection patterns. The high loading SNPs implicated in both PC1 and PC2 may be discoverable using population genetic analyses (i.e. $F_{ST}$ outliers; A. Geraldes *et al.*, unpublished; I. Porth *et al.*, unpublished); however, these markers might not necessarily be retrieved using other methods. For instance, SNP discovery using genome-wide association analyses depends both on phenotyping the corresponding trait and where incorporation of correction methods for population structure do not fully diminish signal from SNPs aligned with population stratification that contribute to the phenotype (A. D. McKown *et al.*, unpublished).

## Trait heritability relationships with geography

Broad-sense heritability assessments ($H^2$) in this study showed that phenology traits had moderate–high $H^2$ estimates, whereas biomass and ecophysiology trait $H^2$ estimates ranged from low to high. As low $H^2$ estimates ($< 0.40$) were observed primarily in ecophysiological traits, we conclude that this is most likely related to some phenotypic plasticity and reflecting the environment in which traits were sampled. This is highlighted by $\delta^{15}N$ ($H^2 = 0.08$), which was expected to have low heritability because of a tendency to track variation in soil $\delta^{15}N$ (Houlton *et al.*, 2007). Our trait heritability results are similar to other *Populus* species where high heritability has been observed in phenology (bud break, leaf flush, height growth cessation, bud set; Bradshaw & Stettler, 1995; Howe *et al.*, 2000; Rae *et al.*, 2004; Rohde *et al.*, 2011; Fabbrini *et al.*, 2012). Notably, this was not necessarily the case for *P. balsamifera*, 'sister' species of *P. trichocarpa* (Keller *et al.*, 2011) or *P. tremula* (Luquez *et al.*, 2008) where bud set had a high heritability but not bud break in both species. Comparatively, other studies in *Populus* have also observed moderate heritability in biomass traits, and many found relatively lower heritability for other traits, particularly ecophysiological traits (Bradshaw & Stettler, 1995; Rae *et al.*, 2004; Luquez *et al.*, 2008; Chamaillard *et al.*, 2011; Keller *et al.*, 2011).

Heritability essentially describes the likelihood that offspring will replicate the parental phenotype, but high heritability may or may not indicate an evolutionary relationship to environment. The strong regressive patterns in *P. trichocarpa* between trait $H^2$ in this study and trait correlation coefficients to geoclimate variables were striking (Fig. 3). Individual traits with higher $H^2$ (phenology, biomass) tended to be both intercorrelated and have strong relationships individually with latitude, daylength and temperature; however, these highly heritable traits were not solely driving the patterns as many low–moderately heritable traits also followed the observed trends. We noted that while the overall patterns between trait heritability and correlation to environment

were clear for most traits, they were not necessarily predictive. Spring traits (bud break, leaf flush, $LMA_{spring}$) had very high $H^2$ (> 0.8) but weak relationships with geoclimate variables included in our study and did not align with the main trends observed between $H^2$ estimates and geoclimate.

This alignment of heritability and correlation of traits to environmental gradients might be driven by high genetic complexity or traits under polygenic control. For instance, critical traits identified for adaptation in trees (including clinal adaptation) are under polygenic control, such as cold tolerance, bud break, bud set, dormancy and growth (Ruttink *et al.*, 2007; Savolainen *et al.*, 2007; Holliday *et al.*, 2008; Ingvarsson *et al.*, 2008; Eckert *et al.*, 2009; Ibáñez *et al.*, 2010; Ma *et al.*, 2010; Rohde *et al.*, 2010; Cooke *et al.*, 2012). In theory, high heritability should reflect spatially variable selection rather than strong directional selection across the entire species range (Mousseau & Roff, 1987) as these might result in selective sweeps and a resulting erosion of genetic variation for the trait. With a larger number of genes and/or varying contributions of gene action, the ability to respond to local environmental heterogeneity or 'tracking' a phenotypic optimum is possible (Savolainen *et al.*, 2007) and selective sweeps are less likely. This is illustrated by *P. balsamifera* accessions where phenology timing shows marked shifts depending on the location of common gardens and results in varied dates for phenology from genetically identical trees (Olson *et al.*, 2012; Soolanayakanahally *et al.*, 2013). Of note, a polygenic hypothesis for high heritability does not adequately describe the scenario where a trait with high heritability may be stochastic and/or does not necessarily reflect obvious selection (i.e. nonadaptive variation). In such a case, the trait might be controlled by one or few genes within a network or with large, epistatic effect (Eckert & Dyer, 2012; Hemani *et al.*, 2013).

## Adaptation in a complex environment

Within *P. trichocarpa*, dioecy and wind pollination/seed dispersal promote high levels of gene flow through outcrossing and migration, but must be mitigated by strong selection as these outcrossing forces should act to oppose differentiation or local adaptation (Farmer, 1996; Kawecki & Ebert, 2004). Geraldes *et al.* (2013) found that range-wide gene flow in *P. trichocarpa* was high and determined low differentiation between provenances (150 km distance). Corresponding studies also detected limited structure within *P. trichocarpa* (Slavov *et al.*, 2010, 2012). Nevertheless, both trait and population structure are largely clinal in *P. trichocarpa*, and thus some components of the genetic and phenotypic variation observed should reflect selective processes.

The relationships between latitude and phenotypic variation (particularly phenology traits such as bud set) have been established previously in other *Populus* species (Luquez *et al.*, 2008; Soolanayakanahally *et al.*, 2009, 2013; Keller *et al.*, 2011; Cooke *et al.*, 2012). Soolanayakanahally *et al.* (2009) suggested that in addition to phenological variation, trait adaptation in northern *P. balsamifera* accessions is largely related to faster growth and higher carbon accumulation to mitigate the shorter growing seasons in northern locales (see Benowicz *et al.*, 2000 for comparable strategies in other hardwood species). We observed similar patterns in *P. trichocarpa* for many phenology traits and traits related to carbon acquisition and growth. For instance, log height growth rates, photosynthesis, stomatal conductance, chlorophyll content and nitrogen-use efficiency were higher in northern accessions of *P. trichocarpa* suggesting faster inherent carbon acquisition and growth capacity. Additionally, we observed that northern trees had greater numbers of leaves in dormant buds which may indicate an increase in the spring canopy and/or faster leaf production rates.

The positional information available for our *P. trichocarpa* accessions allowed us to consider latitude, longitude and elevation, and to estimate climate and photoperiodic components of environments across the species' continental range. Latitude, longitude and elevation are not single elements *per se*, but rather present combinations of these climatic and photoperiodic components. Considering these components individually indicated that maximum daylength (photoperiod) and temperature generally had the highest correlations both to phenotypic traits and PC1 above other components; however, both factors likely present a 'combined' selection pressure. There is some indication that response to one factor may be mitigated or diminished by the other. For instance, while photoperiod remains unchanged from year to year, interannual temperature shifts may modify the timing of phenological events in *P. trichocarpa* (Rohde *et al.*, 2011; McKown *et al.*, 2013). However, photoperiod provides an overriding signal which causes phenological mismatch when trees are moved to latitudes dissimilar from their site of origin (McKown *et al.*, 2013; Soolanayakanahally *et al.*, 2013). We therefore suggest that environmental variation across the range of *P. trichocarpa*, particularly relating to photoperiod and temperature, has played a strong role in shaping both phenotypic trait variation and genetic structure.

## Conclusions

Adaptation in any species requires phenotypic variation in traits, but would not occur without genotypic variation. Within a species, this adaptation can theoretically be maintained through variable selection pressures from heterogeneous environments on a number of genes (Farmer, 1996; Kawecki & Ebert, 2004; Savolainen *et al.*, 2007; Le Corre & Kremer, 2012). Among natural accessions of *Arabidopsis thaliana*, many SNPs involved in adaptation are known to relate both to geography and climate (Fournier-Level *et al.*, 2011). In this study, the genes associated with PC1 likely reflect demographic processes in *P. trichocarpa* along its north–south latitudinal distribution (cf. Neale & Ingvarsson, 2008), but will also include some genes related to environmental gradient selection. The light-associated genes implicated by multivariate analysis may simply reflect the stochastic processes of population history; however, these show significant enrichment in PC1. By comparison, the orthogonal axis is largely unrelated to overall environmental gradients and genes within this axis might vary on a more local basis in *P. trichocarpa*. Considering the genomic variability related to clinal variation, it is likely that a number of loci are acting to respond to strong selection

pressures and that co-varying phenotypic differentiation may be resulting from numerous, small allelic frequency changes in *P. trichocarpa* (cf. Savolainen *et al.*, 2007; Le Corre & Kremer, 2012).

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## Supporting Information

Additional supporting information may be found in the online version of this article.

**Fig. S1** Histogram of SNP eigenvalues contributing to principal component 1 (PC1).

**Table S1** Biogeographical data for *P. trichocarpa* accessions

**Table S2** Phenotypic trait data for *P. trichocarpa* accessions including garden-wide trait metrics and clonal accession means

**Table S3** Projected *P. trichocarpa* accessions using principal components (PC)

**Table S4** Top SNP eigenvalue loadings contributing to both principal components (PC) 1 and 2

**Table S5** Broad-sense heritability values ($H^2$) for traits measured in 448 genotyped *P. trichocarpa* accessions

**Table S6** Full phenotypic trait correlations among all biomass, ecophysiology and phenology traits across multiple years in *P trichocarpa*

**Table S7** All biogeography, climate, PC1, biomass, ecophysiology and phenology trait correlations in *P. trichocarpa*

**Table S8** Linear regressions of $H^2$ vs correlation coefficients ($r$) determined between traits and biogeography variables

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