Research paper

Higher photosynthetic capacity from higher latitude: foliar characteristics and gas exchange of southern, central and northern populations of *Populus angustifolia*

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Narrowleaf cottonwood (*Populus angustifolia* James) is an obligate riparian poplar that is a foundation species in river valleys along the Rocky Mountains, spanning 16° of latitude from southern Arizona, USA to southern Alberta, Canada. Its current distribution is fragmented, and genetic variation shows regional population structure consistent with the effects of geographic barriers and past climate. It is thus very well-suited for investigating ecophysiological adaptation associated with latitude. In other section *Tacamahaca* poplar species, genotypes from higher latitudes show evidence of short-season adaptation with foliar traits that contribute to higher photosynthetic capacity. We tested for similar adaptation in three populations of narrowleaf cottonwoods: from Arizona (south), Alberta (north) and Utah, near the centre of the latitudinal distribution. We propagated 20 genotypes from each population in a common garden in Alberta, and measured foliar and physiological traits after 3 years. Leaves of genotypes from the northern population had higher leaf mass per area (LMA), increased nitrogen (N) content and higher carotenoid and chlorophyll content, and these were associated with higher light-saturated net photosynthesis (A_{sat}). In leaves of all populations the majority of stomata were abaxial, with the proportion of abaxial stomata highest in the southern population. Stomatal conductance (g_s) and transpiration rates were higher in the northern population but water-use efficiency (A_{sat}/g_s) and leaf carbon isotope composition ($\delta^{13}C$) did not differ across the populations. These results (i) establish links between A_{sat} and g_s, N, chlorophyll and LMA among populations within this species, (ii) are consistent with the discrimination of populations from prior investigation of genetic variation and (iii) support the concept of latitudinal adaptation, whereby deciduous trees from higher latitudes display higher photosynthetic capacity, possibly compensating for a shorter and cooler growth season and reduced insolation.

Keywords: adaptation, ecophysiology, leaves, narrowleaf cottonwood, short-season, stomata.

Introduction

Forest trees often show considerable genetic variation among populations, are often locally adapted and frequently show correspondence between gradients of genetic variation and climate variables (Howe et al. 2003, Savolainen et al. 2007, Slavov and Zhelev 2010). Temperature and water availability are two of the most important climatic factors, and the onset of climate warming has focused attention on this adaptation, in both evergreen and deciduous northern trees (Howe et al. 2003, Aitken et al. 2008, Luquez et al. 2008, Soolanayakanahally et al. 2013). Adaptation of phenology to seasonal alternation of temperatures has been the focus of many studies, including those with *Populus* species (Howe et al. 2003), and this may be accompanied by adaptation in photosynthetic and other gas exchange traits that may compensate for differences in season length (Gornall and Guy 2007, Soolanayakanahally et al. 2009,

McKown et al. 2014a). Adaptation in these traits is also seen in response to differences in aridity or temperature (Wright et al. 2004, Grady et al. 2013). Such adaptive differences offer insight into the relationship among traits (leaf economics; Wright et al. 2004) within and among species across the spectrum of climate regimes, and suggest possibilities for selection of appropriate suites of traits in genotypes more suited for warmer or drier future conditions, for afforestation or conservation (Aitken et al. 2008, Grady et al. 2013).

Gradients in seasonal temperatures and aridity often coincide with gradients in latitude or elevation, and so species with wide distributions are well-suited for investigations of associated adaptation. One such species is the narrowleaf cottonwood (*Populus angustifolia* James). It has a wide latitudinal range (Figure 1), and occurs in genetically distinct disjunct populations (Evans et al. 2015). It is of considerable ecological importance (Whitham et al. 2006), and the fate of the southernmost populations in particular is uncertain in the face of climate warming (Aitken et al. 2008, Cayan et al. 2010). There has been no investigation of its gas exchange physiology in the

context of adaptation to season length or aridity. Here we describe photosynthetic physiology and leaf morphology of northern and southern ecotypes of *P. angustifolia* grown in a common garden, and the relationship of those traits with the differing climate conditions of the population origins.

Populus angustifolia is an important foundation species in river valleys in western North America, and has been investigated in relation to ecophysiology (Rood et al. 2010, 2013), genetic variation (Evans et al. 2015) and community ecology (Whitham et al. 2006). It is a riparian (streamside) poplar that is distributed through a 1700 km range of latitude. It belongs to *Populus* section *Tacamahaca* along with *P. balsamifera* L. and *P. trichocarpa* Torr. & A. Gray, but some of its ecophysiological characteristics are closer to those of members of the section *Aigeiros* cottonwoods than to the other section *Tacamahaca* species (Rood et al. 2003). Throughout its range, *P. angustifolia* occurs at higher elevations than *P. deltoides* Bartr. ex. Marsh or *P. fremontii* S. Watson of the section *Aigeiros*, but below *P. trichocarpa* or *P. balsamifera*. This suggests that it is adapted to a relatively narrow and intermediate range of environmental conditions, and consistent with this it is found in discontinuous woodlands along its latitudinal range (Cooke and Rood 2007). This fragmented distribution could increase genetic divergence, and our analysis of genetic polymorphism in neutral markers and candidate phenology genes in narrowleaf cottonwood genotypes from across the latitudinal range has indicated that geographical barriers result in regionally differentiated genetic subpopulations and suggest strong latitudinal clines (Evans et al. 2015).

This evidence of genetic differentiation across latitude suggests that *P. angustifolia* might also be a good candidate for the investigation of corresponding variation in phenology and related physiology, and the association of these traits with climatic factors. In deciduous trees, adaptation in phenology is cued by both temperature and photoperiod (Cooke et al. 2012), and at higher latitudes or higher elevations, the timing of bud flush and bud set define a growth season which is often comparatively cool or short. Within this season, deciduous trees from northern latitudes, including species of *Populus*, may display increased photosynthetic rates as a possible adaptive physiological strategy that compensates for the less favourable conditions (Benowicz et al. 2000, Gornall and Guy 2007, Soolanayakanahally et al. 2009, McKown et al. 2014a). The increased photosynthetic rates are often associated with related foliar parameters, including stomatal conductance (g_s), chlorophyll, nitrogen content (N) and the leaf mass to area ratio (LMA), consistent with such relationships seen in a global spectrum of species (Wright et al. 2004). Trees from southern latitudes, especially if exposed to more arid conditions, may incur net benefit from lower g_s as transpiration will be reduced and water-use efficiency (WUE) may be improved. Such adaptation is seen, for example, in *P. fremontii*, in populations from different elevations rather than different latitudes (Grady et al. 2013).
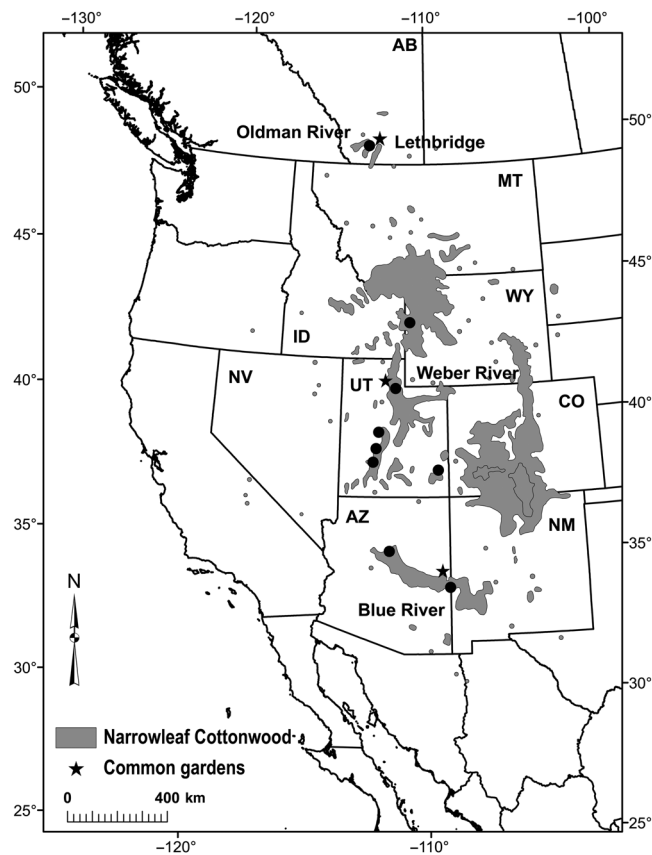

Figure 1. Native distribution of narrowleaf cottonwoods (US Geological Survey 1999) and the locations of the collection sites of all populations (filled circles) and common gardens (Evans et al. 2015) including those used in this study—the northern (Oldman River, AB), central (Weber River, UT) and southern (Blue River, AZ) populations, and the common garden at Lethbridge, AB.

To investigate this adaptation, genotypes originating from different environments are collected, propagated and grown in a common garden, where they experience similar environmental conditions and the phenotypic effects of genetic variation are revealed. Many studies involving *Populus* have taken this approach (e.g., Pauley and Perry 1954, Dunlap et al. 1993, Friedman et al. 2008, Luquez et al. 2008, Fracheboud et al. 2009, Grady et al. 2013, McKown et al. 2014*a*), and for this study and another (Evans et al. 2015) we collected narrowleaf cottonwoods from river valleys along a north–south transect of the Rocky Mountains (Figure 1) and propagated them in three common gardens.

In one of those common gardens we have investigated photosynthetic physiology in three of the populations of *P. angustifolia*, which represented the northern and southern regional populations (Evans et al. 2015) and encompassed the latitudinal range of the species. We analysed many of the foliar morphological and gas exchange characteristics that were investigated for the two other section *Tacamahaca* poplars in similar studies (Gornall and Guy 2007, Soolanayakanahally et al. 2009, McKown et al. 2014*a*). We tested several hypotheses: (i) the discrimination of river valley populations on the basis of variation in foliar physiology and morphology would be consistent with that based on genetic variation as seen in Evans et al. (2015), and in particular that the northern and southern populations would differ; (ii) differences in photosynthetic physiology among populations would be consistent with the concept of latitudinal compensation, i.e., genotypes from river valleys in the higher (northern) latitudes would show higher photosynthetic capacity than those from the south; (iii) the southern populations would show comparatively conservative physiology, with lower g_s and higher WUE than northern populations; and (iv) relationships among leaf economic traits (A_{sat}, g_s, N, chlorophyll and LMA) would be the same among genotypes and populations of *P. angustifolia* as seen in similar studies with other *Populus* species.

Materials and methods

Genotypes and study site

This study involved genotypes from three of the nine populations of narrowleaf cottonwoods (*P. angustifolia* James) included in the study of Evans et al. (2015). These three populations were the most extensively sampled and represented the southern limit of the southern regional population, the centre of the latitudinal distribution and the northern limit of the northern regional population (Figure 1; Evans et al. 2015). They were from the Blue River, Arizona, the Weber River, Utah and the Oldman River, Alberta. Branch cuttings from 20 distinct trees in each of the three populations were collected, and rooted in a greenhouse in Flagstaff, Arizona. Through DNA sequencing of multiple genes, all 60 cottonwoods in this study were determined to be unique genotypes,

and all were *P. angustifolia* rather than interspecific hybrids that co-occur in some populations (Evans et al. 2015).

Subsequently, six saplings from each of the 60 genotypes were transplanted in the spring of 2009 into the common garden in an upland location at the University of Lethbridge in Alberta (49.670°N, 112.863°W). They were distributed among all saplings from the more extensive collection (Evans et al. 2015) in a completely randomized design. In the spring of 2011 half of the saplings were transplanted into an adjacent area to expand spacing and others were replaced with saplings from the same genotype following mortality due to winter kill or browsing by deer. Weeds were mowed and the common garden was occasionally irrigated, particularly in the days prior to each gas exchange measurement, to reduce possible effects of limiting soil moisture.

Leaf sampling

Preliminary measurements of leaf morphology and gas exchange were made in the summer of 2011 and in early July 2012, to determine suitable sampling times, and variation among leaves along the shoot axis. Light-saturated net assimilation rate (A_{sat}) was measured in leaves from a number of genotypes between 08:00 and 16:00 h, using a LI-6400 meter as explained below. It was found to be quite constant for each genotype between 09:30 and 13:30 h, so subsequent measurements were restricted to that interval. In three genotypes from each population, A_{sat} was measured in the sixth to eleventh fully expanded leaves from the base of the shoot, and did not differ among those leaves (analysis of variance, $P = 0.856$), indicating relative uniformity over this portion of the saplings.

Subsequently, more extensive measurements were undertaken during the intervals of 6–13 July and 9–15 August 2012. In July, some of the shoots of trees from Alberta were starting to set buds and some from Utah had stopped elongating, while all from Arizona were still elongating. In August, bud set in trees from Alberta and Utah was advancing while most shoots from Arizona continued to elongate. These differences in phenology, particularly in August, might have resulted in some change in the relative outcomes among the populations for some of the traits (McKown et al. 2013). For the three populations, one apparently healthy and typical clonal sapling was selected for each of the 20 genotypes and a single leaf, typically, from the eighth to tenth position from the base of the tallest stem, was measured. In all populations these leaves were approximately the same age, as there was little difference in the time of bud flush.

Leaf characteristics

For each genotype, the leaf was first sampled for gas exchange (as explained below) and chlorophyll content index (CCI), a measure of relative chlorophyll content calculated from absorbance at 653 and 931 nm, which was measured with a CCM-200 meter (Opti-Sciences, Hudson, NH, USA). Leaves were then harvested and

placed in a cooler before morphological and biochemical measurements. They were scanned at 300 dpi with 16-bit grey scale resolution and analysed with WinFOLIA (Regent Instruments Inc., Québec, QC, Canada) to determine leaf length, maximum width and size. In leaves harvested in July, a 0.7 cm² disk was removed from approximately the same position as CCI was measured, for pigment extraction as indicated below. The leaves were then oven-dried at 80 °C for 48 h and weighed, and leaf mass per area (LMA) was calculated with adjustments for leaf disk removals.

For verification and calibration of the CCI measurements, leaf pigments were extracted with dimethyl sulphoxide (Hiscox and Israelstam 1979). The fresh leaf disk was dark-incubated at 65 °C for 1 h, until it was colourless and the solution was green. Absorbance was then measured at 649 nm (A_{649}), 665 nm and 480 nm relative to a dimethyl sulphoxide blank, using a Spectronic 2000 UV-VIS Spectrophotometer (Bausch & Lomb, Rochester, NY, USA). Chlorophyll a (Chl$_a$, μg ml^{-1}), chlorophyll b (Chl$_b$) and total carotenoid (C_{x+c}) contents were calculated as: Chl$_a$ = 12.19A_{665} − 3.45A_{649}; Chl$_b$ = 21.99A_{649} − 5.32A_{665} and C_{x+c} = (1000A_{480} − 2.14Chl$_a$ − 70.16Chl$_b$)/220 (Wellburn 1994), and then expressed as μg cm^{-2} of leaf surface area.

Separate disks from the same dried leaves harvested in July were observed for stomatal densities on adaxial and abaxial surfaces under a scanning electron microscope (TM-1000, Hitachi High-Technologies Europe, Krefeld, Germany) at 300× magnification. Images were taken at three random locations on both surfaces and analysed with ImageJ 1.20 (National Institutes of Health; http://rsb.info.nih.gov/ij). Stomata were counted, and adaxial and abaxial density, and their ratio, were calculated. These densities in dried leaves would have been ~10% higher than in fresh leaves (S. Woodman and D. Pearce, unpublished data).

Dried leaves from the July sampling were ground to a powder using a ball mill and subsamples of ~4.5 mg were sent to the Stable Isotope Facility at the University of California, Davis, for combustion and elemental analysis. Carbon and nitrogen contents were analysed by an online continuous flow analyser coupled to an isotope ratio mass spectrometer (Europa Scientific Integra, Cheshire, UK). The stable carbon isotope composition (δ^{13}C) of the leaf tissue was calculated as

$$\delta^{13}C\ (‰) = \left(\frac{R_{sample}}{R_{standard}} - 1\right) \times 1000,$$

where R_{sample} and $R_{standard}$ are the molar ratios of ^{13}C/^{12}C in the sample and the standard (Vienna Pee Dee Belemnite), respectively (Lambers et al. 2008). Leaf N content was expressed in terms of leaf mass (N_{mass}, mg N g^{-1} dry mass) or leaf area (N_{area}, g N m^{-2}). For the latter, the leaf area of the sample was estimated from the mass of the sample and the LMA of the leaf.

Gas exchange measurements

A portable LI-6400XT system (LI-COR Instruments, Lincoln, NE, USA) was used for gas exchange measurements. The CO_2 concentration of the inlet air was set to 400 μl l^{-1}, leaf temperature was 26 ± 1 °C and vapour pressure deficit (VPD) was maintained at 2.0 ± 0.1 kPa, except for the preliminary measurements. Leaves were illuminated at 1500 μmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD) to establish a steady-state condition for ~10–15 min prior to each measurement, using a mixed red/blue light emitting diode unit mounted on top of the cuvette. Light-saturated net assimilation rate (A_{sat}, μmol CO_2 m^{-2} s^{-1}), stomatal conductance (g_s, mol H_2O m^{-2} s^{-1}) and transpiration (E, mmol H_2O m^{-2} s^{-1}) were the primary measurements, and because the VPD was held constant, g_s and E were tightly associated. For some analyses the area-based measurement of A_{sat} was also expressed on a mass basis by dividing by LMA, and in those cases is referred to as $A_{sat/mass}$.

The instantaneous intrinsic water-use efficiency (WUE$_i$, μmol CO_2 mol H_2O^{-1}) was calculated as A_{sat}/g_s. For comparison, the foliar carbon isotope composition provided an indication of longer-term WUE (Farquhar and Richards 1984, Farquhar et al. 1989).

Five genotypes from each of the three populations were randomly selected for measurements of light-response curves in August. Rates of net assimilation were measured on the same leaf selected for other measurements, using the LI-6400XT and light intensities of 1800, 1500, 1200, 1000, 800, 600, 400, 300, 200, 100, 50 and then 0 μmol photons m^{-2} s^{-1} PPFD. At each intensity PPFD was held constant until equilibration. Inlet CO_2 was set to 390 μl l^{-1}, leaf temperature at 26 ± 1 °C and VPD at 2.0 ± 0.1 kPa. The flow rate was constant at 500 μmol s^{-1}. From the light-response curves, the maximum CO_2 assimilation point (A_{sat}), the dark respiration rate (R_d), curvature factor (Θ) and the apparent quantum yield (ϕ) were obtained for each individual by fitting the collected data to the nonrectangular hyperbola model (Lambers et al. 2008) using SYSTAT version 10.2 (SYSTAT Software Inc., Chicago, IL, USA) and the following start, minimum and maximum values: A_{sat} (μmol m^{-2} s^{-1}) 25, 1, 60; R_d (μmol m^{-2} s^{-1}) 2, 0.1, 6; Θ 0.8, 0.1, 0.99; and ϕ (mol CO_2 mol^{-1} quanta) 0.05, 0.001, 0.07.

Climate

Estimates of most of the climatic variables for the provenances of the three populations, for the 1971–2000 normal period, were obtained from ClimateWNA (Wang et al. 2012). Values for each genotype, based on its geographic coordinates and elevation, were used to generate an average for each population. Estimates of solar radiation and vapour pressure for years 1981–2010 were obtained from Daymet (Thornton et al. 2012).

Statistics

SPSS (PASW Statistics 18; IBM Corp., Somers, NY, USA) was used for statistical analyses. Generally, univariate analysis of variance (ANOVA) was carried out to compare the individual foliar morphological, biochemical and gas exchange traits among the three populations. Differences among populations were assessed

using post hoc Tukey's HSD tests. Some foliar and gas exchange measures were made in both July and August, and the effects of both population and month were tested in repeated-measures ANOVA. These latter analyses involved slightly reduced sample sizes, because some of the genotypes sampled in July were not suitable for repeated measurement in August. For bivariate associations across the foliar morphological, biochemical and gas exchange traits, Pearson product–moment correlations (r) and coefficients of determination (r^2) were calculated. Analysis of covariance (ANCOVA) was used to test for homogeneity of regression slopes among populations in the comparison of total chlorophyll with CCI.

We used ordination with nonmetric multidimensional scaling (NMDS, Kruskal 1964) to provide an integrative multivariate assessment across the three populations and to further consider the correspondences across the morphological and ecophysiological measures. The results from measurements made in July were used, as those included most physiological parameters as well as stomatal densities. The final data matrix included 49 genotypes (after those with missing values were excluded) and a suite of nine morphological and 11 ecophysiological parameters. A Euclidean distance-based dissimilarity matrix was created, and analysed by NMDS (PC-ORD Version 6, MjM Software Design). A three-dimensional solution was obtained with low stress, and we show the relationship between ordination scores on the two major axes as the population means of scores for genotypes within each of the three populations. We also analysed the transpose matrix by NMDS to show the relationships among the morphological and physiological parameters for the data set.

Results

Climate and physical conditions of source locations

The provenances of the three populations ranged from 34 to 50°N, were of similar longitude (109–113°W) and had mean elevations ranging from 860 m (north) to 2000 m (south) (Table 1). All of the source locations were riparian. The mean annual temperature was higher in the south, and there was little difference in precipitation (Table 1). The climate in Utah (central) was intermediate in some respects but not in others, as reflected in the annual values and also in the growth season temperatures and other climate variables.

For the May–September growth season, the climate at the origin of the northern population was slightly cooler, with lower growing degree days >5 °C (DD5), than at the origin of the southern population (Table 1). The nominal length of the growth season, measured as the number of frost-free days (NFFD) or the frost-free period (FFP), was longer in the north, because of an earlier start. The mean growth season climate at the origin of the central, Utah population, which spanned 960 m of elevation, did not fall between these temperature and season-length

Table 1. Geography and climate of the provenances of *P. angustifolia*, arranged in order of increasing latitude. Southern: Blue River, AZ; central: Weber River, UT; northern: Oldman River, AB. MAT, mean annual temperature; MAP, mean annual precipitation; TD, temperature difference between the warmest and coldest month (continentality); DD > 5, degree days > 5 °C; E_{ref}, Hargreaves reference evapotranspiration; CMD, Hargreaves cumulative moisture deficit (E_{ref} minus precipitation); T_{max}, average monthly maximum temperature; T_{ave}, average monthly temperature; PPT, precipitation; NFFD, number of frost-free days; FFP, frost-free period; vp, daily average partial pressure of water vapour; dayl, daily average daylength; srad, incident shortwave radiation flux density, taken as an average over the daylight period of the day; insol, daily total radiation MJ m^{-2} day^{-1} (srad (W m^{-2}) × dayl (s day^{-1})/1,000,000).

Provenance	Lat (°N)	Long (°W)	Elev (m a.s.l.)	Annual climate							Growth season climate (May–September)									
				MAT (°C)	MAP (mm)	TD (°C)	DD > 5 (°day)	E_{ref} (mm)	CMD (mm)	NFFD	FFP (days)	T_{max} (°C)	T_{ave} (°C)	DD > 5 (°day)	PPT (mm)	E_{ref} (mm)	CMD (mm)	vp (kPa)	dayl (h)	srad (W m^{-2})	insol (MJ m^{-2} d^{-1})
Southern	33.7	109.1	2003	9	481	18.7	2040	1287	806	157	105	26.1	15.9	1679	255	810	555	689	13.5	477	23.2
Central	41.0	111.6	1682	8.2	586	24.5	2144	976	595	188	131	26.2	17.0	1848	188	742	555	785	13.9	451	22.7
Northern	49.7	113.0	859	5.4	402	26.6	1698	676	370	173	123	22.4	14.9	1523	252	568	317	842	14.7	371	19.8

extremes; it was as warm or warmer, and the season was as long or longer, than in the north or south.

In association with the 16° latitudinal difference, the north–south gradient in sunlight resulted in longer days in the north, but with lower daily insolation (Table 1). These differences in both sunlight and temperature are reflected in the Hargreaves reference evaporation (E_{ref}), a measure of potential evapotranspiration, which increased from north to south. Growth season precipitation was low at all locations, and consequently the Hargreaves climatic moisture deficit (CMD) was lowest in the north.

Foliar morphology

Leaves of *P. angustifolia* are typically narrow, but those from the northern population were slightly wider and heavier than those from the more southern populations (see Figure S1, Table S2 available as Supplementary Data at *Tree Physiology* Online). Leaf mass per area was highest for the northern population and lowest for the southern population (Figure 2, Table S2 available as Supplementary Data at *Tree Physiology* Online). This pattern of differences was generally similar in both July and August. Both mass and LMA were higher in August than in July.

The total stomatal densities ranged from 240 to 290 per mm², and were higher in the southern than the central population (Figure 3). The majority of the stomata were on the abaxial leaf surfaces. The northern population had higher adaxial densities and lower abaxial densities than the southern population, and there was greatest asymmetry in the southern population.

Foliar biochemistry

The photosynthetic pigments chlorophyll *a* (Chl$_a$), carotenoids, and total chlorophyll (Chl$_a$ plus Chl$_b$), measured in leaves
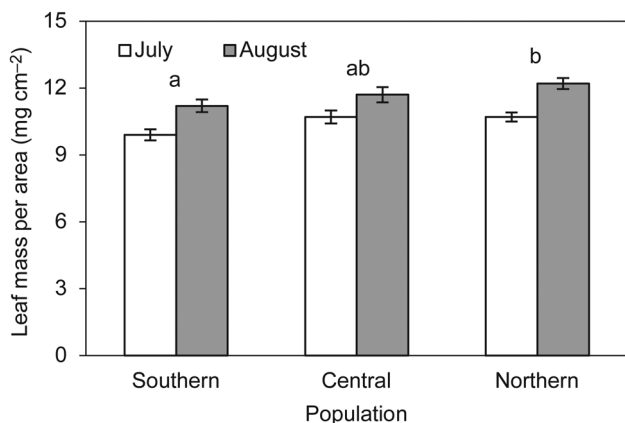

Figure 2. Comparison of LMA in northern, central and southern populations of *P. angustifolia*, grown in a common garden at Lethbridge, AB, and measured in July and August. Means ± SE, $n = 19$–20 for each population. Different letters indicate significant differences among populations in repeated-measures ANOVA (Tukey's HSD, $P < 0.05$; Table S2 available as Supplementary Data at *Tree Physiology* Online). In August, LMA was higher than in July ($P < 0.001$). Significant differences among populations within each of July and August are given in Table S1 available as Supplementary Data at *Tree Physiology* Online.
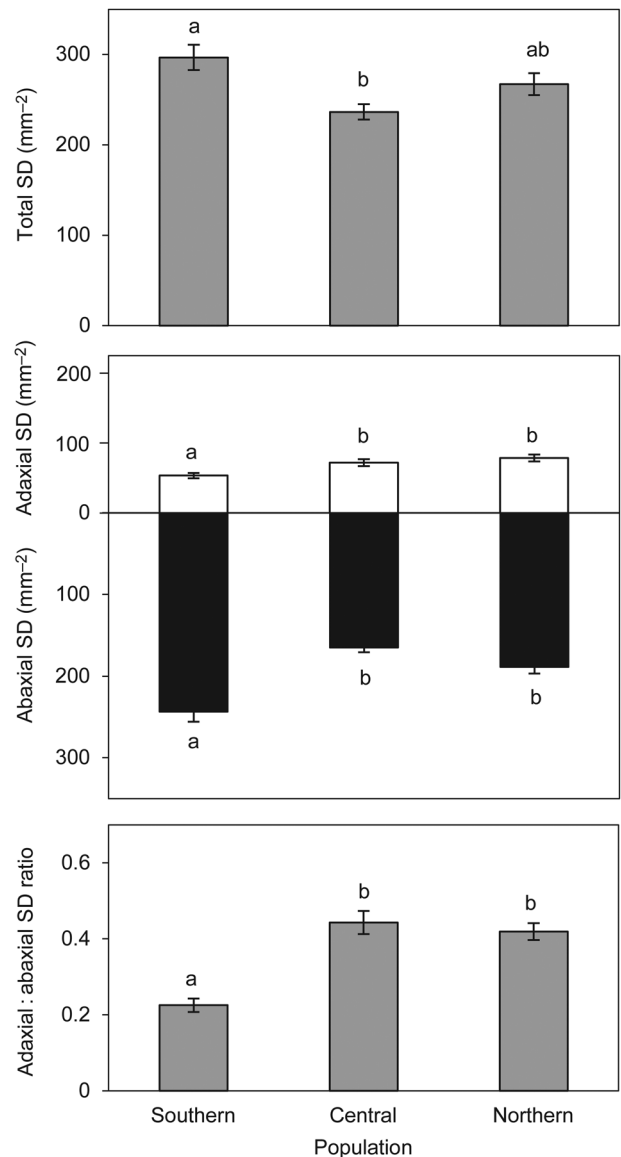

Figure 3. Stomatal density and distribution in leaves of *P. angustifolia* from northern, central and southern populations. Top, total stomatal density (SD); centre, adaxial and abaxial SD; bottom, adaxial : abaxial SD ratio. Means ± SE, $n = 20$. For individual traits different letters indicate significant differences (Tukey's HSD, $P < 0.05$).

harvested in July, were highest for the northern population and similar in the central and southern populations (Table 2). These measurements of extracted total chlorophyll were well-correlated with the CCI determined from the in situ absorbance measurements with the CCM-200 (Figure 4). The CCI was also measured in August, and across both months was higher in the northern population than in the other populations (Figure 5). From the analysis of leaves harvested in July, N_{area} (Figure 5) and N_{mass} (data not shown) were higher for the northern than the central population and the pattern across the populations was similar to that for the extracted chlorophylls from the same leaves.

Table 2. Chlorophyll a, chlorophyll b and carotenoid contents ($\mu g\ cm^{-2}$) of leaves of *P. angustifolia* from southern, central and northern populations. Means ± SE, n = 18–20. Within columns, means followed by different letters are significantly different (Tukey's HSD, $P < 0.05$).

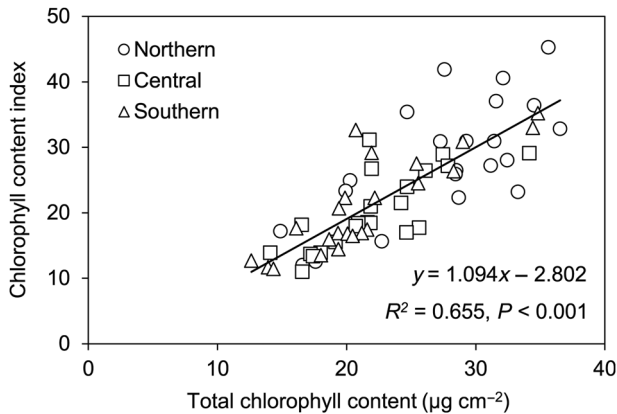| Population	Pigment				
	Chl_a	Chl_b	Total Chl	Carotenoids	Chl_a/Chl_b
Southern	16.2 ± 0.9^a	4.67 ± 0.32	20.9 ± 1.1^a	4.65 ± 0.21^a	3.46 ± 0.19
Central	16.7 ± 0.7^a	4.85 ± 0.28	21.5 ± 0.9^a	4.67 ± 0.17^a	3.58 ± 0.20
Northern	21.2 ± 1.2^b	5.72 ± 0.35	26.9 ± 1.4^b	5.59 ± 0.29^b	3.46 ± 0.10

Figure 4. Linear regression of CCI, measured with the CCM-200, against the total extractable chlorophyll content of leaves (n = 60) of three populations of *P. angustifolia* grown at Lethbridge, AB. The regression slopes for individual populations did not differ (ANCOVA, population × Chl, $P = 0.83$).

Gas exchange

The general pattern for foliar chlorophyll and N among populations was matched by that for the light-saturated photosynthetic rate (A_{sat}), which was higher in the northern population than the central population (Figure 5). Population rankings for A_{sat} were the same for July and August. The light-response study (which involved extended measurements on a subset of those genotypes sampled in August), showed that dark respiration and the apparent quantum yield covaried with A_{sat} ($r^2 > 0.52$, $P < 0.01$), and the curvature tended to decrease from south to north (see Figure S2 available as Supplementary Data at *Tree Physiology* Online), but there were no significant differences among populations for any variable.

Stomatal conductance (g_s) differed across the populations and displayed a similar pattern to A_{sat}, being higher in the genotypes from the northern population (Figure 5); and because the VPD was held constant in the sample cuvette, g_s directly reflected the transpiration rate. Stomatal conductance also varied seasonally, being higher in July than August for all populations (Figure 5). Water-use efficiency (WUE$_i$, A_{sat}/g_s) did not differ across the populations but was higher in August than in July, largely due to lower g_s in August (Figure 6). Consistent with this similarity in WUE$_i$, there was no significant difference in the foliar $\delta^{13}C$ across the three populations (Figure 6).

Correspondence between foliar characteristics and gas exchange

While our study emphasized the comparisons across the three populations, analyses of the individual genotypes also enabled the investigation of correspondences between the foliar and ecophysiological traits across those 60 genotypes. These are shown for leaves measured in July with data from all three populations (Table 3) and in most cases the strongest correlations also held among genotypes within individual populations. A_{sat} was strongly associated with g_s and also strongly positively associated with the CCI and N_{area} or N_{mass} (Table 3, Figure S3 available as Supplementary Data at *Tree Physiology* Online). These correlations are notable since the gas exchange measurements are instantaneous while the other foliar traits have a longer-term basis. Across the genotypes, the CCI and N_{area} or N_{mass} were strongly correlated (Table 3), and the CCI and N were also correlated with $\delta^{13}C$, which was weakly correlated with WUE$_i$. The leaf structure variable LMA was weakly correlated with CCI, N_{area}, A_{sat} and g_s in July, but was not correlated with $A_{sat/mass}$ or N_{mass} (Table 3). We also tested the association of total extracted chlorophyll with all other variables, and correlations were substantially similar to those involving CCI.

For leaves harvested in August (for which there were no accompanying measurements of $\delta^{13}C$ or N), A_{sat} and g_s were strongly correlated, as in July, and A_{sat} was correlated with leaf size. Other correlations, however, were weaker than those found in July. A_{sat} was not correlated with CCI, and was only weakly correlated with leaf size. LMA was not correlated with CCI, or A_{sat} (data not shown).

Total stomatal density (over all genotypes) was negatively correlated with leaf area (Table 3). Notably, density was not correlated with conductance, either among genotypes from all populations or within individual populations. Abaxial density, but not adaxial density, was negatively correlated with leaf area; the adaxial to abaxial ratio was not significantly correlated with any other variable.

Ordination analysis

The multivariate analysis completed using NMDS shows that the populations—northern, central and southern—differed significantly from each other when all traits were analysed together in ordination space (Figure 7). To aid interpretation the ordination
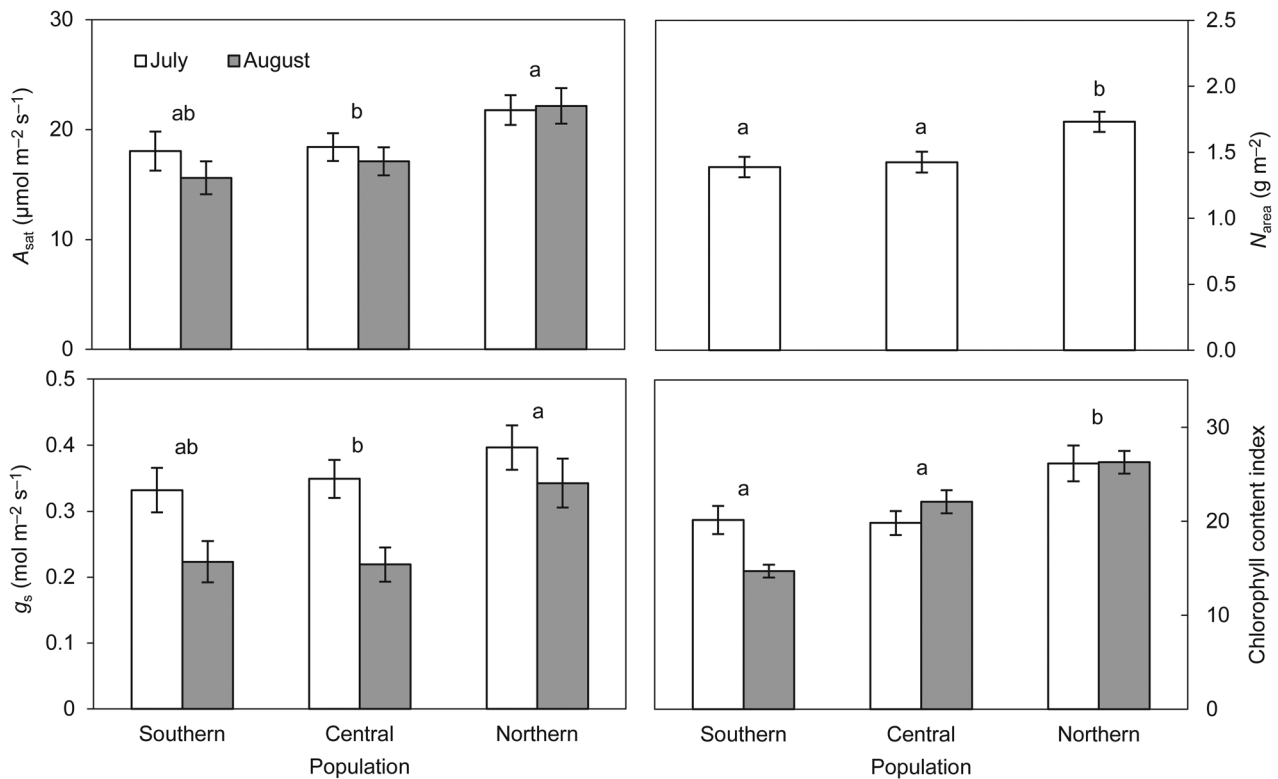
Figure 5. Comparison of light-saturated photosynthesis (A_{sat}), stomatal conductance (g_s), CCI and N content (N_{area}) in leaves of *P. angustifolia* from northern, central and southern populations measured in July and August. Means ± SE, $n = 16–20$. Different letters indicate significant differences among populations in repeated-measures ANOVA (Tukey's HSD, $P < 0.05$; Table S2 available as Supplementary Data at *Tree Physiology* Online). In July, g_s was higher than in August ($P < 0.001$). There was also a seasonal interaction in which the southern population had lower CCI than the central population in August, but not in July (see Table S1 available as Supplementary Data at *Tree Physiology* Online).

diagram has been rigidly rotated to align axis one with leaf N. This axis represents 67% of the variance in the original distance matrix and is strongly positively correlated with all measures of leaf size, and photosynthesis and the related parameters chlorophyll and N (Table 4). Axis two represents 16% of the variance and is strongly positively correlated with adaxial stomatal density and the adaxial : abaxial stomatal ratio. The third axis (not shown) represented 12% of the variance and is most strongly correlated with WUE$_i$.

We also used NMDS to provide an overview of the relationship among all of the morphological and physiological parameters in the genotypes of the three populations. Proximity of points in the ordination pattern (Figure 7) indicates closeness of association, as seen for example in the grouping of A_{sat} with CCI, chlorophyll and N; LMA is somewhat removed from this group.

Discussion

This study involved 60 clonally propagated genotypes of narrowleaf cottonwoods that originated from three populations representing the latitudinal range of the species, grown in a common garden near the northern limit of the range. We measured foliar morphology and gas exchange, and established: (i) collective differences among the populations in leaf morphology

and gas exchange physiology, consistent with the genetic discrimination of populations (Evans et al. 2015); (ii) differences in photosynthetic physiology and leaf morphology among populations consistent with compensating adaptation to differences in climate associated with latitude (and elevation), and in particular, higher A_{sat} in the northern population, which originated from the 'shortest season', and lower g_s in the more southern populations, from the warmer environments; and (iii) links between A_{sat} and physiological (g_s, N and chlorophyll) and morphological (LMA and stomatal density) characteristics, among genotypes and populations, generally consistent with results from similar studies with other *Populus* species, which illustrate leaf economics among populations within this species. These combinations of traits may offer opportunities for selection of appropriately adapted genotypes of *P. angustifolia* for afforestation or conservation.

We found significant differences among our populations for many but not all of the individual traits, and the populations could be discriminated based on the collective results for all traits (Figure 7), generally consistent with the analysis of genetic variation by Evans et al. (2015). On the basis of neutral simple sequence repeats and sequenced neutral loci or potentially adaptive loci, Evans et al. (2015) distinguished three regional populations (northern, central and southern), and substructure

within those groups that corresponded with populations from separate river valleys. The northern regional population included genotypes from both Alberta and the Weber River in Utah (our 'northern' and 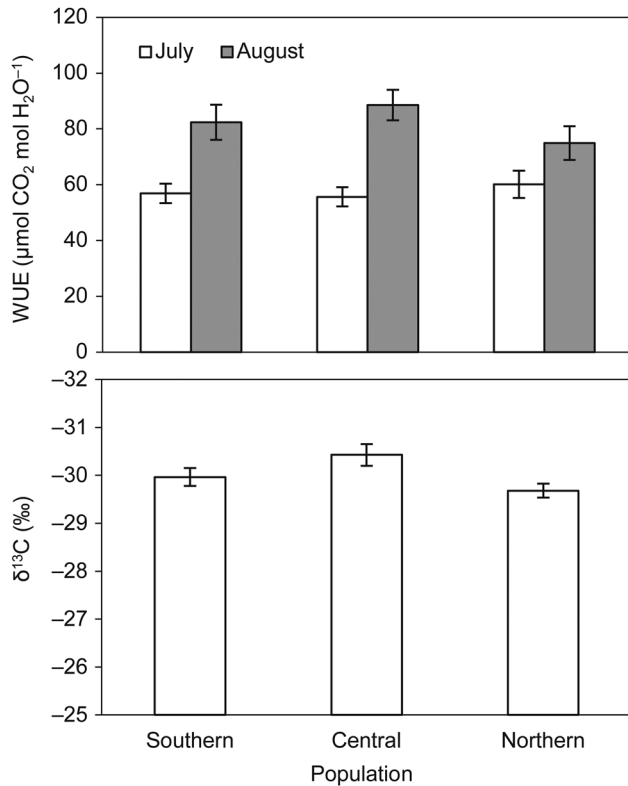'central' populations, respectively), suggesting that they were more closely related than might be inferred from their geographic separation. While we found similarities in some traits between these two populations, our analysis suggested
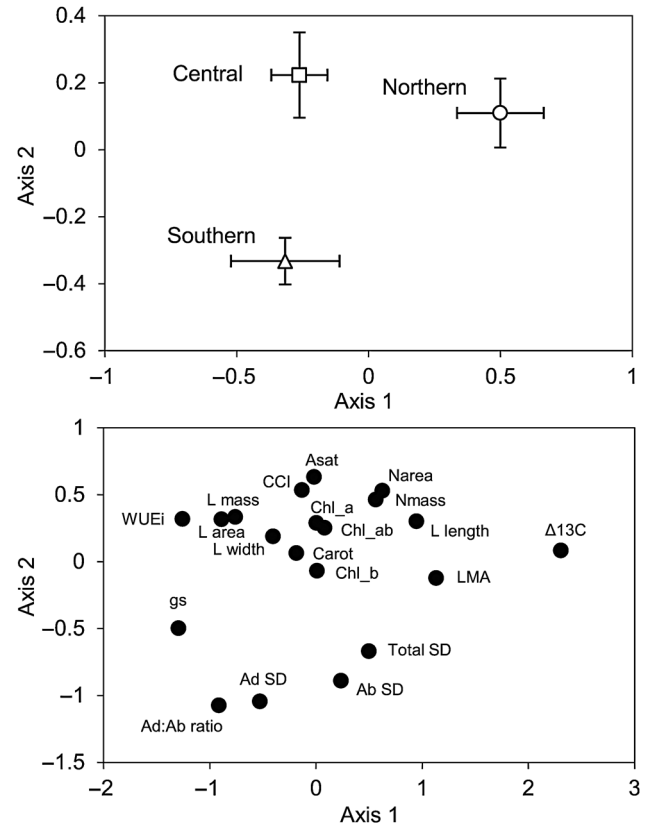

Figure 6. Water-use efficiency (WUE$_i$, A_{sat}/g_s), measured in both July and August, and leaf carbon isotope composition ($\delta^{13}C$), measured in July, for *P. angustifolia* from northern, central and southern populations, Means ± SE, $n = 16–20$. There were no significant differences (Tukey's HSD, $P > 0.05$) among populations for either variable ($\delta^{13}C$, ANOVA; WUE$_i$, repeated-measures ANOVA, Table S2 available as Supplementary Data at *Tree Physiology* Online). WUE$_i$ was higher in August than in July ($P < 0.001$).

Figure 7. Nonmetric multidimensional scaling ordination plots. (Top) Population means (±SE) of ordination scores for genotypes, defined by leaf morphology and physiological characteristics. The two major axes from the ordination are shown, and coefficients of determination, reflecting correlations between the ordination scores on each axis and the various traits, are shown in Table 4. (Bottom) Ordination of the variables, defined by measurements across all genotypes, showing their relatedness.

Table 3. Pearson product–moment correlations between pairs of leaf trait means for genotypes from three populations of *P. angustifolia* grown in a common garden at Lethbridge, AB, and measured in July. $^tP < 0.1$; $*P < 0.05$, $**P < 0.01$; $n = 49–59$.

	LMA	SD	CCI	N_{mass}	N_{area}	$\delta^{13}C$	A_{sat}	g_s	WUE$_i$
Morphology									
Leaf size	0.064	−0.469**	0.636**	0.608**	0.640**	0.309*	0.463**	0.181	0.302*
LMA		−0.029	0.251^t	−0.191	0.227^t	0.045	0.319*	0.268^t	−0.015
Stomatal density (SD)			−0.272^t	−0.286*	−0.277^t	−0.070	−0.110	0.132	−0.342*
Biochemistry									
CCI				0.733**	0.825**	0.366**	0.694**	0.286*	0.375**
N_{mass}					0.894**	0.556**	0.510**	0.058	0.454**
N_{area}						0.556**	0.600**	0.144	0.435**
Carbon isotope composition ($\delta^{13}C$)							0.160	−0.012	0.304*
Gas exchange									
Light-saturated photosynthesis (A_{sat})								0.722**	−0.063
Stomatal conductance (g_s)									−0.550**

Table 4. Coefficients of determination (r^2) for correlations of leaf morphological and physiological variables with ordination axes in Figure 7 (top). Values ≥ 0.5 are emphasized in bold font.

	Axis 1	Axis 2	Axis 3
Leaf mass	**0.637**	0.071	0.094
Leaf area	**0.61**	0.135	0.12
Leaf length	**0.498**	0.046	0.131
Leaf width	**0.589**	0.098	0.094
LMA	0.036	0.099	0.032
Abaxial stomatal density	0.066	0.121	0.116
Adaxial stomatal density	0	**0.668**	0.187
Total stomatal density	0.058	0.002	0.246
Adaxial : abaxial SD ratio	0.008	**0.762**	0.036
CCI	**0.775**	0.013	0.267
Chl$_a$	**0.707**	0.043	0.407
Chl$_b$	0.426	0.138	0.108
Chl$_{ab}$	**0.725**	0.07	0.363
Carotenoids	**0.576**	0.025	0.327
A_{sat}	**0.719**	0	0
N_{area}	**0.699**	0.021	0.351
N_{mass}	**0.548**	0	0.391
g_s	0.205	0.018	0.342
WUE$_i$	0.078	0.05	**0.628**
$\Delta^{13}C$	0.194	0	0.169

that overall they were distinct, and also differed from the southern population (Figure 7).

Of the individual traits, the northern population notably had higher chlorophyll, leaf N, g_s and LMA, than one or other of the more southern populations, and these were associated with higher photosynthesis (A_{sat}). Such correspondence of A_{sat} with latitude is fairly consistent with results from prior common environment studies with the two other *Populus* species in section *Tacamahaca* (the black cottonwood, *P. trichocarpa*, and balsam poplar, *P. balsamifera*; Gornall and Guy 2007, Soolanayakanahally et al. 2009, McKown et al. 2014a). While the use of just one common garden to investigate adaptation in populations from a wide range of climatic origins is typical in studies such as these, the outcomes may be somewhat dependent on the effects of plasticity of some traits in response to the local environment, and a number of ecophysiological traits may show relatively low heritability (e.g., McKown et al. 2014a). Nonetheless, we do note that despite a reversal of the north-to-south latitudinal transfer used in those other studies, we obtained generally similar outcomes, and conclude that these represent evidence of possible adaptation to differing environmental conditions through the native range of the populations. For *P. angustifolia*, the overall climate through the range is continental with differences among provenances reflecting the net effects of contrasting gradients in latitude and elevation (Table 1). The growing season was longer in the north, but with lower maximum temperatures, fewer growing degree days and lower insolation than in the south. Lower production might then be expected in the absence of any compensating adaptive adjustment, such as leaf orientation (Dickmann et al. 1990), or

leaf structure and A_{sat} and associated photosynthetic physiology (Gornall and Guy 2007, Soolanayakanahally et al. 2009, McKown et al. 2014a). Such adaptation may be a generalized pattern for *Populus* and also for other deciduous trees (Benowicz et al. 2000) of western North America.

Higher A_{sat} was closely linked with a number of associated physiological and morphological traits, including chlorophyll, N, g_s and LMA, and relationships among these traits may offer useful insight into such economics (Wright et al. 2004) among populations of *P. angustifolia*. There was higher chlorophyll content in leaves of the narrowleaf cottonwoods from the northern location (Table 2). We also established that this extracted chlorophyll was well-correlated with the CCI (Figure 4), consistent with other such comparisons (e.g., van den Berg and Perkins 2004) and showed that CCI was highest in the northern population (Figure 5). Higher chlorophyll would increase the capacity for light capture, and might be reflected in the trend towards higher apparent quantum yield (see Figure S2 available as Supplementary Data at *Tree Physiology* Online; Lambers et al. 2008). Associated thylakoid proteins and also especially increased levels of RuBisCO and other photosynthetic proteins (Evans 1989, Ripullone et al. 2003) would have contributed partly to the higher leaf N_{area} and A_{sat} in the northern population (Figure 5). Such positive correlations between foliar chlorophyll and N and photosynthetic rates have consistently been observed for *Populus* (Soolanayakanahally et al. 2009) and for other plants (Evans 1989, Reich et al. 1994). Higher A_{sat} in the northern population was also associated with higher g_s, which was also correlated with A_{sat} among genotypes across all populations (Figure 5, Table 3). These observations are again similar to others in *Populus* (Gornall and Guy 2007, McKown et al. 2014a) and other plants (Damour et al. 2010). Leaf mass per area was higher for the northern population than the southern population (Figure 2); but differences in LMA with latitude have not been consistently observed in studies involving the other section *Tacamahaca* poplars (Gornall and Guy 2007, Soolanayakanahally et al. 2009, McKown et al. 2014a). Overall, these associations among populations of A_{sat} with N_{area} and LMA in particular, and also with related traits, are consistent with the comparatively strong relationship between these variables across a much broader range of values in a global spectrum of species (Wright et al. 2004).

Stomatal densities and distributions might also be associated with differences in A_{sat}, through effects on g_s (Pearce et al. 2006, Gornall and Guy 2007). Total stomatal densities differed somewhat among populations (Figure 3), but differences in g_s among populations and over all genotypes were not correlated with differences in stomatal densities (Figure 3, Table 3) in conditions that offered a good opportunity to test this possible relationship in the absence of the potentially confounding influence of varying VPD. While total stomatal density did not differ between northern and southern populations, the adaxial stomatal densities were higher, and the abaxial densities were lower, in

the more northern population than in the southern populations (Figure 3). It is possible that this might be accompanied by associated differences in g_s (and consequently differences in A_{sat}) between adaxial and abaxial surfaces. Consistent with this, in black cottonwoods, the adaxial-to-abaxial ratio also increased with latitude (Gornall and Guy 2007); McKown et al. (2014*b*) have suggested these differences in adaxial densities might reflect trade-offs to differences in selection pressures—for carbon gain in the north, and pathogen resistance in the south.

Latitudinal differences in aridity might also result in adaptive differences among the populations in physiological and morphological traits (Wright et al. 2004, Grady et al. 2013). For a riparian species such as *P. angustifolia* this is less likely, but nonetheless alluvial water supply is sometimes limiting in the north (Rood et al. 2013) and might be more often limiting in the south. While there was little difference in precipitation among the provenances, higher temperature, evaporative demand (E_{ref}) and CMD between north and south (Table 1) might also impose some selective pressure for lower g_s (and so lower transpiration rates) and higher WUE in the more southern populations (Grady et al. 2013). Consistent with these expectations, in association with lower A_{sat}, g_s was lower in the southern populations (Figure 5), and this might confer an adaptive benefit for water conservation. However, despite associated differences in A_{sat} that might have corresponded with differences in leaf structure, N and chlorophyll content, there was little difference in A_{sat}/g_s (WUE$_i$) among populations (Figure 6) in July or August. One explanation of these results, consistent with common models of regulation of g_s (Damour et al. 2010), is that stomata were differentially regulated dependent on A_{sat} and water supply, such that A_{sat}/g_s remained relatively constant for all populations, at either of the times measured.

Just as there was no difference among populations in WUE$_i$ (instantaneous A_{sat}/g_s), there was also little difference in leaf δ^{13}C, the longer-term measure of WUE. It should be noted, however, that in our study the majority of values fell within only narrow ranges of δ^{13}C and WUE$_i$, and were comparatively low, consistent with the moderate conditions that prevailed. These outcomes are consistent with those from *P. trichocarpa* (Gornall and Guy 2007, McKown et al. 2014*a*), where neither δ^{13}C nor WUE$_i$ was correlated with latitude, but differ from that in the greenhouse study with *P. balsamifera* (Soolanayakanahally et al. 2009), where both WUE$_i$ and δ^{13}C were positively correlated with latitude. In this latter study, while A_{sat} increased with latitude g_s did not, and it was concluded that high A was supported partly by increased mesophyll conductance.

We also considered features of leaf morphology that might have been related to WUE, including leaf size, stomatal density and stomatal distribution. Given that the northern population originated from somewhat cooler conditions than the other two populations, one might expect larger leaves with lower stomatal densities (Dunlap and Stettler 2001), and a higher adaxial-to-abaxial stomatal ratio (Foster and Smith 1986). While the direction of differences

in leaf size and width among the populations in this study was consistent with expectations, the magnitude of the differences was small (see Figure S1 available as Supplementary Data at *Tree Physiology* Online). Across all populations total stomatal densities were negatively correlated with leaf size (Table 3) as expected but differences in densities among all three populations were not clearly related to provenance aridity (Figure 3, Table 1). Similarly, while the abaxial-to-adaxial stomatal ratio was higher in the northern than the southern population, among all populations there was no clear relationship with climate. Thus, although leaf size and stomatal characteristics likely reflected some degree of acclimation to the common garden, overall we found no compelling evidence of differences in leaf size or stomatal distribution or density that might have been related to adaptation to water use at the origins of the three populations. It seems probable that the mostly favourable soil moisture of the native riparian habitats, together with temperature and aridity conditions that are comparatively similar among the populations, has resulted in little differential selective pressure for leaf dimensions or stomatal density, or WUE, among these populations of *P. angustifolia*.

In summary, we have shown that that the three populations of *P. angustifolia* show collective differences in leaf morphology and photosynthetic physiology, which might be adaptive for differences in growth season length, temperature and insolation. These outcomes suggest possibilities for selection of appropriately adapted genotypes with suites of ecophysiological characteristics for enhanced productivity, or for afforestation or conservation in the face of drier or warmer conditions that will likely be experienced in the future. Such management may help avert potential decline and collapse of communities as seen in the recent past in other riparian *Populus* populations in western North America (Rood et al. 2003).

Supplementary data

Supplementary data for this article are available at *Tree Physiology* Online.

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Conflict of interest

None declared.

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