



Tree Physiology 37, 604–616
doi:10.1093/treephys/tpx004



Research paper

Divergences in hydraulic architecture form an important basis for niche differentiation between diploid and polyploid *Betula* species in NE China

Wei-Wei Zhang^{1,†}, Jia Song^{1,2,†}, Miao Wang¹, Yan-Yan Liu^{1,2}, Na Li¹, Yong-Jiang Zhang³, N. Michele Holbrook^{3,4} and Guang-You Hao^{1,5}

¹CAS Key Laboratory of Forest Ecology and Management, Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang 110016, Liaoning, China; ²University of Chinese Academy of Sciences, Beijing 100049, China; ³Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, MA 02138, USA; ⁴The Arnold Arboretum of Harvard University, Boston, MA 02131, USA; ⁵Corresponding author (haogy@iae.ac.cn)

Received October 15, 2016; accepted January 25, 2017; published online February 13, 2017; handling Editor Guillermo Goldstein

Habitat differentiation between polyploid and diploid plants are frequently observed, with polyploids usually occupying more stressed environments. In woody plants, polyploidization can greatly affect wood characteristics but knowledge of its influences on xylem hydraulics is scarce. The four *Betula* species in NE China, representing two diploids and two polyploids with obvious habitat differentiation, provide an exceptional study system for investigating the impact of polyploidization on environmental adaptation of trees from the point view of xylem hydraulics. To test the hypothesis that changes in hydraulic architecture play an important role in determining their niche differentiation, we measured wood structural traits at both the tissue and pit levels and quantified xylem water transport efficiency and safety in these species. The two polyploids had significantly larger hydraulic weighted mean vessel diameters than the two diploids (45.1 and 45.5 vs 25.9 and 24.5 μm) although the polyploids are occupying more stressed environments. As indicated by more negative water potentials corresponding to 50% loss of stem hydraulic conductivities, the two polyploids exhibited significantly higher resistance to drought-induced embolism than the two diploids (−5.23 and −5.05 vs −3.86 and −3.13 MPa) despite their larger vessel diameters. This seeming discrepancy is reconciled by distinct characteristics favoring greater embolism resistance at the pit level in the two polyploid species. Our results showed clearly that the two polyploid species have remarkably different pit-level anatomical traits favoring greater hydraulic safety than their congeneric diploid species, which have likely contributed to the abundance of polyploid birches in more stressed habitats; however, less porous inter-conduit pits together with a reduced leaf to sapwood area may have compromised their competitiveness under more favorable conditions. Contrasts in hydraulic architecture between diploid and polyploid *Betula* species suggest an important functional basis for their clear habitat differentiation along environmental gradients in Changbai Mountain of NE China.

Keywords: cavitation, embolism, inter-conduit pit, polyploidy, stress tolerance, xylem hydraulics.

Introduction

Polyploidy, a highly ubiquitous and dynamic process, is a major mechanism for plant evolution and diversification (Jiao et al. 2011, Soltis et al. 2014); however, knowledge regarding the impact of polyploidization on plant physiology is scarce (Soltis

et al. 2010). Polyploids typically differ from diploids in a suite of morphological and anatomical traits that may have significant consequences for plant physiological performances, such as tolerance to environmental stresses (Levin 2002, Ramsey 2011).

[†]These authors contributed equally to this study.

Polyploid plants often have larger stomata, higher photosynthetic rates and higher water-use efficiencies in drought-stressed conditions (Beaulieu et al. 2008, Parisod 2012, Jordan et al. 2015). The frequency of polyploids in flowering plants usually increases in stressed environments (Parisod et al. 2010, Moghe and Shiu 2014), indicating greater ability to withstand harsh climate conditions relative to their diploid counterparts (Levin 1983, Manzaneda et al. 2012). Other studies, however, have demonstrated that polyploids have equal physiological tolerance to stresses, such as low temperature and drought, and thus are not necessarily more successful than their diploid relatives in more stressed environments (Dubcovsky and Dvorak 2007, Martin and Husband 2009, 2013, Thompson et al. 2015). Due to the scarcity of physiological studies on polyploid plants and discrepancies between published results, further comparative studies between polyploid plants and their closely associated taxa are needed to identify potential adaptive differences and the underlying physiological mechanisms.

Niche differentiation or differences in spatial distribution between polyploids and their diploid relatives are frequently observed (Levin 1983, Soltis et al. 2010) but may not necessarily indicate differences in adaptation. For example, one explanation for the high incidence of polyploidy at high latitudes, high altitudes and recently glaciated areas is related to the tendency of harsh environmental conditions to induce polyploid formation rather than indicating its adaptive significance in these stressed environments (Otto and Whitton 2000). On the other hand, stronger drought and cold tolerances have been reported in some polyploids compared with their diploid relatives in wild herbaceous and woody species (Li et al. 2009, Van Laere et al. 2011, Hao et al. 2013), which suggests that niche differentiation between diploid and polyploid plants can reflect differences in environmental adaptation. Further, it has been suggested that greater adaptation to harsh or novel environments in polyploids has benefited from their larger genetic buffering capacity (Hollister 2015), which enables them to have higher phenotypic plasticity in morphology, phenology and physiology (Levin 2002, Leitch and Leitch 2008). Although many studies have found higher tolerance to abiotic stresses in polyploids compared with their diploid relatives, the underlying physiological mechanisms remain poorly understood (Soltis et al. 2010). Regardless of the origin of polyploids, i.e., either originated in stressed conditions or expanded into stressed environments and succeeded there owing to their stronger stress tolerances (Otto and Whitton 2000, Madlung 2013), the physiological mechanisms underlying niche differentiation between diploid and polyploid plants warrant further investigation.

Some recent studies have shown that polyploidization in plants significantly affects xylem structure and function, and thus changes in hydraulic architecture related to polyploidy can play an important role in determining the environmental adaptation of

polyploid plants (Maherali et al. 2009, Hao et al. 2013). Polyploidization can cause significant anatomical alterations in stem xylem, such as vessels size and cell wall thickness (Nassar et al. 2008, Maherali et al. 2009), which can potentially result in substantial changes in xylem water transport efficiency and safety against embolism (Tyree and Sperry 1988, Zanne et al. 2010). One study reported that the increase in vessel diameter appeared to be responsible for the increase of stem hydraulic conductivity of polyploid *Chamerion angustifolium* compared with its diploid cytotype (Maherali et al. 2009). In contrast, polyploid *Atriplex canescens* showed lower hydraulic conductivity and greater resistance to drought-induced hydraulic failure compared with the diploid cytotype (Hao et al. 2013). No consensus regarding the influence of polyploidization on stem xylem hydraulic efficiency and safety has been achieved. Notably, polyploidization has been found to cause significant increase in vessel diameters that seemed to be counterintuitive when higher resistance to embolism in polyploids was observed (Hao et al. 2013). Our speculation was that higher resistance in polyploids, despite their larger vessel diameters, involves structural adaptations at the pit level, which is yet to be proved.

Changes in important leaf functional traits due to polyploidization, such as size and thickness, can also greatly influence plant water relations and hydraulics at the leaf level (Markesteijn et al. 2011, Sessa and Givnish 2014). For example, polyploids typically have fewer but larger stomata, resulting in lower transpiration rates (Li et al. 1996). These fundamental physiological differences between diploids and polyploids at the leaf level form another important basis for ecological differentiation from the point view of plant water relations. Notably, leaves can be considered as hydraulic bottlenecks on the whole-plant water transport pathway due to their high resistance to water transport (Sack and Holbrook 2006, Brodribb et al. 2010, Lens et al. 2013, Schenk et al. 2015, Wang et al. 2016). Moreover, leaves have been shown to be more vulnerable to drought-induced hydraulic dysfunction and to exhibit narrower hydraulic safety margins compared with the stem (Hao et al. 2008, Liu et al. 2015). Therefore, investigations on water relations of polyploid plants also need to involve measurements at the leaf level.

To unravel the adaptive significance of polyploid plants, the best approach is to conduct comparative studies between closely related polyploid and diploid taxa. The genus *Betula*, with tree species of different ploidy levels that show obvious niche differentiation, provides an unusual study system for investigating environmental adaptations of woody polyploid species by comparing with congeneric diploid species. In the temperate forest of Changbai Mountain of NE China, *Betula platyphylla* Suk. (2x) and *Betula costata* Trautv. (2x) are two fast-growing species that are widely distributed in habitats of relatively low elevations. In contrast, *Betula ermanii* Cham. (4x) occurs in subalpine habitats or on the ridges on high mountains of NE China, where it usually becomes dense, pure forests of bushes at the

timberline. *Betula dahurica* Pall. (10x) usually occurs on dry mountain slopes, mountain ridges and stone crevices that have relatively low water availability. The goal of the present study was to investigate the differences in leaf and stem hydraulics related to ploidy levels that may underlie the obvious habitat differences of *Betula* species differing in ploidy levels. Specifically, we hypothesized that: (i) the two fast-growing diploid *Betula* species that are dominant in less stressed habitats would have higher xylem hydraulic conductance; (ii) the two polyploid *Betula* species dominant in more stressed environments (drought, lower temperature, winter frost drought, etc.) would have greater resistance to drought-induced xylem embolism than the two congeneric diploid species in both stems and leaves; and (iii) higher embolism resistance in polyploid *Betula* species, despite their larger vessel diameters, would be explained by pit-level adaptations. To test these hypotheses, stem and leaf level hydraulic conductance, resistance to drought-induced hydraulic failure, as well as tissue and pit-level xylem anatomical characteristics were compared among the four *Betula* species differing in ploidy levels in the temperate forests of Changbai Mountain in NE China.

Materials and methods

Study site and plant materials

The study was performed at National Reserve of Changbai Mountain (42°42'N–42°10'N, 127°38'E–127°10'E, altitude 720–2691 m), Jilin Province, NE China. This region has a typical temperate continental monsoon climate with long and cold winters and short and cool summers. The mean annual precipitation is ~700 mm at lower elevations and increases to ~1400 mm at the top of the mountain, and it is strongly influenced by monsoon with ~60–70% of the annual precipitation occurring from June to September. Average annual air temperature falls from 3.5 to –7.4 °C with the increase of altitude from 500 to 2744 m (Liu et al. 2015). The Changbai Mountain Natural Reserve has obvious vertical vegetation zones, including Korean pine and broad-leaf mixed forests at low altitude (720–1100 m), spruce and fir conifer forests at middle altitude (1100–1800 m), Erman's birch (*B. ermanii*) forests in the subalpine zone at high altitude (1800–2100 m) and alpine tundra at elevations above 2100 m (Sun et al. 2006).

Betula platyphylla (2x), *B. costata* (2x), *B. ermanii* (4x) and *B. dahurica* (10x) are four native tree species commonly occur in this region. The two diploid species are fast-growing pioneer species that usually occupy humid low lands. In contrast, *B. ermanii* (4x), a dominant subalpine treeline tree species, is mainly restricted to the high elevations ranging from 1800 m to the upper limit of forest distribution at 2100 m. *Betula dahurica* (10x), a main companion species in Korean pine and broad-leaved mixed forests of the low elevations, is widely distributed in drier soil and exposed rocky slopes. *Betula platyphylla*,

B. costata and *B. dahurica* trees used for the present study are from the mixed forest habitat (~730 m altitude) where they commonly occur. *Betula ermanii* trees normally only occur at much higher elevations and thus samples were taken from Erman's birch forests at 1900–2000 m altitude. For each species, we randomly selected six mature individuals (diameter at breast height ranging from 13 to 20 cm) in their respective habitats. Only sun-exposed branches or leaves were sampled for the measurements. Replicates for stem and leaf trait measurements were sampled from different individuals in each species. To minimize the impact of sampling height on plant functional traits, all the samples were taken at heights 3–5 m above the ground.

Stem hydraulic conductivity

For hydraulic conductivity measurements, six branches ~1.5 m in length with each from a different individual were collected for each species at predawn in August 2014. Branches were re-cut immediately under water (5 cm removed) and wrapped in plastic bags. With the cutting ends submerged in water, branches were transported to the laboratory at the Research Station of Changbai Mountain Forest Ecosystems of the Chinese Academy of Sciences within 1 h.

Stem segments without side branches of 20 cm in length and 0.8–1.0 cm in diameter, cut off from the middle part of the sampled branches under water, were used for hydraulic conductivity measurement. Leaves terminal to each stem segments were collected for area measurements. Stem segments were first flushed with filtered (<0.20 µm pore diameter) and degassed 20 mM KCl solution at 0.1 MPa for 20 min to remove air bubbles from the embolized vessels (Tyree and Sperry 1988). After shaving both ends of the segments with a sharp razor blade, the flushed segments were connected to an apparatus with degassed and filtered 20 mM KCl solution for maximum hydraulic conductivity ($K_{\max}$) determination. A hydrostatic pressure generated by a hydraulic head of 50 cm was used to drive water flow through the stem segments. Stem hydraulic conductivity (K_s , kg m⁻¹ s⁻¹ MPa⁻¹) was defined as the mass flow rate of measure solution through an excised stem segment per unit pressure gradient. Sapwood area (SA) was determined at 1 cm from both ends of a stem segment using the dye staining method with 0.1% toluidine blue solution and the two values were averaged (Sperry et al. 1988).

Leaves distal to the stem segment were scanned with a digital scanner for total leaf area (LA) distal to the segment measurement using ImageJ software (US National Institutes of Health, Bethesda, MD, USA). Sapwood-specific hydraulic conductivity (K_s , kg m⁻¹ s⁻¹ MPa⁻¹) and leaf-specific hydraulic conductivity (K_l , kg m⁻¹ s⁻¹ MPa⁻¹) were calculated as $K_{\max}$ divided by SA and LA, respectively. Leaf to sapwood area ratio (LA/SA) was determined for each segment used for hydraulic conductivity measurement. Leaves were oven-dried at 60 °C for at least 72 h

before determination of dry mass to the nearest 0.001 g. Leaf mass per area (LMA) was calculated as total leaf dry mass divided by LA of a branch.

Stem xylem structural characteristics

To measure wood density (ρ_{wood}), the volume of 3-cm long stem segments sampled from the middle part of the stems for hydraulic conductivity measurements (with bark and pith removed) was measured by the water displacement method. The sapwood was then oven-dried for 72 h at 60 °C to for dry mass determination using a four-digit analytical balance (CPA225D, Sartorius, Inc., Göttingen, Germany). Wood density (g cm^{-3}) was calculated as dry mass divided by fresh volume. Maximum vessel lengths (MVL) in four species were measured by air infiltration method along the entire length of large stems (Ewers and Fisher 1989). The distal end of a long branch (>1.2 m) was immersed in water and forced by air at a pressure of 100 kPa through the basal end. The branch was successively cut back by 1-cm segments from the distal end until air bubbles occurred. The remaining sample length was defined as the MVL (Zimmermann and Jeje 1981). Six branches were measured for each species.

For xylem anatomical measurements, stem cross-sections ~20 μm thick from four segments (0.8–1.0 cm in diameter) per species were made with a sliding microtome (Model 2010–17, Shanghai Medical Instrument Corp., Shanghai, China). Stem cross-sections were stained with 0.1% toluidine blue to increase visual contrasts. Cross-sections were examined with a light microscope with digital camera (Leica ICC50, Wetzlar, Germany) and images were taken under magnifications of $\times 100$ with an inbuilt digital camera for vessel density (VD) and vessel diameter calculation, respectively. For each transverse section, two images were taken under each of the two magnifications in the outer sapwood representing early wood of two most recent growth rings avoiding tension wood. Vessel density (no. mm^{-2}) was calculated as the number of vessels per unit cross-sectional area using the 'analyze particles' function of ImageJ software (US National Institutes of Health). The hydraulic mean diameter (D_H) was calculated for each image as follows:

$$D_H = \left(\sum D^4 / N \right)^{1/4}, \quad (1)$$

where D is the mean vessel diameter and N is the number of vessels in the image (Choat et al. 2007).

Scanning electron microscopy

Fresh stems ~1 cm in diameter were cut into 1.5-cm-long segments and then tangential sections ~25 μm in thickness were made with a sliding microtome (Model 2010–17, Shanghai Medical Instrument Corp.) avoiding tension wood. Section samples were subsequently dehydrated through an ethanol series (30–50–70–90%) for 10 min in each solution, immersed in

100% ethanol overnight and finally air-dried. Samples were fixed to aluminum stubs with electron conductive carbon cement (Neubauer Chemikalien, Münster, Germany) and coated with platinum using a Quorum SC7620 sputter coater (Quorum Technologies Ltd, East Sussex, UK) for 2 min. Samples were observed with a Quanta™250 environmental scanning electron microscope (FEI, Hillsboro, OR, USA) at an accelerating voltage of 20 kV. Pictures of pits were taken under $\times 8000$ and $\times 15,000$ magnifications for pit density calculation and dimension measurements, respectively. Pit parameters were measured using ImageJ software (freeware available from website <https://imagej.nih.gov/ij>). About 90 individual pits from at least 20 different pit fields in 6 segments, each from a different tree, were measured for each birch species. Individual pit-level measurements were averaged for each stem segment analyzed.

Stem vulnerability curves

Stem vulnerability curves were constructed using the air-injection method (Sperry and Saliendra 1994). Stem segments ~20 cm in length, previously flushed to achieve maximum hydraulic conductivity, and were fixed into 3.5-cm-long double-sleeved air-injection chambers. When stem segments used are shorter than MVL, the air-injection method may overestimate the xylem vulnerability to cavitation (Ennajeh et al. 2011). However, we aimed at studying the comparative vulnerability to cavitation among different *Betula* species rather than getting absolute P50 values in the present study. Further, the sample length effects on vulnerability curves generated by the air-injection method have been shown to be relatively minor for *Betula* species with relatively short vessels; P50 differed only ~0.2 MPa between 17.5 and 37.5-cm-long stem segments for *Betula pendula* Roth (Ennajeh et al. 2011). Therefore, we believe that the vessel length-associated artifacts would not significantly influence the comparison between diploid and polyploid *Betula* species in the present study. Bark of each of the stem segments was removed before installing into the air-injection chamber to facilitate air entrance. As the segments were exposed to progressively increased air-injection pressures ranging from 0.5 to 7.0 MPa, K_h values after each injection treatment were measured. Pressure was held constant for 5 min at each pressure level and then allowed 10–30 min for equilibration under atmospheric pressure before determination of K_h . The percent loss of hydraulic conductivity (PLC) after air injection at each pressure level was calculated as follows:

$$\text{PLC} = 100(1 - K_h/K_{\text{max}}), \quad (2)$$

where K_{max} is the maximum hydraulic conductivity measured on flushed segments before any air-injection treatment. The vulnerability curve was plotted as PLC against air-injection pressure for each individual segment and was fitted using a sigmoid function ($y = a/(1 + \exp(-(x - x_0)/b))$).

Leaf vulnerability curves

Leaf hydraulic vulnerability curves were determined by measuring small shoot hydraulic conductance (K_{leaf}) at different water potentials using the partial rehydration method (Brodribb and Holbrook 2003). Branches 0.5–1.0 m long were collected at predawn, sealed into double-black plastic bags with moist paper towels and then transported to the laboratory within an hour. Branches were dehydrated on the bench top for varying lengths of time before they were sealed into double-black plastic bags and then kept in the dark for at least 1 h for equilibration. To get relatively high initial water potentials, some branches were rehydrated. Immediately after initial water potential measurement of a branch on two leaves, another two leaves were cut under water at the same time and allowed to absorb water for a certain period ranging from 20 to 60 s. After absorbing the surface water, rehydrated leaves were sealed into plastic bags with wet paper towels for 10 min before final water potential measurements. Pressure–volume curve measurements were conducted to calculate leaf capacitance and leaf water fraction needed for K_{leaf} calculation (Hao et al. 2008). The K_{leaf} ($\text{mmol m}^{-2} \text{s}^{-1} \text{MPa}^{-1}$) values were plotted against initial water potentials and the data were fitted using an exponential sigmoid model to estimate the pressure at 50% loss of conductivity ($P_{50_{\text{leaf}}}$) (Hao et al. 2013).

Midday leaf water potential

In mid-August 2014, midday leaf water potential (Ψ_{md}) was measured using a ZLZ-4 pressure chamber (Lanzhou University, Lanzhou, China). Measurements were made on two fully developed mature leaves from sun-exposed terminal branches of different individuals for each species at 12:00–14:00 h on sunny days. After excision, samples were immediately sealed in plastic ziplock bags containing moist paper towels and kept in a cooler before transportation to the laboratory. All water potential measurements were determined in the laboratory within 1 h of sample collection. To evaluate the risk of xylem hydraulic dysfunction caused by cavitation, safety margin in stem was calculated as the difference between the midday leaf water potential and xylem water potential corresponding to 50% loss of hydraulic conductivity ($P_{50_{\text{stem}}}$). Safety margin in leaves was quantified as the difference between midday leaf water potential and leaf water potential at 50% loss of K_{leaf} ($P_{50_{\text{leaf}}}$).

Stomatal characteristics

Guard cell length (GCL) and stomatal density (SD) were measured using the nail polish impression method (Maherali et al. 2009). Measurements were made under magnifications of $\times 100$ using a light microscope (Leica ICC50). Two mature leaves on each of six individuals for each species were measured. Epidermal impressions were made by applying a thin pellicle of transparent fingernail polish on the abaxial leaf surface in the mid-area between the central vein and the leaf edge. The

impressions were removed from the leaf after ~ 15 min with clear adhesive tape and glued on a microscope slide. The GCL (μm) was defined as the length between the junctions of the guard cells at each end of the stoma. Stomatal density (mm^{-2}) was calculated by counting the number of stomata in a view with known area. Stomatal pore area index (SPI) was calculated as $\text{SD} \times \text{GCL}^2$ (Sack et al. 2003).

Statistical analysis

Stem and leaf vulnerability curves were fitted with an exponential sigmoid model using Sigmaplot v10.0 (Systat Software, Inc., San Jose, CA, USA). Prior to any statistical analysis, all data were tested for normality and variance constant. Comparisons of parameters among species were performed using one-way ANOVA. Post hoc comparisons were conducted using Tukey's honestly significant difference (HSD) to test for differences between species (SPSS 16.0 software package, SPSS, Inc., Chicago, IL, USA).

Results

Tissue and pit-level xylem anatomical traits

Clear patterns were shown in comparing the wood anatomical traits between the diploid and polyploid birch species at both the tissue and pit levels. Mean hydraulic vessel diameter was significantly larger in the two polyploid species than the two diploid species, i.e., 45.1 and 45.5 μm in *B. ermanii* (4x) and *B. dahurica* (10x) vs 25.9 and 24.5 μm in *B. platyphylla* and *B. costata* (Table 1). Although sampled trees of *B. ermanii* (4x) were growing at a much higher elevation, it still showed much larger vessel diameters than the diploid species, suggesting that differences in genetics rather than growth conditions might be playing a major role in determining these xylem anatomical traits. The xylem of the two polyploid species also included a wider range of vessel diameter than the two diploid species; *B. ermanii* (4x) and *B. dahurica* (10x) had vessels up to 70.1 and 87.3 μm in diameter while no vessels larger than 50 μm in diameter were observed in the diploid birch species (Figure 1). As expected, vessel densities were significantly lower in the polyploid species. *Betula ermanii* (4x) from the subalpine timberline forest ecosystems had significantly smaller MVL than the other three species that are most common at lower elevations (Table 1). All the pit-level anatomical characteristics differed strikingly between the diploid and the polyploid species (Figure 2 and Table 2). The pit membrane and pit aperture sizes were apparently independent of the vessel diameter; significantly larger pit membrane and aperture areas were observed in the two diploid birch species with smaller vessel diameters (Figure 2 and Table 2). The shape of the outer pit aperture was markedly different between the polyploid and diploid birch species as indicated by significantly larger diameter ratio measured following the longest and the shortest axis ($D_{\text{PA ratio}}$; $P < 0.05$, Table 2).

Table 1. Functional traits for *B. platyphylla* (2x), *B. costata* (2x), *B. ermanii* (4x) and *B. dahurica* (10x). P50_{stem}, xylem water potential at 50% loss of stem hydraulic conductivity; P50_{leaf}, leaf water potential at 50% loss of maximum K_{leaf} ; ρ_{wood} , wood density; D_H , hydraulic mean diameter; SPI calculated as $SD \times GCL^2$; Ψ_{md} , midday leaf water potential. Different lower case letters following the values represent significant differences between species ($P < 0.05$, Tukey's HSD post hoc test, one-way ANOVA). Values represent means $\pm$ 1 SE ($n = 6$).

	<i>B. platyphylla</i> (2x)	<i>B. costata</i> (2x)	<i>B. ermanii</i> (4x)	<i>B. dahurica</i> (10x)
P50 _{stem} (MPa)	$-3.86 \pm 0.13b$	$-3.13 \pm 0.16b$	$-5.23 \pm 0.25a$	$-5.05 \pm 0.12a$
P50 _{leaf} (MPa)	-1.70	-1.53	-1.68	-1.20
ρ_{wood} (g cm ⁻³)	$0.46 \pm 0.01ab$	$0.42 \pm 0.01b$	$0.50 \pm 0.02a$	$0.48 \pm 0.01a$
D_H (μm)	$25.9 \pm 0.7b$	$24.5 \pm 0.9b$	$45.1 \pm 0.93a$	$45.5 \pm 0.3a$
VD (no. mm ⁻²)	$254.2 \pm 11.1b$	$313.5 \pm 14.5a$	$165.6 \pm 4.1c$	$174.6 \pm 11.7c$
MVL (cm)	$82.8 \pm 6.2a$	$76.3 \pm 4.1ab$	$60.6 \pm 3.8b$	$80.4 \pm 4.7a$
Leaf size (cm ²)	$31.9 \pm 2.0a$	$26.6 \pm 2.1ab$	$19.5 \pm 2.4b$	$21.5 \pm 3.1b$
Leaf thickness (mm)	$0.16 \pm 0.01b$	$0.24 \pm 0.01a$	$0.21 \pm 0.01a$	$0.24 \pm 0.01a$
LMA (g m ⁻²)	$62.7 \pm 2.5a$	$66.8 \pm 4.4a$	$70.8 \pm 3.9a$	$57.2 \pm 4.8a$
GCL (μm)	$34.3 \pm 1.4a$	$32.1 \pm 1.0a$	$43.2 \pm 0.8b$	$41.9 \pm 1.3b$
SD (no. mm ⁻²)	$172.5 \pm 4.9a$	$148.0 \pm 12.8a$	$166.7 \pm 2.6a$	$157.1 \pm 3.9a$
Ψ_{md} (MPa)	$-1.31 \pm 0.05a$	$-1.45 \pm 0.02a$	$-1.39 \pm 0.07a$	$-1.41 \pm 0.02a$
SPI	$0.25 \pm 0.01a$	$0.19 \pm 0.01a$	$0.38 \pm 0.01b$	$0.34 \pm 0.01b$

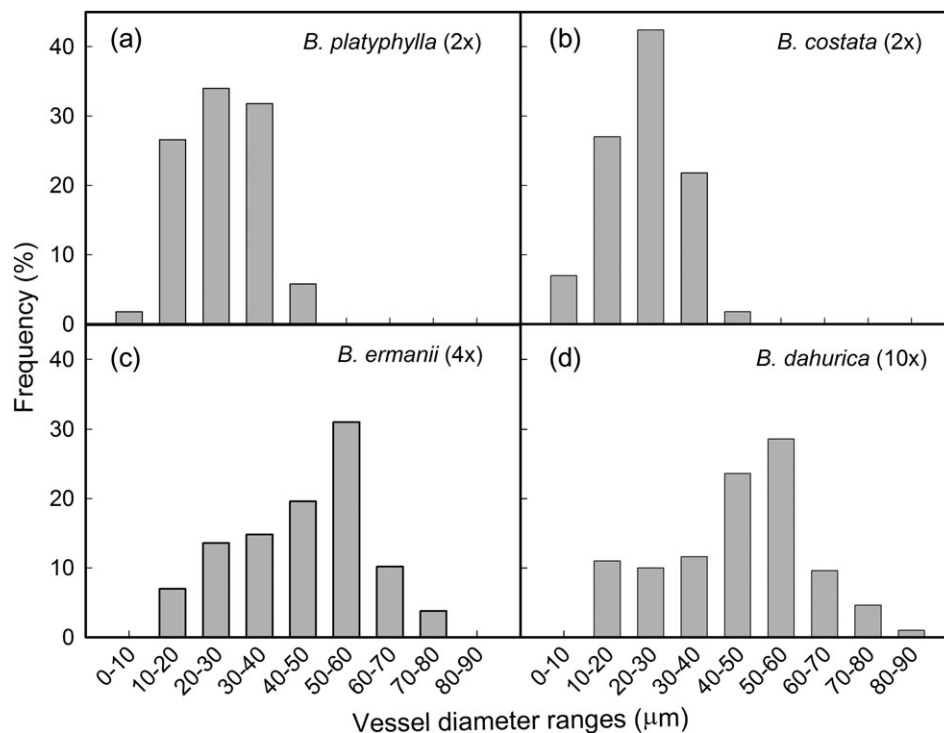


Figure 1. Xylem vessel lumen diameter frequency distribution in stems of (a) *B. platyphylla* (2x), (b) *B. costata* (2x), (c) *B. ermanii* (4x) and (d) *B. dahurica* (10x) showing larger average vessel diameters in the polyploid than the diploid species.

Stem hydraulic efficiency and safety against embolism

When stem hydraulic conductivity was expressed on an SA basis, there was no consistent pattern in comparing the diploid and the polyploid birch species; *B. dahurica* (10x) had substantially higher K_s than any of the other three species, while K_s of *B. ermanii* (4x) showed no significant difference with the two diploid species (Figure 3a). Rather, contrasts in hydraulic conductivity emerged between the polyploid and diploid species when it was expressed on an LA basis, i.e., the K_l values in

B. ermanii (4x) and *B. dahurica* (10x) were ~79% higher than those in the diploid species (Figure 3b). Differences in comparing K_s and K_l between diploid and polyploid species primarily result from the two polyploid species having substantially lower leaf to SA ratios than the diploid species (Figure 3c).

Analyses of the stem vulnerability curves showed that the two polyploid species had significantly lower (more negative) P50_{stem} values than the diploid species (Table 1 and Figure 4), indicating higher resistance to drought-induced embolism in the

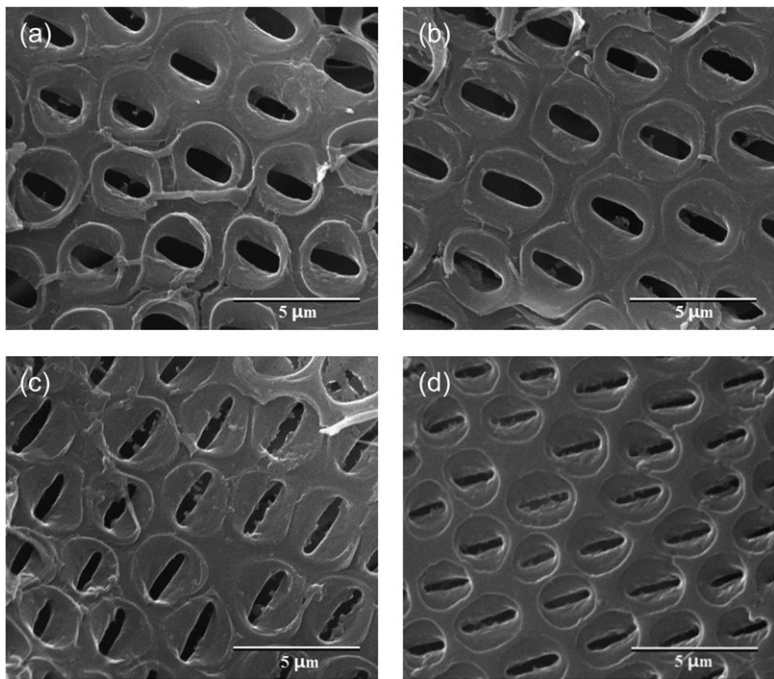


Figure 2. Scanning electron micrographs of intervessel bordered pits viewed on radial walls in stems of (a) *B. platyphylla* (2x), (b) *B. costata* (2x), (c) *B. ermanii* (4x) and (d) *B. dahurica* (10x), showing contrasts in size and shape of pit membrane and aperture between diploid and polyploid species.

Table 2. Pit characteristics of *B. platyphylla* (2x), *B. costata* (2x), *B. ermanii* (4x) and *B. dahurica* (10x) measured from SEM micrographs. N_P , pit density; A_{PIT} , intervessel pit surface area or intervessel pit membrane area; A_{PA} , pit aperture surface area; F_{AP} , aperture fraction, pit aperture area per pit membrane area; D_M , horizontal pit membrane diameter at its widest point; D_{PAL} and D_{PAS} , diameter of the outer pit aperture as measured following the longest and the shortest axis, respectively; $D_{PA\ ratio}$, pit aperture shape, ratio of long axis of outer pit aperture over short axis. Different lower case letters following the values represent significant differences between species ($P < 0.05$, Tukey's HSD post hoc test, one-way ANOVA). Values are means $\pm$ 1 SE ($n = 6$).

	<i>B. platyphylla</i> (2x)	<i>B. costata</i> (2x)	<i>B. ermanii</i> (4x)	<i>B. dahurica</i> (10x)
N_P (no. mm ⁻²)	95.7 $\pm$ 5.5b	85.5 $\pm$ 2.1b	121.4 $\pm$ 4.2a	167.5 $\pm$ 5.5a
A_{PIT} (μ m ²)	8.41 $\pm$ 0.04a	8.94 $\pm$ 0.05a	4.27 $\pm$ 0.03b	4.75 $\pm$ 0.03b
A_{PA} (μ m ²)	1.60 $\pm$ 0.06a	1.57 $\pm$ 0.02a	0.83 $\pm$ 0.02b	0.77 $\pm$ 0.01b
F_{AP}	0.31 $\pm$ 0.00a	0.28 $\pm$ 0.00a	0.41 $\pm$ 0.00b	0.43 $\pm$ 0.00b
D_M (μ m)	3.26 $\pm$ 0.04a	3.37 $\pm$ 0.05a	2.32 $\pm$ 0.01b	2.46 $\pm$ 0.03b
D_{PAL} (μ m)	1.98 $\pm$ 0.02a	2.02 $\pm$ 0.03a	1.93 $\pm$ 0.02a	1.91 $\pm$ 0.03a
D_{PAS} (μ m)	0.97 $\pm$ 0.03a	1.01 $\pm$ 0.04a	0.48 $\pm$ 0.01b	0.50 $\pm$ 0.02b
$D_{PA\ ratio}$	2.05 $\pm$ 0.04b	2.08 $\pm$ 0.07b	4.03 $\pm$ 0.06a	3.91 $\pm$ 0.08a

polyploid birch species despite their larger vessel diameters. No significant differences were found in $P50_{stem}$ either between the two diploid species, i.e., -3.86 MPa in *B. platyphylla* (2x) and -3.53 MPa in *B. costata* (2x), or between the two polyploid species, i.e., -5.23 MPa in *B. ermanii* (4x) and -5.05 MPa in *B. dahurica* (10x). Midday leaf water potentials were around -1.50 MPa for all the four species, which was substantially higher than xylem tension causing 50% loss of hydraulic conductivity in stems (Figure 4).

Leaf functional traits

The two polyploid birch species also exhibited significant differences compared with the diploid species in leaf characteristics. Leaves tended to be smaller in the two polyploid species

(Table 1). The most significant differences were the larger SPI and stomatal sizes in the two polyploid than in the two diploid birch species (Table 1), i.e., larger GCL in *B. ermanii* and *B. dahurica* than in *B. platyphylla* and *B. costata*. However, no significant difference in SD was found among the four birch species. Physiological characteristics calculated from the pressure–volume relations did not show a consistent pattern in comparing the two diploid and two polyploid birch species (Table S1 available as Supplementary Data at [Tree Physiology Online](#)). In contrast to stems, leaves were much more vulnerable to drought-induced loss of hydraulic function in all the four species (Figure 5). Mean $P50_{leaf}$ ranged from -1.70 MPa in *B. platyphylla* (2x) to -1.20 MPa in *B. dahurica* (10x), and did not show a clear pattern between species of different ploidy levels. In

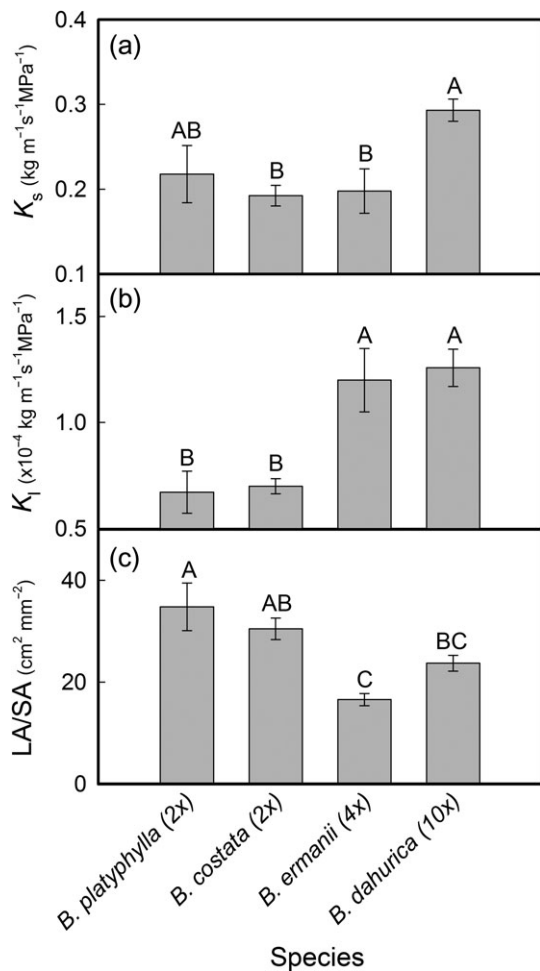


Figure 3. (a) Sapwood-specific hydraulic conductivity (K_s), (b) leaf-specific hydraulic conductivity (K_l) and (c) LA/SA for *B. platyphylla* (2x), *B. costata* (2x), *B. ermanii* (4x) and *B. dahurica* (10x). Values are means $\pm$ 1 SE ($n = 6$). For each graph, different upper case letters represent significant differences between species ($P < 0.05$, Tukey's HSD post hoc test, one-way ANOVA).

B. dahurica, $\Psi_{50\text{leaf}}$ was even less negative than Ψ_{md} , resulting in a negative leaf hydraulic safety margin (Figure 5d).

Discussion

Our data show that the polyploid birch species differ from diploid species of the same genus in water transport efficiency and safety. The two polyploid species have significantly wider vessels in their sapwood but sapwood-specific hydraulic conductivity did not show a consistent trend with increase of ploidy level. Branches of polyploid birch species bear leaves of significantly reduced total area per SA, thus resulting in higher leaf-specific hydraulic conductivity than the two diploid species. The polyploids exhibit significantly higher resistance to drought-induced hydraulic dysfunction and larger safety margins at the stem level than the two diploid species, which are consistent with a suite of pit-level characteristics adapted to greater resistance to embolism propagation between conduits. Our results provide a

potential physiological explanation for the commonly observed habitat differentiation between the diploid and polyploid birch species in the temperate forests of NE China.

Hydraulic efficiency and safety in species of different ploidy levels

Larger vessel diameters in the two polyploid birch species did not result in consistently higher sapwood-specific hydraulic conductivity but were associated with significantly higher resistance to drought-induced hydraulic dysfunction, which seemed to contradict with the trade-off between hydraulic efficiency and safety mediated partially by variation in xylem conduit size (Wheeler et al. 2005, Maherali et al. 2009). Consistent with this study, a previous investigation in comparing different cytotypes of *A. canescens* also found that significantly wider vessels in polyploids was associated with lower sapwood-specific hydraulic conductivity and higher resistance to drought-induced xylem embolism (Hao et al. 2013). It has been found that one of the most immediate biological consequences of polyploidy on plants is the increase in cell sizes, including guard cell, leaf mesophyll cell and xylem conduits (Li et al. 1996, Nassar et al. 2008, Maherali et al. 2009). Therefore, larger vessel diameters in polyploid plants most likely represent the strong positive correlation between chromosome size and overall plant cell size (Lomax et al. 2014, Jordan et al. 2015), rather than indicating an adaptation for a higher water demand.

The apparent decoupling between vessel diameter and sapwood-specific hydraulic conductivity in comparing the diploid and polyploid birch species suggests that hydraulic conductivity of stems cannot simply be predicted from the Hagen–Poiseuille law (Zimmermann 1983); other vessel characteristics besides diameter can also play important roles in determining xylem hydraulic efficiency. For example, vessel length and pit characteristics that vary substantially among species, between individuals of the same species, and even within an individual can become strong determinates of xylem water transport efficiency (Sano 2005, Domec et al. 2010). The tetraploid species *B. ermanii* has significantly shorter MVL compared with other birch species, which is expected to be adaptive in the subalpine habitats where smaller vessel lumen volume strongly contributes to reduced risk of embolism due to freeze–thaw and frost drought stresses (Hacke and Sperry 2001, Lens et al. 2013). More importantly, unlike ideal pipes assumed by the Hagen–Poiseuille law, vessel or tracheid hydraulic conductivity can also be determined, to a large degree, by the porosity of the inter-conduit pits (Lens et al. 2011, Christman et al. 2012, Hacke 2014). Although wider vessels are more likely to contain larger maximum pit membrane pores (Wheeler et al. 2005), our comparisons in xylem anatomy between the diploid and polyploid *Betula* species show clearly that vessel diameter and pit structural characteristics can be independent of each other, i.e., larger vessel diameters could even be associated with smaller and less porous

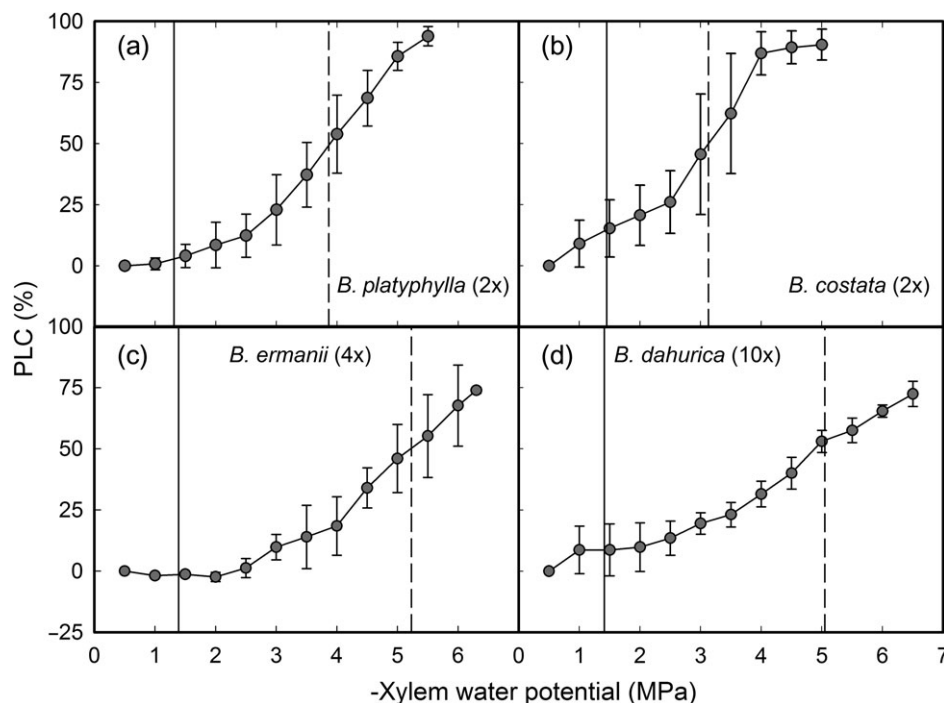


Figure 4. Percent loss of hydraulic conductivity (PLC) in response to xylem water potential change (interpreted from air-injection pressure) in stems of (a) *B. platyphylla* (2x), (b) *B. costata* (2x), (c) *B. ermanii* (4x) and (d) *B. dahurica* (10x). Values are means $\pm$ 1 SE ($n = 6$). Data were fitted to sigmoid function ($y = a/(1 + \exp(-(x - x_0)/b))$). Mean water potential at 50% loss of stem hydraulic conductivity ($P50_{\text{stem}}$) and midday leaf water potential (Ψ_{md}) are indicated by vertical dashed lines and solid lines, respectively.

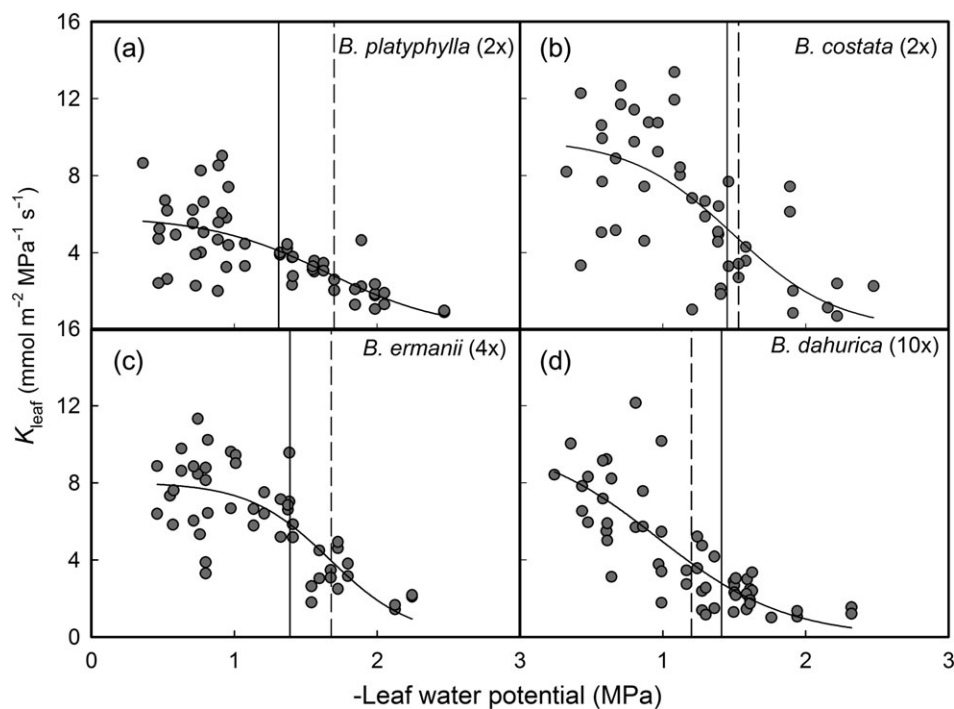


Figure 5. Leaf hydraulic conductance (K_{leaf}) as a function of leaf water potential for (a) *B. platyphylla* (2x), (b) *B. costata* (2x), (c) *B. ermanii* (4x) and (d) *B. dahurica* (10x). Leaf water potential at 50% loss of maximum K_{leaf} ($P50_{\text{leaf}}$) and midday leaf water potential (Ψ_{md}) are indicated by vertical dashed lines and solid lines, respectively.

pits. This finding at least partially explained the lack of consistent difference in sapwood hydraulic efficiency between diploid and

polyploid birch species despite the fact that polyploid species have substantially larger vessel diameters.

The strikingly different pit characteristics between diploid and polyploid birch species may reflect very different selective pressure on xylem hydraulics in their respective environments. On one hand, pit characteristics such as larger pit membrane area, larger aperture and higher aperture fraction can contribute to the increase in sapwood hydraulic conductivity as proved by inter-specific comparative studies in angiosperms (Lens et al. 2011, Jacobsen et al. 2016). On the other hand, these characteristics associated with higher porosity to water flow at the pit level in diploid birch species unavoidably compromise their ability to resist air seeding through inter-conduit pits and hence drought-induced xylem embolism (Hacke and Sperry 2001, Christman et al. 2012, Lens et al. 2013). Moreover, the contrastingly different pit aperture shape has likely contributed to differences in drought-induced embolism between the diploid and polyploid *Betula* species. Pit aperture shape has been found to be correlated with embolism resistance, with cavitation-resistant species showing narrower and more elliptical pit apertures (Lens et al. 2011, Scholz et al. 2013). Consistently, more elliptical pit apertures in polyploid birch species are associated with higher cavitation resistance compared with the two diploid species. Contrasts in pit characteristics between diploid and polyploid birches found in the present study thus reflect a trade-off at the pit level between requirements for hydraulic safety and efficiency (Hacke and Sperry 2001, Hacke et al. 2006, Lens et al. 2011), which is apparently independent of vessel diameter variations.

The overall small leaf hydraulic safety margin in all the four studied species irrespective of ploidy level was largely due to the much greater vulnerability of the leaf hydraulic system than that of the stems (Nardini and Luglio 2014, Sperry and Love 2015), which in combination with stomatal regulations may represent a common adaptive strategy taken by tree species to balance requirements for hydraulic safety and at the same time avoiding too much restriction on CO₂ uptake for carbon assimilation (Brodribb and Holbrook 2003, Delzon and Cochard 2014). The greater susceptibility of leaf hydraulic system could act as 'hydraulic fuse' in protecting the stem xylem system under unfavorable water conditions (Zufferey et al. 2011, Johnson et al. 2012, Liu et al. 2015). Recent studies have suggested that declines in K_{leaf} in response to decreased water potential could be attributed to cell shrinkage (Scoffoni et al. 2014), conduit collapse (Zhang et al. 2014, 2016), cell turgor loss (Brodribb and Holbrook 2006) and/or decreased membrane permeability related to aquaporin (Cochard et al. 2007), which are more easily reversible than cavitation and could be used by plants to amplify the hydraulic signal for stomatal closure and to protect xylem vessels from embolism. Unsurprisingly, leaf hydraulic capacity has been shown to be highly plastic in response to environmental conditions (Sack and Holbrook 2006, Sack and Scoffoni 2013). The overall greater vulnerability, dynamic nature and higher developmental plasticity may have altogether contributed to the overall lack of consistent

differences in leaf hydraulics between diploid and polyploid birch species, which is considered to have adaptive significance to different cytotypes of *A. canescens* in the Chihuahuan Desert (Hao et al. 2013).

Hydraulics and niche differentiation

Stem hydraulic properties have been shown to have strong influences on environmental adaptation of trees and could be one important physiological basis for niche differentiation among species (Cavender-Bares and Holbrook 2001, Hao et al. 2008, 2013). The obvious differences in stem hydraulic traits between polyploid and diploid birch species seem to be an important underlying mechanism for their obvious habitat differentiation. The two polyploid birch species that occur in more stressed environment, i.e., timberline habitat for *B. ermanii* (4x) and drought-prone habitat for *B. dahurica* (10x), showed higher resistance to drought-induced embolism than the two diploid species growing in more favorable conditions. Although *B. ermanii* (4x) may not normally be drought stressed during the growing season, freeze-thaw cycles and winter desiccation at the sub-alpine timberline habitats can be a great threat to the integrity of stem hydraulic function (Mayr et al. 2002, Schreiber et al. 2013, Granda et al. 2014). The greater success of these two polyploid birch species in these stressed conditions has likely benefited from stronger resistance to xylem embolism, which provides a mechanistic explanation for the long-held hypothesis that polyploids are more stress tolerant than diploids (Martin and Husband 2009, Thompson et al. 2015).

Besides embolism resistance, polyploid *Betula* species also differ in other important aspects of hydraulic architecture from their diploid counterparts, which may also contribute to their niche differentiation. Smaller LA/SA consequently higher leaf-specific hydraulic conductivity in polyploid species could allow them to maintain more secure water supply under more stressed conditions. In addition, smaller leaves and lower LA/SA contribute to higher efficiency in controlling water loss under drought and are often associated with increased water deficits (Zhang et al. 2009). However, smaller leaves and lower LA/SA mean smaller surface area for carbon assimilation, and less carbon return per investment in stem hydraulic systems. Therefore, the hydraulic architecture associated with drought resistance in polyploid species could incur a physiological cost in terms of carbon economy, which may limit their physiological performances and thus lead to a disadvantage in competing with diploid species under more favorable conditions.

Conclusions

Our results suggest that differences in hydraulic traits may, at least partially, be responsible for habitat differentiation between the four birch species of different ploidy levels in the study area of NE China. Greater resistance to embolism formation permitted

by pit-level adaptive traits may have contributed to the dominance of *B. ermanii* (4x) in the timberline forest ecosystem and the success of *B. dahurica* (10x) in the drought-prone habitats. However, high hydraulic resistance at the pit level and reduced LA per SA may have limited their physiological performance in terms of carbon assimilation under more favorable conditions. At the genus level, species with multiple ploidy levels that function differently in plant–environment interactions may have contributed to the overall success of the taxa by allowing sister species to colonize heterogeneous environments.

Supplementary Data

Supplementary Data for this article are available at [Tree Physiology](#) Online.

Acknowledgments

We thank the staff at the Research Station of Changbai Mountain Forest Ecosystems for supporting this study. Guanhua Dai, Luxin Zhao, Luxiang Zhao and Lufu Zhao are acknowledged for field assistances. We thank the two anonymous reviewers for very helpful comments.

Conflict of interest statement

None declared.

Funding

This work was supported by the National Natural Science Foundation of China (31500222, 31670412), the key project of Ministry of Science and Technology of China (2016YFA0600803), the Hundred-Talents Program and the project QYZDJ-SSW-DQC027 from the Chinese Academy of Sciences.

References

- Beaulieu JM, Leitch IJ, Patel S, Pendharkar A, Knight CA (2008) Genome size is a strong predictor of cell size and stomatal density in angiosperms. *New Phytol* 179:975–986.
- Brodribb TJ, Holbrook NM (2003) Stomatal closure during leaf dehydration, correlation with other leaf physiological traits. *Plant Physiol* 132: 2166–2173.
- Brodribb TJ, Holbrook NM (2006) Declining hydraulic efficiency as transpiring leaves desiccate: two types of response. *Plant Cell Environ* 29: 2205–2215.
- Brodribb TJ, Feild TS, Sack L (2010) Viewing leaf structure and evolution from a hydraulic perspective. *Funct Plant Biol* 37:488–498.
- Cavender-Bares J, Holbrook NM (2001) Hydraulic properties and freezing-induced cavitation in sympatric evergreen and deciduous oaks with contrasting habitats. *Plant Cell Environ* 24:1243–1256.
- Choat B, Sack L, Holbrook NM (2007) Diversity of hydraulic traits in nine *Cordia* species growing in tropical forests with contrasting precipitation. *New Phytol* 175:686–698.
- Christman MA, Sperry JS, Smith DD (2012) Rare pits, large vessels and extreme vulnerability to cavitation in a ring-porous tree species. *New Phytol* 193:713–720.
- Cochard H, Venisse JS, Barigah TS, Brunel N, Herbette S, Guilliot A, Tyree MT, Sakr S (2007) Putative role of aquaporins in variable hydraulic conductance of leaves in response to light. *Plant Physiol* 143: 122–133.
- Delzon S, Cochard H (2014) Recent advances in tree hydraulics highlight the ecological significance of the hydraulic safety margin. *New Phytol* 203:355–358.
- Domec J-C, Schäfer K, Oren R, Kim HS, McCarthy HR (2010) Variable conductivity and embolism in roots and branches of four contrasting tree species and their impacts on whole-plant hydraulic performance under future atmospheric CO₂ concentration. *Tree Physiol* 30: 1001–1015.
- Dubcovsky J, Dvorak J (2007) Genome plasticity a key factor in the success of polyploid wheat under domestication. *Science* 316: 1862–1866.
- Ennajeh M, Simões F, Khemira H, Cochard H (2011) How reliable is the double-ended pressure sleeve technique for assessing xylem vulnerability to cavitation in woody angiosperms? *Plant Physiol* 142: 205–210.
- Ewers FW, Fisher JB (1989) Variation in vessel length and diameter in stems of six tropical and subtropical lianas. *Am J Bot* 76:1452–1459.
- Granda E, Scoffoni C, Rubio-Casal AE, Sack L, Valladares F (2014) Leaf and stem physiological responses to summer and winter extremes of woody species across temperate ecosystems. *Oikos* 123: 1281–1290.
- Hacke UG (2014) Variable plant hydraulic conductance. *Tree Physiol* 34:105–108.
- Hacke UG, Sperry JS (2001) Functional and ecological xylem anatomy. *Perspect Plant Ecol* 4:97–115.
- Hacke UG, Sperry JS, Wheeler JK, Castro L (2006) Scaling of angiosperm xylem structure with safety and efficiency. *Tree Physiol* 26: 689–701.
- Hao GY, Hoffmann WA, Scholz FG, Bucci SJ, Meinzer FC, Franco AC, Cao KF, Goldstein G (2008) Stem and leaf hydraulics of congeneric tree species from adjacent tropical savanna and forest ecosystems. *Oecologia* 155:405–415.
- Hao GY, Lucero ME, Sanderson SC, Zacharias EH, Holbrook NM (2013) Polyploidy enhances the occupation of heterogeneous environments through hydraulic related trade-offs in *Atriplex canescens* (Chenopodiaceae). *New Phytol* 197:970–978.
- Hollister JD (2015) Polyploidy: adaptation to the genomic environment. *New Phytol* 205:1034–1039.
- Jacobsen AL, Tobin MF, Toschi HS, Percolla MI, Pratt RB (2016) Structural determinants of increased susceptibility to dehydration-induced cavitation in post-fire resprouting chaparral shrubs. *Plant Cell Environ* 39:2473–2485.
- Jiao YN, Wickett NJ, Ayyampalayam S et al. (2011) Ancestral polyploidy in seed plants and angiosperms. *Nature* 473:97–100.
- Johnson DM, McCulloh KA, Woodruff DR, Meinzer FC (2012) Hydraulic safety margins and embolism reversal in stems and leaves: why are conifers and angiosperms so different? *Plant Sci* 195:48–53.
- Jordan GJ, Carpenter RJ, Koutoulis A, Price A, Brodribb TJ (2015) Environmental adaptation in stomatal size independent of the effects of genome size. *New Phytol* 205:608–617.
- Leitch AR, Leitch IJ (2008) Genomic plasticity and the diversity of polyploid plants. *Science* 320:481–483.
- Lens F, Sperry JS, Christman MA, Choat B, Rabaey D, Jansen S (2011) Testing hypotheses that link wood anatomy to cavitation resistance and hydraulic conductivity in the genus *Acer*. *New Phytol* 190: 709–723.

- Lens F, Tixier A, Cochard H, Sperry JS, Jansen S, Herbette S (2013) Embolism resistance as a key mechanism to understand adaptive plant strategies. *Curr Opin Plant Biol* 16:287–292.
- Levin DA (1983) Polyploidy and novelty in flowering plants. *Am Nat* 122:1–25.
- Levin DA (2002) The role of chromosomal change in plant evolution. Oxford University Press, New York, NY.
- Li WD, Biswas DK, Xu H, Xu CQ, Wang XZ, Liu JK, Jiang GM (2009) Photosynthetic responses to chromosome doubling in relation to leaf anatomy in *Lonicera japonica* subjected to water stress. *Funct Plant Biol* 36:783–792.
- Li WL, Berlyn GP, Ashton PMS (1996) Polyploids and their structural and physiological characteristics relative to water deficit in *Betula papyrifera* (Betulaceae). *Am J Bot* 83:15–20.
- Liu YY, Song J, Wang M, Li N, Niu CY, Hao GY (2015) Coordination of xylem hydraulics and stomatal regulation in keeping the integrity of xylem water transport in shoots of two compound-leaved tree species. *Tree Physiol* 35:1333–1342.
- Lomax BH, Hilton J, Bateman RM, Upchurch GR, Lake JA, Leitch IJ, Cromwell A, Knight CA (2014) Reconstructing relative genome size of vascular plants through geological time. *New Phytol* 201:636–644.
- Madlung A (2013) Polyploidy and its effect on evolutionary success: old questions revisited with new tools. *Heredity* 110:99–104.
- Maherali H, Walden AE, Husband BC (2009) Genome duplication and the evolution of physiological responses to water stress. *New Phytol* 184:721–731.
- Manzaneda AJ, Rey PJ, Bastida JM, Weiss-Lehman C, Raskin E, Mitchell-Olds T (2012) Environmental aridity is associated with cytotype segregation and polyploidy occurrence in *Brachypodium distachyon* (Poaceae). *New Phytol* 193:797–805.
- Markestijn L, Poorter L, Paz H, Sack L, Bongers F (2011) Ecological differentiation in xylem cavitation resistance is associated with stem and leaf structural traits. *Plant Cell Environ* 34:137–148.
- Martin SL, Husband BC (2009) Influence of phylogeny and ploidy on species ranges of North American angiosperms. *J Ecol* 97:913–922.
- Martin SL, Husband BC (2013) Adaptation of diploid and tetraploid *Chamerion angustifolium* to elevation but not local environment. *Evolution* 67:1780–1791.
- Mayr S, Wolfschwenger M, Bauer H (2002) Winter-drought induced embolism in Norway spruce (*Picea abies*) at the Alpine timberline. *Plant Physiol* 115:74–80.
- Moghe GD, Shiu SH (2014) The causes and molecular consequences of polyploidy in flowering plants. *Ann NY Acad Sci* 1320:16–34.
- Nardini A, Luglio J (2014) Leaf hydraulic capacity and drought vulnerability: possible trade-offs and correlations with climate across three major biomes. *Funct Ecol* 28:810–818.
- Nassar NMA, Graciano-Ribeiro D, Fernandes SDC, Araujo PC (2008) Anatomical alterations due to polyploidy in cassava, *Manihot esculenta* Crantz. *Genet Mol Res* 7:276–283.
- Otto SP, Whitton J (2000) Polyploid incidence and evolution. *Annu Rev Genet* 34:401–437.
- Parisod C (2012) Polyploids integrate genomic changes and ecological shifts. *New Phytol* 193:297–300.
- Parisod C, Holderegger R, Brochmann C (2010) Evolutionary consequences of autopolyploidy. *New Phytol* 186:5–17.
- Ramsey J (2011) Polyploidy and ecological adaptation in wild yarrow. *Proc Natl Acad Sci USA* 108:7096–7101.
- Sack L, Holbrook NM (2006) Leaf hydraulics. *Annu Rev Plant Biol* 57:361–381.
- Sack L, Scoffoni C (2013) Leaf venation: structure, function, development, evolution, ecology and applications in the past, present and future. *New Phytol* 198:983–1000.
- Sack L, Cowan PD, Jaikumar N, Holbrook NM (2003) The 'hydrology' of leaves: coordination of structure and function in temperate woody species. *Plant Cell Environ* 26:1343–1356.
- Sano Y (2005) Inter- and intraspecific structural variations among intervascular pit membranes, as revealed by field-emission scanning electron microscopy. *Am J Bot* 92:1077–1084.
- Schenk HJ, Steppe K, Jansen S (2015) Nanobubbles: a new paradigm for air-seeding in xylem. *Trends Plant Sci* 20:199–205.
- Scholz A, Rabaey D, Stein A, Cochard H, Smets E, Jansen S (2013) The evolution and function of vessel and pit characters with respect to cavitation resistance across 10 *Prunus* species. *Tree Physiol* 33:684–694.
- Schreiber SG, Hamann A, Hacke UG, Thomas BR (2013) Sixteen years of winter stress: an assessment of cold hardiness, growth performance and survival of hybrid poplar clones at a boreal planting site. *Plant Cell Environ* 36:419–428.
- Scoffoni C, Vuong C, Diep S, Cochard H, Sack L (2014) Leaf shrinkage with dehydration: coordination with hydraulic vulnerability and drought tolerance. *Plant Physiol* 164:1772–1788.
- Sessa EB, Givnish TJ (2014) Leaf form and photosynthetic physiology of *Dryopteris* species distributed along light gradients in eastern North America. *Funct Ecol* 28:108–123.
- Soltis DE, Buggs RJA, Doyle JJ, Soltis PS (2010) What we still don't know about polyploidy. *Taxon* 59:1387–1403.
- Soltis PS, Liu XX, Marchant DB, Visger CJ, Soltis DE (2014) Polyploidy and novelty: Gottlieb's legacy. *Philos Trans R Soc Lond B Biol Sci* 369:20130351.
- Sperry JS, Love DM (2015) What plant hydraulics can tell us about responses to climate-change droughts. *New Phytol* 207:14–27.
- Sperry JS, Saliendra NZ (1994) Intra- and inter-plant variation in xylem cavitation in *Betula occidentalis*. *Plant Cell Environ* 17:1233–1241.
- Sperry JS, Donnelly JR, Tyree MT (1988) A method for measuring hydraulic conductivity and embolism in xylem. *Plant Cell Environ* 11:35–40.
- Sun SC, Jin DM, Shi PL (2006) The leaf size-twigs size spectrum of temperate woody species along an altitudinal gradient: an invariant allometric scaling relationship. *Ann Bot* 97:97–107.
- Thompson KA, Husband BC, Maherali H (2015) No influence of water limitation on the outcome of competition between diploid and tetraploid *Chamerion angustifolium* (Onagraceae). *J Ecol* 103:733–741.
- Tyree MT, Sperry JS (1988) Do woody plants operate near the point of catastrophic xylem dysfunction caused by dynamic water stress? Answers from a model. *Plant Physiol* 88:574–580.
- Van Laere K, Franca SC, Vansteenkiste H, Van Huylbroeck J, Steppe K, Van Labeke M-C (2011) Influence of ploidy level on morphology, growth and drought susceptibility in *Spathiphyllum wallisii*. *Acta Physiol Plant* 33:1149–1156.
- Wang AY, Wang M, Yang D, Song J, Zhang WW, Han SJ, Hao GY (2016) Responses of hydraulics at the whole-plant level to simulated nitrogen deposition of different levels in *Fraxinus mandshurica*. *Tree Physiol* 36:1045–1055.
- Wheeler JK, Sperry JS, Hacke UG, Hoang N (2005) Inter-vessel pitting and cavitation in woody Rosaceae and other vesselless plants: a basis for a safety versus efficiency trade-off in xylem transport. *Plant Cell Environ* 28:800–812.
- Zanne AE, Westoby M, Falster DS, Ackerly DD, Loarie SR, Arnold SEJ, Coomes DA (2010) Angiosperm wood structure: global patterns in vessel anatomy and their relation to wood density and potential conductivity. *Am J Bot* 97:207–215.
- Zhang YJ, Meinzer FC, Hao GY et al. (2009) Size-dependent mortality in a Neotropical savanna tree: the role of height-related adjustments in hydraulic architecture and carbon allocation. *Plant Cell Environ* 32:1456–1466.

- Zhang YJ, Rockwell FE, Wheeler JK, Holbrook NM (2014) Reversible deformation of transfusion tracheids in *Taxus baccata* is associated with a reversible decrease in leaf hydraulic conductance. *Plant Physiol* 165:1557–1565.
- Zhang YJ, Rockwell FE, Graham AC, Alexander T, Holbrook NM (2016) Reversible leaf xylem collapse: a potential 'circuit breaker' against cavitation. *Plant Physiol* 172:2261–2274.
- Zimmermann MH (1983) Xylem structure and the ascent of sap. Springer-Verlag, Berlin, Germany.
- Zimmermann MH, Jeje AA (1981) Vessel-length distribution in stems of some American woody plants. *Can J Bot* 59:1882–1892.
- Zufferey V, Cochard H, Ameglio T, Spring J-L, Viret O (2011) Diurnal cycles of embolism formation and repair in petioles of grapevine (*Vitis vinifera* cv. Chasselas). *J Exp Bot* 62:3885–3894.