LETTER

Climate determines vascular traits in the ecologically diverse genus *Eucalyptus*

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Abstract

Current theory presumes that natural selection on vascular traits is controlled by a trade-off between efficiency and safety of hydraulic architecture. Hence, traits linked to efficiency, such as vessel diameter, should show biogeographic patterns; but critical tests of these predictions are rare, largely owing to confounding effects of environment, tree size and phylogeny. Using wood sampled from a phylogenetically constrained set of 28 *Eucalyptus* species, collected from a wide gradient of aridity across Australia, we show that hydraulic architecture reflects adaptive radiation of this genus in response to variation in climate. With increasing aridity, vessel diameters narrow, their frequency increases with a distribution that becomes gradually positively skewed and sapwood density increases while the theoretical hydraulic conductivity declines. Differences in these hydraulic traits appear largely genotypic in origin rather than environmentally plastic. Data reported here reflect long-term adaptation of hydraulic architecture to water availability. Rapidly changing climates, on the other hand, present significant challenges to the ability of eucalypts to adapt their vasculature.

Keywords

Anatomy, angiosperm tree, aridity, conduit diameter, hydraulic conductivity, vessel frequency distribution, water availability, wood density, xylem.

INTRODUCTION

Availability of water is critical for the survival of most life forms and especially for sessile and long-lived organisms such as trees. Trees use water to meet transpiration demand, keep tissues functional, translocate nutrients and hormones and support biochemical processes. In sapwood of trees, water is passively drawn through a network of interlinked vessels from roots to leaves. In angiosperm trees, the diameter of these vessels can vary between 10 and 500 μm (Tyree & Zimmermann 2002). According to Hagen–Poiseuille's law of laminar flow, the capacity of a single vessel to conduct water increases with the fourth power of vessel diameter, markedly increasing the vessel area-specific efficiency of transporting water (Sperry *et al.* 2006). This relationship between vessel diameter and conductivity has been demonstrated for many temperate and tropical tree species (e.g. Tyree *et al.* 1994; Sperry *et al.* 2006; McCulloh *et al.* 2010; Meinzer *et al.* 2010). However, wider vessels in some taxa are prone to losing conductivity (e.g. Hargrave *et al.* 1994; Maherali *et al.* 2006; Cai & Tyree 2010; Hajek *et al.* 2014; Guet *et al.* 2015) when the tension to pull water through the stem exceeds physical limits, resulting in air seeding and subsequent formation of air emboli. Forming a vessel network that is capable of transporting sufficient water to maintain physiological functioning of leaves while minimising risks of loss of conductivity, is a key measure of the 'fitness' of tree species. This trade-off is captured in ecological theory by the safety vs. efficiency framework of xylem vessel networks (e.g. Tyree & Zimmermann 2002; Hacke *et al.* 2006; Sperry *et al.* 2008; Meinzer *et al.* 2010).

Increasing tension of water in vessels in trees can be the effect of two causes: (1) limited availability of water in soil and (2) transport of water into increasingly tall canopies. Evolutionary forces have seemingly resulted in 'strategies' that limit risks of hydraulic failure in angiosperm trees. Leaf deciduousness is an effective mechanism to increase survival during periods of limited availability of water. While some evergreen angiosperm species adjust canopy leaf area in accordance with water availability (e.g. Specht & Specht 1989), a more widespread feature of evergreen angiosperms is the ability to tolerate high tension in vessels when water availability is low (Maherali *et al.* 2004). Tolerating increasing tension while maintaining hydraulic function requires modification of hydraulic architecture, including vascular traits as well as tree height.

Empirical measurements show that density of sapwood increases as availability of water declines (Hacke *et al.* 2001; Poorter *et al.* 2010) and species with dense sapwood usually have a larger capacity to withstand tension in xylem vessels (Hacke *et al.* 2001). This increased hydraulic safety mostly coincides with high relative frequency of narrow vessels (Wheeler *et al.* 2007; Sperry *et al.* 2008; Brodribb *et al.* 2012), but comes at a cost of low canopy conductance and limited uptake of CO_2 (Santiago *et al.* 2004), resulting in slower growth and shorter stature (Poorter *et al.* 2010). Curiously, in some angiosperm species vessel diameter alone has a weak influence on the capacity to resist increasing xylem tension

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(e.g. Hacke *et al.* 2001; Sperry *et al.* 2005; Lens *et al.* 2011), while in other species that same trait has a major influence (e.g. Maherali *et al.* 2006; Hajek *et al.* 2014; Guet *et al.* 2015). Low correlation coefficients between these traits are generally attributed to the abundance and architecture of intervessel pits (e.g. Wheeler *et al.* 2005; Choat *et al.* 2008; Lens *et al.* 2011), the capacity of a species to reverse embolism (Bucci *et al.* 2003) and other structural and physiological factors (see Meinzer *et al.* 2010). Thus, although there is wide acknowledgement that vessel diameter is linked to conductivity, both within and among species, available evidence on the impact of vessel diameter on hydraulic safety points to considerable inconsistency across taxa with divergent hydraulic anatomy.

Under water-limited conditions, selection should favour species that develop hydraulically safe features (e.g. tolerance of high xylem tension, short stature) (e.g. Tyree *et al.* 1994; Moles *et al.* 2009; Carlquist 2012). By contrast, trees in mesic environments are more likely to be light- rather than water limited (Lines *et al.* 2012). To overcome increasing pressure from competition for light and limit tension of water in apical meristems (Koch *et al.* 2004), the vasculature of tall trees must be effective at transporting large amounts of water to the top of their crowns. A network composed of wide vessels over a large distance of the vertical transport pathway would theoretically meet these requirements. This is consistent with observations that taller tree species often have wider vessels at their stem base as a result of vessel widening over a long vertical distance (e.g. Petit *et al.* 2010; Poorter *et al.* 2010; Olson *et al.* 2014). A logical prediction from safety vs. efficiency trade-off theory would be that as a result of high selective pressures, trees in arid environments would on average have narrower vessels (to lessen risk of embolism) and trees in increasingly mesic environments would have wide vessels (to enable plants to be competitive for light and carbon uptake).

We still lack critical tests of this prediction. More specifically, we lack strong evidence of aridity being a major driver of the evolution of hydraulic traits, in large part due to the absence of suitable, phylogenetically constrained comparisons. Previous analyses have been predominantly derived from taxa that vary widely in evolutionary history and have contrasting xylem anatomy (Tyree *et al.* 1994; Lens *et al.* 2004; Wheeler *et al.* 2007; Carlquist 2012; Olson & Rosell 2013; Olson *et al.* 2014). It thus remains problematic to attribute differences in hydraulic traits among taxa to the effects of climate, tree height or contrasting phylogenetic conservatism. Here, we overcome some of these difficulties by testing the relation of hydraulic traits to environmental factors using phylogenetically constrained data obtained from species of the genus *Eucalyptus* growing along a well-defined aridity gradient. Fossil records for eucalypts date back *c.* 25 M years (Crisp *et al.* 2004) and today more than 700 species of *Eucalyptus* (Ladiges 1997) have colonised a wide range of habitats throughout Australia and nearby islands. Present day diversity of eucalypts reflects adaptive radiation to cope with a wide array of environmental conditions, including precipitation (*P*) that ranges from below 200 to above 3000 mm annually and equally variable potential evapotranspiration (*ET*). The long evolutionary history – uninterrupted by more recent glacial periods (Petherick *et al.* 2013) – makes this taxon ideal for investigating climatic adaptation in vascular traits.

We assessed vascular traits in 105 mature trees of 28 *Eucalyptus* species selected to cover a wide gradient of aridity across continental Australia, which is represented by the ratio of precipitation to potential evapotranspiration (*P*/*ET*; Fig. 1b, Table S1; see also Gleason *et al.* 2012). We hypothesised that, across this gradient, wood and vessel anatomical traits (diameter, frequency, wood density, vessel void-to-wood ratio, theoretical sapwood conductivity) would vary in a manner consistent (Fig. 1a) with the prediction of increasing hydraulic efficiency with increasing *P*/*ET*. We also tested if climate or tree height is a better predictor of vascular traits. Finally, by comparing seven species each grown in contrasting wetter and drier sites, we assessed the degree to which observed differences in vascular traits result from phenotypic responses to local environments, genotypic differences or both. This test provided a measure of the degree of plasticity in hydraulic vasculature, which can be indicative when projecting hydraulic function under changing climates for the genus *Eucalyptus*.

MATERIAL AND METHODS

Study sites and species

Between 2009 and 2014 we collected stem sapwood from 105 mature trees from 28 species of *Eucalyptus* (*n* = 3 trees for each species) at 21 locations across SE, E and SW Australia (Table S1). Spread over a distance of *c.* 3500 km, sample sites were arrayed along two geographic gradients, spanning in total a range of mean annual precipitation (250–1800 mm, MAP) and temperature (15–24 °C, MAT); they covered an elevation gradient of > 1200 m and included Australian ecosystems such as mountain ash, peppermint and jarrah forests and box-ironbark and mallee woodlands. The majority of sampled species originated from ecosystems in SE and E Australia (19 species), covering woodlands and forests that dominate dry, temperate Mediterranean-type and sub-alpine climate regions. A smaller set of species was sampled from arid and semi-arid woodlands of SW Australia (9 species). We excluded species with permanent access to shallow groundwater as well as those that regularly experience sub-zero air temperatures. Both conditions could lead to formation of specialised vascular networks that represent niche adaptation, rather than broader scale trait evolution.

Sample collection, preparation and analyses

To test the premise that tree height is a dominant driver in shaping vascular traits compared to climate, we assessed the effect of both drivers on hydraulic traits: (1) at the base of stems (i.e. main bole) and (2) at a standard height (8 m) below the apex.

At each site, the diameter distribution of trees was assessed and sampled trees covered the range present. Height of 85 of the 105 sampled trees was measured. Only mature, dominant trees without visible injuries or deformations were selected. Tension wood was avoided. Samples of sapwood (increment cores and wood wedges) were extracted at the basipetal region of trunks, *c.* 100–130 cm above ground. Increment cores were extracted using a motorised (TED-262R; Tanaka, Japan) TRE-COR® (CSIRO, Canberra, Australia) and a hand-operated
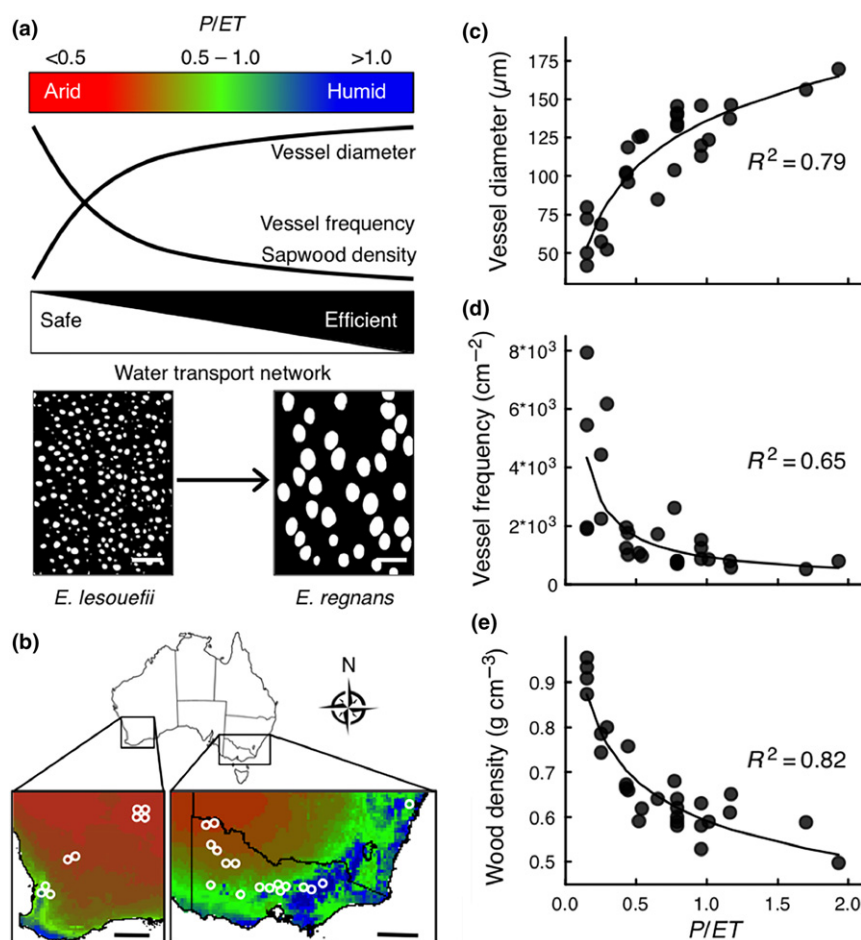
Figure 1 Conceptual framework of predicted changes and empirical observations of hydraulic traits in 28 species of *Eucalyptus*. (a) Conceptual framework of predicted changes in hydraulic traits along a gradient of aridity, represented as ratio of annual precipitation (P) over potential evapotranspiration (ET); also shown are binary micrographs of diffuse porous sapwood collected from the base of two eucalypt species that grew at the driest (left) and wettest site (right; scale bar = 200 μm). (b) Site locations in Australia: colours in detailed cut-outs represent long-term P/ET conditions and correspond with panel A (scale bar = 200 km). (c–e) Change in hydraulic traits along a gradient of water availability (i.e. P/ET). Graphs display empirical measurements and mean values for all 28 species ($n = 3$ trees per species); black lines showed best-fit functions for log-transformed data (all variables except mean vessel diameter). Coefficients of determination are shown.

increment corer (SUUNTO, Vantaa, Finland). Sapwood wedges were cut with a hand- or chainsaw. After extraction, samples were stored in sealed containers to keep fresh. Each of seven species that span a wide gradient of aridity and tree height were sampled at two sites (42 trees) that differed in water availability to assess potential intraspecific and site-related variation in hydraulic traits (see Table S1 for species). In 10 species (30 trees) that originated from widely divergent climates, we collected sapwood samples 8 m below the apex after trees were felled (see Table S1 for species). Height of these trees ranged from 10 to more than 70 m. These wood samples were used to test for the effect of tree height/stem diameter on vessel diameters.

Sample cubes (transversal plane of *c.* 0.5 cm^2) of the outermost 1 cm of sapwood were prepared to determine fresh weight and volume (immersion technique), before drying at 105 °C for 48 h to determine dry weights. The dry weight and volume of each cube was used to calculate sapwood density (g cm^{-3}). A second set of fresh wood cubes was used to

prepare three transversal sections (25–45 μm thickness depending on species) using a rotary (Leica RM2255; Leica Microsystems, Wetzlar, Germany) or a sliding microtomes (Leica SM2010R; Leica Microsystems). All sections were stained with safranin solution (1%) to enhance contrast of wood against void space of vessels. Following the staining, sections were cleared and prepared for microscopic analyses (aqueous embedding on glass slides).

Depending on vessel size and abundance, three to eight digital images were taken at random locations at a range of magnifications (5, 10, 20×) using a transmission light microscope (Leica DM2500M; Leica Microsystems) equipped with a high-resolution digital camera (Leica DFC 500; Leica Microsystems). A total of 450 images were generated. Where necessary the images were manually corrected if vessel walls became detached during preparation. Data of vessel area and abundance, as well as image area were generated from each corrected image using automated analysis software (Leica Application Suite, Version 3.8; Leica Microsystems) or ImageJ

(National Institutes of Health, Bethesda, MD). Incomplete vessels (i.e. edge effect) were excluded from the analyses. These analyses generated information for 23 948 vessels.

Calculation of hydraulic properties

Measurements of vessel area and abundance were used to calculate hydraulic properties of sapwood for each species and site. Mean hydraulically weighted vessel diameter (D_H) for each species and site was calculated, weighting individual vessel diameters according to their hydraulic conductance (Tyree & Zimmermann 2002):

$$D_H = \left[\frac{\sum D^4}{N} \right]^{1.25} \quad (1)$$

where D is the equivalent circular diameter of vessels and N is the number of vessels measured. The D_H is biased towards wider vessels that conduct the majority of water to account for the implications of Hagen–Poiseuille's law. Furthermore, average vessel frequency was calculated per image by division of the sum of vessel count and the image area. Resulting vessel frequencies were scaled to a unified area basis (i.e. vessels per cm^2 sapwood) before averaging vessel frequency per species and site. In a similar fashion the vessel void-to-wood ratio was calculated by dividing the sum of vessel lumen area by image area before averaging per species and site. For each species we calculated the theoretical hydraulic conductivity (K_{th}, kg s^{-1} m^{-1} MPa^{-1}) of sapwood using D_H (m):

$$K_{th} = \frac{D_H^4 \pi}{128 \eta} \times VF \times 1000 \quad (2)$$

where η represents the viscosity of water at 20 °C (1.002×10^{-9}, MPa s), VF is vessel frequency (m^{-2}). The final multiplication by a factor of 1000 is necessary to convert m^3 of water to kg.

Climate data

Long-term, grid-interpolated climate data for each sample location were obtained from SILO Data Drill$^{©}$ (State of Queensland, Department of Environment and Resource Management). Each dataset listed daily observations for 100 years prior to the date of sample collection for each species. These measurements were generated using an anomaly interpolation method that uses available climate data from nearby weather stations. The following climate parameters were used: daily maximum air temperature (T_{max}, °C), rainfall (P, mm) and potential evapotranspiration (ET, mm). For each site we calculated an index of water availability as ratio P/ET. This index denotes the degree of aridity, ranging from hyper-arid to wet with increasing index scores (UNEP 1997). According to the day when sapwood was collected, the calculated 100-year intervals used daily data ranging from March 1909 to April 2014.

Statistical analysis

Data were aggregated from tree- to species-level in testing for trends across the entire gradient of water availability (results were similar using individual trees as the observations). Non-

linear regression analyses were used to evaluate relations of vascular traits, namely the relation of mean vessel or the hydraulically weighted vessel diameter to vessel frequency, sapwood density and the vessel void-to-wood ratio. Data for all variables, except for mean vessel diameter, were log-transformed prior to determining best-fit functions. Univariate modelling and relative frequency distributions of vessel diameters were used to further explore the data.

To assess phenotypic plasticity of *Eucalyptus* species to adjust hydraulic traits to growing conditions at a drier or wetter site, we log–log-transformed vessel diameter and P/ET data before fitting a linear regression model first to all 28 species to obtain the slope value. The identical transformation and regression procedures were then applied to data of seven species that were sampled at both wet sites and dry sites. A fourth model assessed the average change in log vessel diameter when comparing a species from a drier to a more mesic site. The first three models ('all 28 species', '7 species – dry', '7 species – wet') examine the proportional change in vessel diameter that could result from fixed genotypic differences among species, plastic phenotypic differences within species or both. The fourth model ('7 species – dry vs. wet') includes plastic phenotypic differences within species and fixed genotypic differences among populations. Thus, comparison of the fourth model with the vessel–climate relationships of the other models provides a mean to detect the presence of 'within' species constraints on hydraulic traits.

Aabel™ software (V2.0; Gigawiz Ltd. Co., Tulsa, USA) and JMP (V11; SAS Institute Inc., Cary, USA) were used for statistical tests and graphic display of data. We note that region of collection of samples in eastern or western Australia had no effect on results (i.e. geographic domain was neither significant as a main effect nor interacted with climate variables in influencing vascular traits), although sites encompassed soils belonging to seven different orders. Thus, species data from across regions were pooled for analyses shown here.

RESULTS

Measured vascular traits of vessel diameter and frequency and sapwood density show strong patterns among species across the water availability gradient (Fig. 1). Vessel diameter (~ 10 to > 300 μm), vessel frequency (360–9070 vessels per cm^2 cross-sectional surface area) and wood density (0.47–0.96 g cm^{-3}) at the base of trees correlated strongly with site water availability (Fig. 1c–e). Vessel diameter declined markedly with increasing aridity, while vessel frequency and wood density increased. Across all species, mean vessel diameter declined with increasing vessel frequency and increasing wood density (Fig. 2a–b). The summed 'vessel void space' did not vary systematically with site climate and averaged around 15% of cross-sectional sapwood area (Fig. 2c). Although the vessel void space was similar for species growing in arid and mesic environments, the estimated conductivity of sapwood K_{th} showed a clear positive relationship with site water availability (Fig. 3). Theoretical conductivity of sapwood was lowest (9.73 ± 2.05 (± 1 SE) kg m^{-1} s^{-1} MPa^{-1}) in a species (*E. salubris*) growing at the driest site ($P/ET = 0.15$) and was more than 20-fold greater in trees growing at the wettest

site (*E. regnans*; $P/ET = 1.93$; 228.97 ± 47.03 kg m^{-1} s^{-1} MPa^{-1}).

Statistical analyses showed strong relationships of vascular traits to water availability even after accounting for covariation in tree height. While K_{th} increased with availability of water, so did tree height (curvilinear increase, $R^2 = 0.88$, data not shown). Regression models showed that both P/ET and tree height influence mean and hydraulically weighted vessel diameters. Height had reduced predictive power compared to P/ET and lower *t*- and *F*-ratios (Table S2A, B). More importantly, relationships of vascular traits to aridity remained robust even after removing effects of height, demonstrating that for eucalypts, climate is a strong driver of hydraulic properties and that species growing in areas of greater water availability have greater water transport capacity as a result of their hydraulic traits.

Vascular traits measured equidistant from tree apices support interpretations based on measures at tree bases. Hydraulic properties of wood sampled from 8 m below apices (to account for species differences in tree height and potential taper in vessel diameter) strongly mirrored patterns found at the bases of tree trunks. Mean vessel diameter in wood 8 m below the apex ranged threefold, from 50 to 157 μm (absolute min/max = 11/245 μm), and correlated strongly with climate (Fig. 4a) and not stem diameter (Fig. 4b). All related statistical tests show that climate and not height had a significant effect on mean vessel diameter 8 m below tree apices (Table S2C, D). The dominant effect of climate is underpinned by trait correlations that help separate effects of climate and tapering of vessel diameters (Fig. 5). Taller trees towards the mesic end of our environmental gradient displayed a more marked acropetal taper of vessels – as is visible
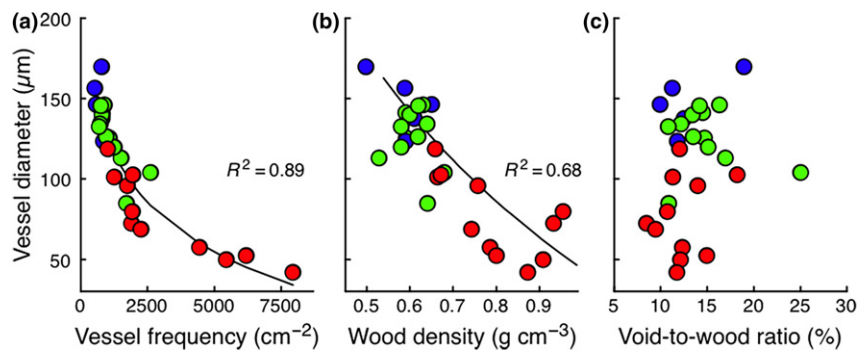

Figure 2 Relationships between vessel frequency (count per cm^2) (a), sapwood density (b) and the ratio of vessel void-to-wood (c) and mean vessel diameter in sapwood of 28 *Eucalyptus* species (*n* = 3 trees per species) across a wide gradient of water availability in Australia. The ratio of annual precipitation over evapotranspiration (P/ET) is used to represent water availability, displayed as colours where red = $P/ET < 0.5$, green = $P/ET > 0.5 < 1$ and blue = $P/ET > 1$. Lines display fit functions where data for vessel frequency and wood density were log-transformed. Coefficients of determination are shown.
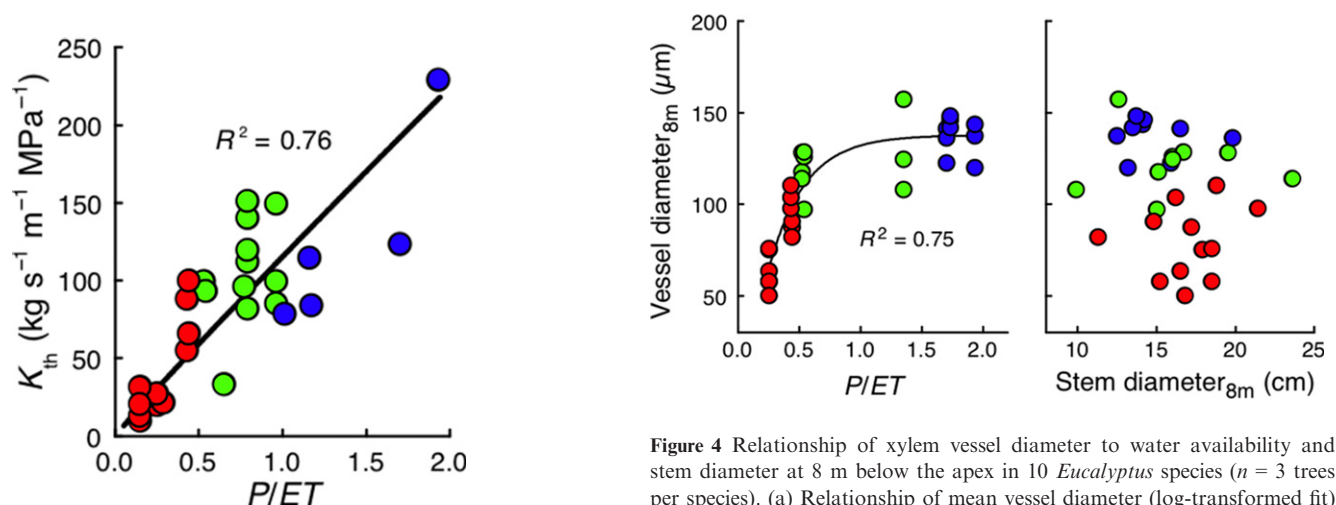

Figure 3 Relationship of theoretical hydraulic conductivity (K_{th}) of sapwood to water availability, represented as ratio of precipitation (*P*) over evapotranspiration (*ET*) of 28 species of *Eucalyptus* (*n* = 3 trees per species). Line shows best-fit function; colour-coding follows Fig. 2; coefficient of determination is shown.

Figure 4 Relationship of xylem vessel diameter to water availability and stem diameter at 8 m below the apex in 10 *Eucalyptus* species (*n* = 3 trees per species). (a) Relationship of mean vessel diameter (log-transformed fit) to water availability, which is represented as ratio of precipitation (*P*) over evapotranspiration (*ET*) for each site. (b) Relationship between mean vessel diameter and stem diameter at 8 m below the apex of individual sample trees which ranged from under 10 to over 70 m in total height (*n* = 30). The line shows the best fit function; coefficient of determination is shown.

in their offset below the 1 : 1 line for the relationship between vessel diameter at 8 m below tree apex, and that at the stem base (Fig. 5a). Although for the tallest trees vessel frequency decreased only slightly towards the stem base (Fig. 5b), their wider vessel diameters (at the base) resulted in a pronounced increase in theoretical conductivity relative to that assessed 8 m below their apex (Fig. 5c).

Evidence for the impact of climate on vessel diameter also comes from analyses of relative frequency distributions of vessel diameter within species. This evidence suggests different strategies to cope with growing conditions across climatically divergent sites. Such analyses are particularly powerful when assessing vessel anatomy of diffuse porous species – like eucalypts – which consists of different sized vessels throughout their annuli. Our data showed that along the aridity gradient, trees maintained a wide range of vessel diameters. On average, the range in vessel diameters *in each species* spanned *c.* 65% of the total range observed *across all sampled trees*. Minimum, mean, maximum and hydraulically weighted vessel diameter all increased from arid towards mesic environments, across species (Fig. 6a). Moreover, classifying and aggregating measurements of individual vessel diameters of all species into three broad contrasting categories of water availability [*arid* ($P/ET < 0.5$), *dry temperate* ($P/ET > 0.5 < 1$), *humid* ($P/ET > 1$)] revealed distinct shifts in frequency distributions of vessel diameters that aligned with putative ecological adaptation (Fig. 6b).

Vessel systems of *Eucalyptus* species from *arid* environments had a median vessel diameter of 62 μm and a positively skewed distribution (Fig. 6b). In contrast, *Eucalyptus* species from *humid* environments developed hydraulic networks of mostly wide and only few narrow vessels (negatively skewed distribution), with a median diameter of 152 μm. *Eucalyptus* species from *dry-temperate* environments exhibited an intermediate median value with a near-normal distribution of vessel diameters spanning the range of values.

The skewness of vessel diameter distributions shown in Fig. 6b provided further insight into how availability of water helps dictate construction of vascular networks in tree stems. *Eucalyptus* species from arid environments can still form vessels with diameters greater than 150 μm, whereas species from mesic environments also form very narrow vessels.

Data for seven species growing at both a drier and a wetter site showed no evidence of phenotypic plasticity in vascular traits (Fig. S1). Unlike clear general increases in D_H across environmental gradients (whether for all 28 species or just the seven studied at two sites), contrasts within species across environmental gradients did not result in increasing D_H (or mean vessel diameter) when water availability increased. The proportional change in D_H was negative when comparing dry with wet site data. This evidence suggests vessel diameters are not smaller in drier conditions due to either phenotypic plasticity or ecotypic variation within species.

DISCUSSION

Our phylogenetically constrained dataset developed from measurements of nearly 24 000 vessel diameters from a broad range of *Eucalyptus* species arrayed along a climate gradient enabled a test of the effect of water availability on vascular traits. Similarly constrained datasets have been published for both *Acer* (Lens *et al.* 2011) and *Prunus* (Scholz *et al.* 2013), albeit over much narrower gradients of water availability. However, these studies did not assess the effect of water availability on sapwood traits. Our data clearly showed that climate is a dominant factor controlling the diameter and frequency of vessels in sapwood of eucalypts. Although both tree height and vessel diameter decline with increasing aridity, our analyses (Table S2) showed that tree height has only an ancillary effect on vessel diameter and frequency.

In contrast to our within-genus results, recent meta-analyses across diverse taxa suggested that vessel diameter is a function of tree height and not climate (Olson & Rosell 2013; Olson *et al.* 2014). Height-dependent vessel tapering is regarded as the cause, resulting in a vessel diameter – stem diameter relationship that arises from a proportional relationship between
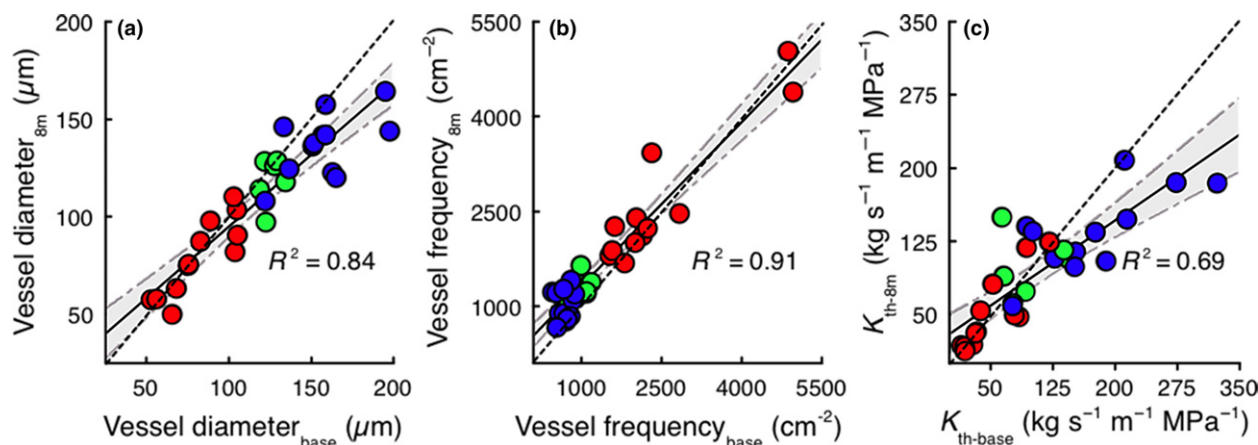

Figure 5 Relationship of hydraulic traits in sapwood sampled at the stem base and at a standardised distance of 8 m below the stem apex of 10 *Eucalyptus* species (*n* = 3 trees per species). (a) Relationship of mean vessel diameter between sampling heights. (b) Relationship of mean vessel frequency between sampling heights. (c) Relationship of theoretical hydraulic conductivity (K_{th}) between sampling heights. Solid lines show linear fits, dotted lines 1 : 1 relations and dot-dashed lines and grey shaded areas indicate 95% confident intervals; colour-coding follows Fig. 2; coefficients of determination are shown.
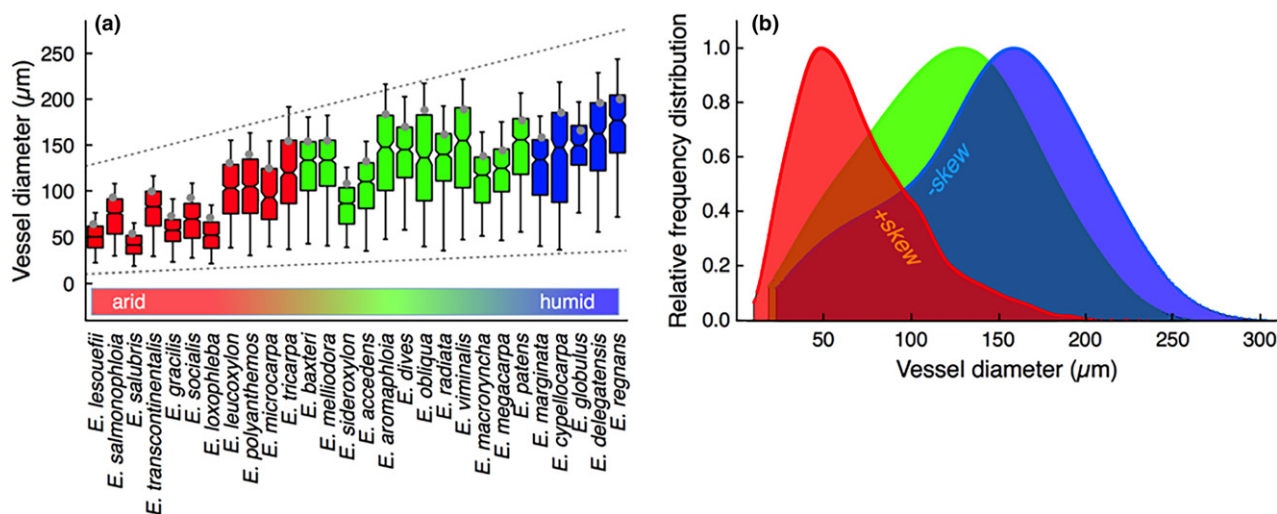
Figure 6 Size and frequency distribution of vessel diameters in stem sapwood of 28 *Eucalyptus* species ($n = 3$ trees per species). (a) Range of vessel diameters measured in each species; notch indicates median, whiskers show 5th and 95th percentiles; grey dots show hydraulically weighted vessel diameters; dotted lines show linear trends of minimum ($y = 0.83x + 11.69$; $R^2 = 0.66$) and maximum mean vessel diameters ($y = 4.98x + 128.16$; $R^2 = 0.65$). (b) Relative frequency distribution of vessel diameters; data were binned into three categories, namely *arid* ($n = 9287$), *dry-temperate* ($n = 5863$) and *humid* ($n = 1544$); distribution of vessel diameters in species from *arid* environments was positively skewed (γ_1: +0.95), that of species sampled in *humid* environments was negatively skewed (γ_1: −0.31), and approached a normal distribution in species from *dry-temperate* environments (γ_1: +0.02); highest vessel diameter in each group was set at 1; colour-coding follows Fig. 2.

stem diameter at the base of a tree and total height (stem length), with vessels tapering as a function of conductive path length. These broad comparisons among species led the authors to suggest that correlations of vessel diameter with climate are an indirect effect of variation in tree size (height in particular) rather than a direct response to aridity (Olson & Rosell 2013; Olson *et al.* 2014). Such conclusions conflict with other evidence that suggests (at least some) hydraulic divergence along climate gradients (Carlquist 1989; De Micco *et al.* 2008; Gleason *et al.* 2012) in accordance with theory on tree hydraulic architecture (Wheeler *et al.* 2007; Sperry *et al.* 2008; Chave *et al.* 2009; Brodribb *et al.* 2012; Carlquist 2012).

Ours is among few studies that assess sapwood traits above the stem base when evaluating relationships of vessel traits and tree height (e.g. Litunen & Kallikoski 2010). Our analyses suggest increasing tree height and associated vessel taper partially influence climate–vessel diameter relationships determined at the stem base. This height-taper effect is revealed in the comparison of sapwood collected from the stem base with samples collected from a fixed distance below the apex. By standardising for path length, and despite taper effects on vessel diameter, our analysis revealed strong climate–vessel diameter relationships and no relationship with stem diameter.

In mesic climates we found that sapwood of eucalypt trees was dominated by wide vessel diameters likely to facilitate transport of large volumes of water necessary to minimise hydraulic constraints on photosynthesis (Mencuccini *et al.* 2007). A positive relationship between vessel diameter and growth rates is observed across species (e.g. Poorter *et al.* 2010), resulting in tall trees with less dense wood. As water availability declines, *Eucalyptus* trees are progressively shorter in stature (Givnish *et al.* 2014 and our study). We found that as sapwood density increased, so did vessel frequency while mean vessel diameter declined. Interestingly, the general

increase in vessel frequency was insufficient to compensate for effects on theoretical hydraulic conductivity of narrowing of vessel diameters. K_{th} declined with increasing aridity, yet the vessel void-to-wood ratio remained relatively unchanged.

Low variation in vessel void-to-wood ratios compared to highly variable diameter and frequency of vessels in *Eucalyptus* is comparable with global data (several thousand angiosperm species; Zanne *et al.* 2010). The average vessel void-to-wood ratio of around 15% observed here is very close to the global average of 14% for angiosperms (Zanne *et al.* 2010). Consistent vessel void-to-wood ratios over a large range of water availability – that resulted in a twofold difference in wood density and a theoretical fourfold difference in hydraulic conductivity – suggests strong interspecific differentiation in traits involved in wood formation in *Eucalyptus*. It has previously been shown that the proportion of tracheids increases as vessel diameters decline (Jacobsen *et al.* 2005). Hence, the proportion of lignified wood matrix (and resulting wood density) can increase even when the overall vessel void-to-wood ratio remains constant. Indeed, for a wide range of angiosperm species it was shown that wood density was affected by the fibre wall fractions and fibre lumina (Ziemińska *et al.* 2015).

We observed marked differences in relative frequency distributions of vessel diameters, while the ratio of vessel void-to-wood remained relatively constant along the climate gradient. This type of information is often lost in reports of mean vessel diameters or D_H. We speculate that observed abundances of comparatively narrow vessels and tracheids in species from arid climates constitute a safeguard for continuous acropetal water transport during periods of low water availability. Embolism of wide vessels would thus not be catastrophic. At the mesic end of the water availability spectrum, wide-diameter vessel systems transport large quantities of water, while a 'safety net' of narrow vessels and tracheids

help maintain limited hydraulic function during short, hot and dry summer periods.

We found no indication of plasticity of vessel development to prevailing but contrasting climates in mature trees within single species. Vessels of seven species sampled at paired drier and wetter sites were not wider at the wetter sites. Given the lack of evidence within a given species for smaller vessel diameters in drier conditions, it seems more likely that genotypic rather than environmental differences are responsible for the smaller vessel diameters of species growing in more arid environments. We cautiously interpret this as an adaptive feature of the 28 study species. Caveats to our interpretation include the effects of other site-related edaphic factors, such as soil properties on growth and wood traits. However, despite the fact that the study sites encompassed a wide range of parent materials and soil orders, climate-driven water availability was the dominant environmental factor related to vascular traits.

A strong genetic influence on the 'shape' of vascular networks raises important questions about the capacity of mature trees to adjust their xylem anatomy to changing environmental conditions. Increasing temperatures and reductions and/or seasonal shifts in precipitation (IPCC 2013) may pose a considerable risk of hydraulic failure, particularly as trees operate with slim safety margins against this failure (Choat *et al.* 2012). Our analysis of ecological wood anatomy of the genus *Eucalyptus* supports the idea that vascular networks and associated traits exhibit adaptive variation. Some tree species display the capacity to adjust vascular traits in response to annual variability of water (e.g. Bryukhanova & Fonti 2012). Yet, our study in the diverse genus *Eucalyptus* shows that vascular traits and wood characteristics appear to be 'hard-wired'. This places eucalypts – and possibly other tree species – at risk to keep pace with anticipated rapid changes in climate.

AUTHORSHIP

S.P. and M.A.A. designed the study; S.P., M.H. and C.M. collected field data and samples; S.P., R.S., M.H. and A.W. analysed samples and collated the database of vessel traits and environmental variables; S.P., M.T. and P.R. provided statistical analyses; S.P. wrote the first draft of the manuscript and all authors contributed to data interpretation and manuscript editing.

REFERENCES

Brodribb, T.J., Pittermann, J. & Coomes, D.A. (2012). Elegance versus Speed: examining the competition between conifer and angiosperm trees. *Int. J. Plant Sci.*, 173, 673–694.

Bryukhanova, M. & Fonti, P. (2012). Xylem plasticity allows rapid hydraulic adjustment to annual climatic variability. *Trees*, 27, 485–496.

Bucci, S.J., Scholz, F.G., Goldstein, G., Meinzer, F.C. & Sternberg, L.D.L. (2003). Dynamic changes in hydraulic conductivity in petioles of two savanna tree species: factors and mechanisms contributing to the refilling of embolized vessels. *Plant, Cell Environ.*, 26, 1633–1645.

Cai, J. & Tyree, M.T. (2010). The impact of vessel size on vulnerability curves: data and models for within-species variability in saplings of aspen, *Populus tremuloides* Michx. *Plant, Cell Environ.*, 33, 1059–1069.

Carlquist, S. (1989). Adaptive wood anatomy of chaparral shrubs. In: *The California Chaparral: Paradigms Re-examined* (ed Keely, J.E.). Los Angeles Country Museum of Natural History Contributions, Los Angeles, pp. 25–35.

Carlquist, S. (2012). How wood evolves: a new synthesis. *Botany*, 90, 901–940.

Chave, J., Coomes, D., Jansen, S., Lewis, S.L., Swenson, N.G. & Zanne, A.E. (2009). Towards a worldwide wood economics spectrum. *Ecol. Lett.*, 12, 351–366.

Choat, B., Cobb, A.R. & Jansen, S. (2008). Structure and function of bordered pits: new discoveries and impacts on whole-plant hydraulic function. *New Phytol.*, 177, 608–626.

Choat, B., Jansen, S., Brodribb, T.J., Cochard, H., Delzon, S., Bhaskar, R. *et al.* (2012). Global convergence in the vulnerability of forests to drought. *Nature*, 491, 752–755.

Crisp, M., Cook, L. & Steane, D. (2004). Radiation of the Australian flora: what can comparisons of molecular phylogenies across multiple taxa tell us about the evolution of diversity in present-day communities? *Phil. Trans. Roy. Soc. B.*, 359, 1551–1571.

De Micco, V., Aronne, G. & Baas, P. (2008). Wood anatomy and hydraulic architecture of stems and twigs of some Mediterranean trees and shrubs along a mesic-xeric gradient. *Trees*, 22, 643–655.

Givnish, T.J., Wong, S.C., Stuart-Williams, H., Holloway-Phillips, M. & Farquhar, G. (2014). Determinants of maximum tree height in *Eucalyptus* species along a rainfall gradient in Victoria, Australia. *Ecology*, 95, 2991–3007.

Gleason, S.M., Butler, D.W., Ziemińska, K., Waryszak, P. & Westoby, M. (2012). Stem xylem conductivity is key to plant water balance cross Australian angiosperm species. *Funct. Ecol.*, 26, 343–352.

Guet, J., Fichot, R., Lédée, C., Laurans, F., Cochard, H., Delzon, S. *et al.* (2015). Stem xylem resistance to cavitation is related to xylem structure but not to growth and water-use efficiency at the within-population level in *Populus nigra* L. *J. Exp. Bot.*, doi:10.1093/jxb/erv232.

Hacke, U.G., Sperry, J.S., Pockman, W.T., Davis, S.D. & McCulloh, K.A. (2001). Trends in wood density and structure are linked to prevention of xylem implosion by negative pressure. *Oecologia*, 126, 457–461.

Hacke, U.G., Sperry, J.S., Wheeler, J.K. & Castro, L. (2006). Scaling of angiosperm xylem structure with safety and efficiency. *Tree Physiol.*, 26, 689–701.

Hajek, P., Leuschner, C., Hertel, D., Delzon, S. & Schuldt, B. (2014). Trade-offs between xylem hydraulic properties, wood anatomy and yield in *Populus*. *Tree Physiol.*, 34, 744–756.

Hargrave, K.R., Kolb, K.J., Evers, F.W. & Davis, S.D. (1994). Conduit diameter and drought-induced embolism in *Salvia mellifera* Greene (Labiatae). *New Phytol.*, 126, 695–705.

IPCC (2013). *Climate Change 2013: The Physical Science Basis*. Cambridge University Press, Cambridge.

Jacobsen, A.L., Ewers, F.W., Pratt, R.B., Paddock, W.A. III & Davis, S.D. (2005). Do xylem fibers affect vessel cavitation resistance? *Plant Physiol.*, 139, 546–556.

Koch, G.W., Sillett, S.C., Jennings, G.M. & Davies, S.D. (2004). The limits to tree height. *Nature*, 428, 851–854.

Ladiges, P.Y. (1997). *Phylogenetic History and Classification of Eucalypts*. Cambridge University Press, Cambridge.

Lens, F., Luteyn, J.L., Smets, E. & Jansen, S. (2004). Ecological trends in the wood anatomy of Vaccinioideae (Ericaceae s.l.). *Flora*, 199, 309–319.

Lens, F., Sperry, J.S., Christman, M.A., 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.

Lines, E.R., Zavala, M.A., Purves, D.W. & Coomes, D.A. (2012). Predictable changes in aboveground allometry in trees along gradients of temperature, aridity and competition. *Global Ecol. Biogeogr.*, 21, 1017–1028.

Litunen, A. & Kallikoski, T. (2010). The effect of tree architecture on conduit diameter and frequency from small distal roots to tips in *Betula pendula*, *Picea abies* and *Pinus sylvestris*. *Tree Physiol.*, 30, 1433–1447.

Maherali, H., Moura, C.F., Caldeira, M.C., Willson, C.J. & Jackson, R.B. (2004). Functional coordination between leaf gas exchange and vulnerability to xylem cavitation in temperate forest trees. *Plant, Cell Environ.*, 29, 571–583.

Maherali, H., Moura, C.F., Caldeira, M.C., Willson, C.J. & Jackson, R.B. (2006). Functional coordination between leaf gas exchange and vulnerability to xylem cavitation in temperate forest trees. *Plant, Cell Environ.*, 29, 571–583.

McCulloh, K., Sperry, J.S., Lachenbruch, B., Meinzer, F.C., Reich, P.B. & Voelker, S. (2010). Moving water well: comparing hydraulic efficiency in twigs and trunks of coniferous, ring-porous, and diffuse-porous saplings from temperate and tropical forests. *New Phytol.*, 186, 439–450.

Meinzer, F.C., McCulloh, K.A., Lachenbruch, B., Woodruff, D.R. & Johnson, D.M. (2010). The blind men and the elephant: the impact of context and scale in evaluating conflicts between plant hydraulic safety and efficiency. *Oecologia*, 164, 287–296.

Mencuccini, M., Hölttä, T., Petit, G. & Magnani, F. (2007). Sanio's laws revisited: size-dependent changes in the xylem architecture of trees. *Ecol. Lett.*, 10, 1084–1093.

Moles, A.T., Warton, D.I., Warman, L., Swenson, N.G., Laffan, L.W., Zanne, A.E. *et al.* (2009). Global patterns in plant height. *J. Ecol.*, 97, 923–932.

Olson, M.E. & Rosell, J.A. (2013). Vessel diameter – stem diameter scaling across woody angiosperms and the ecological causes of xylem vessel diameter variation. *New Phytol.*, 197, 1204–1213.

Olson, M.E., Anfodillo, T., Rosell, J.A., Petit, G., Crivellaro, A., Isnard, S. *et al.* (2014). Universal hydraulics of the flowering plants: vessel diameter scales with stem length across angiosperm lineages, habits and climates. *Ecol. Lett.*, 17, 988–997.

Petherick, L., Bostock, H., Cohen, T.J., Fitzsimmons, K., Tibby, J., Fletcher, M.-S. *et al.* (2013). Climatic records over the past 30 ka from temperate Australia – a synthesis from the Oz-INTIMATE workgroup. *Quaternary Sci Rev.*, 74, 58–77.

Petit, G., Pfautsch, S., Anfodillo, T. & Adams, M.A. (2010). The challenge of tree height in *Eucalyptus regnans*: when xylem tapering overcomes hydraulic resistance. *New Phytol.*, 187, 1146–1153.

Poorter, L., McDonald, I., Alarcón, A., Fichtler, E., Licona, J.-C., Peña-Carlos, M. *et al.* (2010). The importance of wood traits and hydraulic conductance for the performance and life history strategies of 42 rainforest tree species. *New Phytol.*, 185, 481–492.

Santiago, L.S., Goldstein, G., Meinzer, F.C., Fisher, J.B., Machado, K., Woodruff, D. *et al.* (2004). Leaf photosynthetic traits scale with hydraulic conductivity and wood density in Panamanian forest canopy trees. *Oecologia*, 140, 543–550.

Scholz, A., Rabaey, D., Stein, A., Cochard, H., Smets, E. & Jansen, S. (2013). The evolution and function of vessel and pit characteristics with respect to cavitation resistance across 10 *Prunus* species. *Tree Physiol.*, 33, 684–694.

Specht, R.L. & Specht, A. (1989). Canopy structure in *Eucalyptus*-dominated communities in Australia along climatic gradients. *Acta Oecol. Oecol. Plant.*, 10, 191–213.

Sperry, J.S., Hacke, U.G. & Wheeler, J.K. (2005). Comparative analysis of end wall resistivity in xylem conduits. *Plant, Cell Environ.*, 28, 456–465.

Sperry, J.S., Hacke, U.G. & McCulloh, K.A. (2006). Size and function in conifer tracheids and angiosperm vessels. *Am. J. Bot.*, 93, 1490–1500.

Sperry, J.S., Meinzer, F.C. & McCulloh, K.A. (2008). Safety and efficiency conflicts in hydraulic architecture: scaling from tissues to trees. *Plant, Cell Environ.*, 31, 632–645.

Tyree, M.T. & Zimmermann, M.H. (2002). *Xylem Structure and the Ascent of Sap*. Springer, New York.

Tyree, M.T., Davis, S.D. & Cochard, H. (1994). Biophysical perspectives of xylem evolution: is there a tradeoff of hydraulic efficiency for vulnerability to dysfunction? *IAWA J.*, 15, 335–360.

UNEP (1997). *World Atlas of Desertification*. United Nations Environmental Program, Nairobi, Kenya.

Wheeler, J.K., Sperry, J.S., Hacke, U.G. & Hoang, N. (2005). Inter-vessel pitting and cavitation in woody Rosaceae and other vesselled plants: a basis for a safety versus efficiency trade-off in xylem transport. *Plant, Cell Environ.*, 28, 800–812.

Wheeler, E.A., Baas, P. & Rodgers, S. (2007). Variations in dicot wood anatomy: a global analysis based on the InsideWood database. *IAWA J.*, 28, 229–258.

Zanne, A.E., Westoby, M., Falster, D.S., Ackerly, D.D., Loarie, S.R., Arnold, S.E.J. *et al.* (2010). Angiosperm wood structure: global patterns in vessel anatomy and their relation to wood density and potential conductivity. *Am. J. Bot.*, 97, 207–215.

Ziemińska, K., Westoby, M. & Wright, I.J. (2015). Broad anatomical variation within a narrow wood density range – a study of twig wood across 69 Australian angiosperms. *PLoS ONE*, 10, e0124892.

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