

Xylem function of arid-land shrubs from California, USA: an ecological and evolutionary analysis

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ABSTRACT

Xylem traits were examined among 22 arid-land shrub species, including measures of vessel dimensions and pit area. These structural measures were compared with the xylem functional traits of transport efficiency and safety from cavitation. The influence of evolution on trait relationships was examined using phylogenetic independent contrasts (PICs). A trade-off between xylem safety and efficiency was supported by a negative correlation between vessel dimensions and cavitation resistance. Pit area was correlated with cavitation resistance when cross species data were examined, but PICs suggest that these traits have evolved independently of one another. Differences in cavitation resistance that are not explained by pit area may be related to differences in pit membrane properties or the prevalence of tracheids, the latter of which may alter pit area through the addition of vessel-to-tracheid pits or through changes in xylem conduit connectivity. Some trait relationships were robust regardless of species ecology or evolutionary history. These trait relationships are likely to be the most valuable in predictive models that seek to examine anatomical and functional trait relationships among extant and fossil woods and include the relationship among hydraulic conductivity and vessel diameter, between vessel diameter and vessel length, and between hydraulic conductivity and wood density.

Key-words: cavitation resistance; chaparral; coastal sage; ecological wood anatomy; hydraulic conductivity; Mojave Desert; pit area hypothesis.

INTRODUCTION

A central hypothesis of vascular transport is that xylem safety from water stress-induced failure (i.e. cavitation resistance) comes at the cost of reduced efficiency of the hydraulic transport system. In many instances, plants appear to have efficient xylem vessels suggesting that there is selection against inefficient combinations of traits. For instance, in a data set of 29 angiosperm species, vessel length was found to increase with increasing vessel diameter, resulting in a nearly constant end-wall limitation on

the total vessel conductivity (Hacke *et al.* 2006). However, at least evolutionarily, it appears that ancient versus more recently derived lineages may display different scaling patterns and potentially less efficient trait combinations (Sperry *et al.* 2007).

Large vessels, which maximize conductivity, also tend to be more vulnerable to water stress-induced cavitation (Salleo & Lo Gullo 1989; Hargrave *et al.* 1994; Pockman & Sperry 2000; Martinez-Vilalta *et al.* 2002; Tyree & Zimmermann 2002; Wheeler *et al.* 2005); however, the strength of the trade-off between these traits is inconsistent. As there is no direct causal link between vessel diameter and the putative mechanism of cavitation by water stress (the air seeding hypothesis), relationships among these traits are likely caused by other changes in vessel anatomy that occur concomitantly with changes in vessel diameter. The 'pit area hypothesis' provides a basis for the trade-off often found between transport safety and efficiency, because larger vessels tend to have more collective pit area than small ones. Vulnerability to water stress-induced cavitation is correlated to the diameter of the largest pore in inter-vessel pit membranes. Vessels that contain a large amount of pit area have a greater probability of having a large pit pore (Choat *et al.* 2005). Experimental data show a strong inverse relationship between the total inter-vessel pit area per vessel (A_P) and cavitation resistance across a large number of species (Wheeler *et al.* 2005; Hacke *et al.* 2006). However, the pit area hypothesis focuses on inter-vessel pits and has not been tested in many species with highly diverse xylem including species with vasicentric or vascular tracheids. It is not clear whether pits at the interface between vessels and tracheids may also act as potential air-seeding sites, thereby altering or weakening the relationship of cavitation pressure with A_P . Additionally, few studies that have examined the relationship among these traits have taken into account the evolutionary history of species, which may also influence trait correlations.

Woody shrub species dominate several plant communities occurring in the winter rainfall region of southern California. Shrub species from these communities show a wide range of wood anatomical traits (Carlquist & Hoekman 1985). A prevalent feature of the woody angiosperm flora of southern California is the presence of vascular, vasicentric or true tracheids occurring concomitantly with vessels (Carlquist 2001). In wood with fibres or fibre-tracheids, vascular

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tracheids are present only in the last layers of a growth ring (Carlquist & Hoekman 1985). By contrast, vascentric tracheids are intermixed with vessels and occur throughout a growth ring. When true tracheids are present they are the sole imperforate tracheary element type in the woods in which they occur. Great diversity among these shrub species exists in terms of tracheid type and other basic features such as vessel diameters, vessel grouping, vessel sculpturing and number of vessels per square millimetre (Carlquist & Hoekman 1985; Carlquist 2001). Such variation in wood anatomy, particularly in vessel dimensions, likely impacts xylem functional traits in these species and may account for the variety of relationships found among xylem functional traits in species from these communities.

Species from three southern Californian communities, chaparral, coastal sage scrub and Mojave Desert scrub, display non-convergent patterns in water use particularly in relation to how plants in these communities respond to seasonal water stress (Jacobsen *et al.* 2008). These species are morphologically similar (shrub habit and sclerophyllous leaves) consistent with a pattern of structural convergence in response to similar seasonal rainfall patterns. However, similarity in plant form does not coincide with similar water relations and functional traits. Species from the chaparral, coastal sage scrub and Mojave Desert scrub communities differ in the relationships between minimum seasonal water potential, xylem pressure associated with 50% loss of hydraulic conductivity (P_{50}), xylem specific hydraulic conductivity (K_s) and xylem density (Jacobsen *et al.* 2007a,b, 2008) suggesting that they may also diverge in the relationship between their xylem functional and structural traits.

Here we measured key wood anatomical parameters of 22 arid-land shrub species from these three dominant southern Californian plant communities. Anatomical parameters were compared with transport efficiency and safety from cavitation to test whether non-convergent water-use patterns correspond with non-convergent structure–function correlations among these diverse species. By limiting comparisons with these species which are functionally, evolutionarily and anatomically diverse but which are from semi-arid winter rainfall communities (see Jacobsen *et al.* 2008 for more detailed data on study site climatic similarities and differences) and are similar in form, stature and habit, we attempted to minimize the potential for changes in structure–function relationships related to habitat (i.e. riparian versus upland) or form (i.e. shrubs versus trees), etc. The influence of species phylogenetic relationships was examined by analysing data using phylogenetic independent contrasts (PICs; Felsenstein 1985). Species examined in the present study form a fully resolved and well-supported phylogeny. If relationships could be identified between wood anatomical traits and transport physiology that are robust both within lineages and across evolutionarily diverse groups and irrespective of functional strategy, such traits could prove useful in predicting plant ecological and physiological traits based on living or fossil wood specimens.

MATERIALS AND METHODS

Plant material

The 22 species included in this study (Table 1) represent a subset of species studied by Jacobsen *et al.* (2007a, 2008). Species showed diverse xylem anatomy with regards to vessel size and clustering as well as the tissue surrounding the vessels. In most of the study species, vessels were associated with vascentric (and sometimes vascular or true) tracheids (Carlquist & Hoekman 1985; Carlquist 2001). *Atriplex canescens* is distinct because of its successive cambia (Carlquist 2001). Wood anatomical parameters were measured in the present study on the stems of the same plants or plant populations whose hydraulic conductivities and cavitation resistances were measured previously (Jacobsen *et al.* 2007a). Stems were cut to segments 4–9 mm in diameter and 25–35 cm in length. Segments were placed in plastic bags with moist paper towels, placed in a cooler and shipped to the University of Alberta for anatomical analysis.

Vessel diameter measurements

Transverse sections of five to six stem segments per species were prepared with razor blades or with a sliding microtome. Sections were stained with toluidine blue for 3 min, rinsed in water and mounted on glass slides with glycerin. Photographs of the outer two to three growth rings were taken with a Leica DFC420 C digital camera mounted on a DM3000 microscope (Leica Microsystems, Wetzlar, Germany). Magnification was usually 200 \times . The diameter of each vessel lumen in a radial sector of functional xylem was measured with image analysis software (ImagePro Plus version 6.1, Media Cybernetics, Silver Spring, MD, USA). A minimum of three radial sectors per stem was measured to obtain an average based on a minimum of 100 vessel diameters per stem. Based on the Hagen–Poiseuille equation, the diameter of the average vessel of a stem segment was calculated as $D = [(\Sigma d^4)/n]^{1/4}$, where n is the number of vessels measured, and d is the individual vessel lumen diameter. Hence, D represents the diameter of a vessel of average lumen conductivity (Tyree & Zimmermann 2002; Wheeler *et al.* 2005). The species mean was the mean of five to six segments.

Vessel length

The mean vessel length (L) was measured on a set of five stems per species by using the silicone injection method (Sperry, Hacke & Wheeler 2005; Hacke *et al.* 2007). Stem segments typically had a minimum length of 25 cm. The basal end of each stem was trimmed with a fresh razor blade and fitted to plastic tubing with gaskets made from rubber tubing. Stems were then flushed with filtered (0.2 μm) 20 mM KCl solution to remove embolism. Flushing proceeded at a water pressure of 50 kPa for 20 min. The basal end of the segments was injected with silicone at 50 kPa for

Table 1. Species included in this study, the site and the species' figure symbols

Species/site	Figure symbol	Family
Chaparral – Cold Creek Canyon Preserve, Santa Monica Mountains, CA, USA (34.5 N 118.4 W)		
<i>Adenostoma fasciculatum</i> Hook & Arn.	Af	Rosaceae
<i>Arctostaphylos glandulosa</i> Eastw.	Ag	Ericaceae
<i>Ceanothus cuneatus</i> (Hook.) Nutt.	Cc	Rhamnaceae
<i>Ceanothus megacarpus</i> Nutt.	Cm	Rhamnaceae
<i>Ceanothus oliganthus</i> Nutt.	Co	Rhamnaceae
<i>Ceanothus spinosus</i> Nutt.	Cs	Rhamnaceae
<i>Quercus berberidifolia</i> Liebm.	Qb	Fagaceae
<i>Rhus ovata</i> S. Watson	Ro	Anacardiaceae
Coastal sage scrub – preserve located at Pepperdine University, Malibu, CA, USA (34.2 N 118.4 W)		
<i>Artemisia californica</i> Less.	Ac	Asteraceae
<i>Eriogonum cinereum</i> Benth.	Ec	Polygonaceae
<i>Hazardia squarrosa</i> (Hook. & Arn.) E. Greene	Hsq	Asteraceae
<i>Lotus scoparius</i> (Nutt.) Ottley	Lsc	Fabaceae
<i>Malosma laurina</i> (Nutt.) Abrams	Ml	Anacardiaceae
<i>Salvia leucophylla</i> E. Greene	Sl	Lamiaceae
<i>Salvia mellifera</i> E. Greene	Sm	Lamiaceae
Mojave Desert scrub – Red Rock Canyon State Park, CA, USA (35.2 N 117.6 W)		
<i>Ambrosia dumosa</i> (A. Gray) Payne	ad	Asteraceae
<i>Atriplex canescens</i> (Pursh) Nutt.	aca	Chenopodiaceae
<i>Coleogyne ramosissima</i> Torrey	cr	Rosaceae
<i>Hymenoclea salsola</i> A. Gray	hs	Asteraceae
<i>Isomeris arborea</i> Nutt.	ia	Capparaceae
<i>Larrea tridentata</i> (DC.) Cov.	lt	Zygophyllaceae
<i>Lepidospartum squamatum</i> (A. Gray) A. Gray	ls	Asteraceae

24 h. A 10:1 silicone-hardener mixture was used (Rhodorsil RTV 141, Bluestar Silicones, distributed by Skycon, Toronto, ON, Canada). Because the elastomer is transparent, it was mixed with a soluble fluorescent whitening agent (Uvitex OB, Ciba Specialty Chemicals, Tarrytown, NY, USA). The Uvitex was dissolved in chloroform (1% w/w), and one drop was added per gram of the silicone-hardener mix (Hacke *et al.* 2007; Sperry *et al.* 2007). After the injection was completed, segments were left at room temperature for at least 48 h to allow the silicone to cure. Stems were then sectioned at several distances from the injection surface. As most vessels were short, an exponential scale was used (Wheeler *et al.* 2005). Cross-sections were observed using a fluorescence microscope (same equipment as described earlier). The length distribution of the cross-sectional sample of vessels was measured by using the exponential decay function described by Wheeler *et al.* (2005), (their eqns 2 and 3). The L used to represent a segment corresponded to the mean of the log-transformed vessel length distribution. A Weibull function should be used to represent tracheid distributions, because they have a minimum length, which the exponential decay function does not consider (Sperry *et al.* 2007). However, vessel distributions can usually be described by the simpler and more widely used exponential decay function. A comparison in four species (*Ceanothus spinosus*, *Lotus scoparius*, *Salvia leucophylla*, *Lepidospartum squamatum*) yielded a maximum difference of 4.5% between the mean L determined by Weibull versus exponential functions.

Pit area

The A_P was measured as described by Wheeler *et al.* (2005). The A_P was estimated as the product of the fraction of inter-vessel pit area per vessel surface area (F_P) and the surface area of the average vessel ($A_P = F_P \pi D L$). The F_P was the product of the average vessel contact fraction and pit-field fraction. The contact fraction is the fraction of the vessel surface area in pitted contact with adjacent vessels. This was estimated from the ratio of contact perimeter to total vessel perimeter, after being summed across all vessels in a xylem sector. Tracheids adjacent to vessels differed widely in their pit contacts and were not included in measurements of contact fraction. Contact fractions were measured in the same transverse sections used for measuring D , and are means of five segments per species. As only a fraction of the contact area between vessels is occupied by pits, we measured the pit-field fraction. This is the fraction of the total inter-vessel pit area per unit contact area. It was measured in hand-cut longitudinal sections of a subsample of the stems used for determining D . A 100 \times magnification oil immersion lens (Leica) was used to measure this parameter.

Mean cavitation pressure

Cavitation resistance of stem segments was measured previously (Jacobsen *et al.* 2007a). Vulnerability curves for each species were generated with the centrifuge method (Alder

et al. 1997). Vulnerability curves indicate the loss of hydraulic conductivity associated with progressively more negative xylem pressure. Measurements were conducted during the wet season. Curves of *C. ramosissima* and *A. canescens* were re-measured in May 2008 because of consistently high levels of xylem fatigue in these species that made estimates of cavitation resistance difficult. Vulnerability curves were scaled to the percent loss of hydraulic conductivity at a xylem pressure of -0.25 MPa to correct for cavitation fatigue (Hacke *et al.* 2001b). After fitting the scaled curves of each species with a Weibull function, cavitation resistance was expressed with the mean cavitation pressure (Hacke *et al.* 2006). The mean cavitation pressure is the mean of the Weibull probability density function. In perfectly sigmoidal curves, the mean cavitation pressure equals the P50.

Specific conductivity

The xylem area-specific conductivity (K_s) data were measured previously by Jacobsen *et al.* (2007a, 2008). In brief, stems were collected from the field during the wet season. Stems were cut under water in the field and then transported to the laboratory where they were trimmed under water from both ends until a segment 4–8 mm in diameter and 14 cm in length was obtained. Stems were then connected to a tubing system and flushed for 1 h at 100 kPa and the maximum hydraulic conductivity (K_{hmax}) of stems was measured gravimetrically (Sperry, Donnelly & Tyree 1988) using an analytical balance (CPI24S, Sartorius, Goettingen, Germany). The K_{hmax} was divided by the xylem area (minus the pith) to obtain the xylem specific conductivity.

Wood density

Wood density data were taken from Jacobsen *et al.* (2008). Stem segments c. 5 cm long and 6–8 mm in diameter were split longitudinally and their pith and bark removed. Xylem segments were then soaked in degassed water adjusted to a pH of 2 overnight. Wood density was determined as described in Hacke *et al.* (2001a) and Jacobsen *et al.* (2008).

Use of log–log plots to evaluate scaling relationships

Assuming that D and A_P were linearly related to the size of the largest pore of a vessel, axes were log-transformed to linearize the regression functions (Fig. 1). Power functions of the form $\log(y) = a + b \log(x)$ were used. Thus, the mean cavitation pressure changes in proportion to x^b , where x represents the anatomical parameter. The relationships of K_s with D (Martinez-Vilalta *et al.* 2002; Sperry, Hacke & Pittermann 2006) and A_P (Wheeler *et al.* 2005) can also be described by power functions (Fig. 2). A log–log plot was also used to evaluate the scaling of vessel diameter with length (Wheeler *et al.* 2005). Standardized major axis (SMA) slope-fitting was used for all analyses, because we

were interested in estimating the scaling best describing the bivariate scatter of the variables, which were both subject to measurement error (Niklas 1994; Warton *et al.* 2006). Regression slopes and all other statistical parameters were calculated using the computer package SMATR 2.0 (Warton *et al.* 2006). Only regressions significant at $P \leq 0.05$ are shown in the graphs.

PICs

PICs were calculated for measured parameters using published phylogenetic data. The phylogeny was constructed by hand using recent molecular-based phylogenies (Morgan, Soltis & Robertson 1994; Bayer & Starr 1998; Hardig, Soltis & Soltis 2000; Goertzen *et al.* 2003; Hilu *et al.* 2003; Urbatsch, Roberts & Karaman 2003; Soltis & Soltis 2004; Soltis *et al.* 2005). As we combined several trees to create this phylogeny we lacked branch length information; therefore, all PIC analyses were run assuming equal branch lengths (Ackerly 2000). PICs were calculated using COMPARE (Martins 2004).

Principal component analysis

Principal component analysis was used to assess the relationships among measured plant structural and functional traits of species from different communities or among species differing in presence and type of xylem tracheids. The xylem traits of mean cavitation pressure, K_s , A_P , wood density and D were included in this analysis. Tracheid presence and type follow those reported in Carlquist & Hoekman (1985). Differences between principle components among communities and tracheid type were examined using analysis of variance.

RESULTS

Wider vessels were associated with more vulnerable xylem across all species (Fig. 1a, solid regression line), and within chaparral (Fig. 1a, dash-dotted line) and coastal sage species (Fig. 1a, dashed line). Mean vessel diameters ranged from $17.7 \mu\text{m}$ in *I. arborea* to $57.8 \mu\text{m}$ in *M. laurina* (Fig. 1a). The slopes of the regression lines of cavitation resistance and vessel diameter were not different for chaparral and coastal sage communities; however, these traits were not correlated among Mojave Desert species. Some Mojave species like *I. arborea*, *C. ramosissima*, *A. canescens* and *A. dumosa* had relatively narrow vessels yet were not very resistant to cavitation, a combination of traits not prevalent in the other two communities. Vessel length was correlated with mean cavitation pressure only within chaparral species, with longer vessels being more vulnerable than shorter vessels (Fig. 1b). Inter-vessel pit area showed a correlation with mean cavitation pressure across all communities (Fig. 1c, solid line) and within chaparral species (Fig. 1c, dash-dotted line).

Vessel length, diameter and pit area were not correlated with mean cavitation pressure when data were analysed

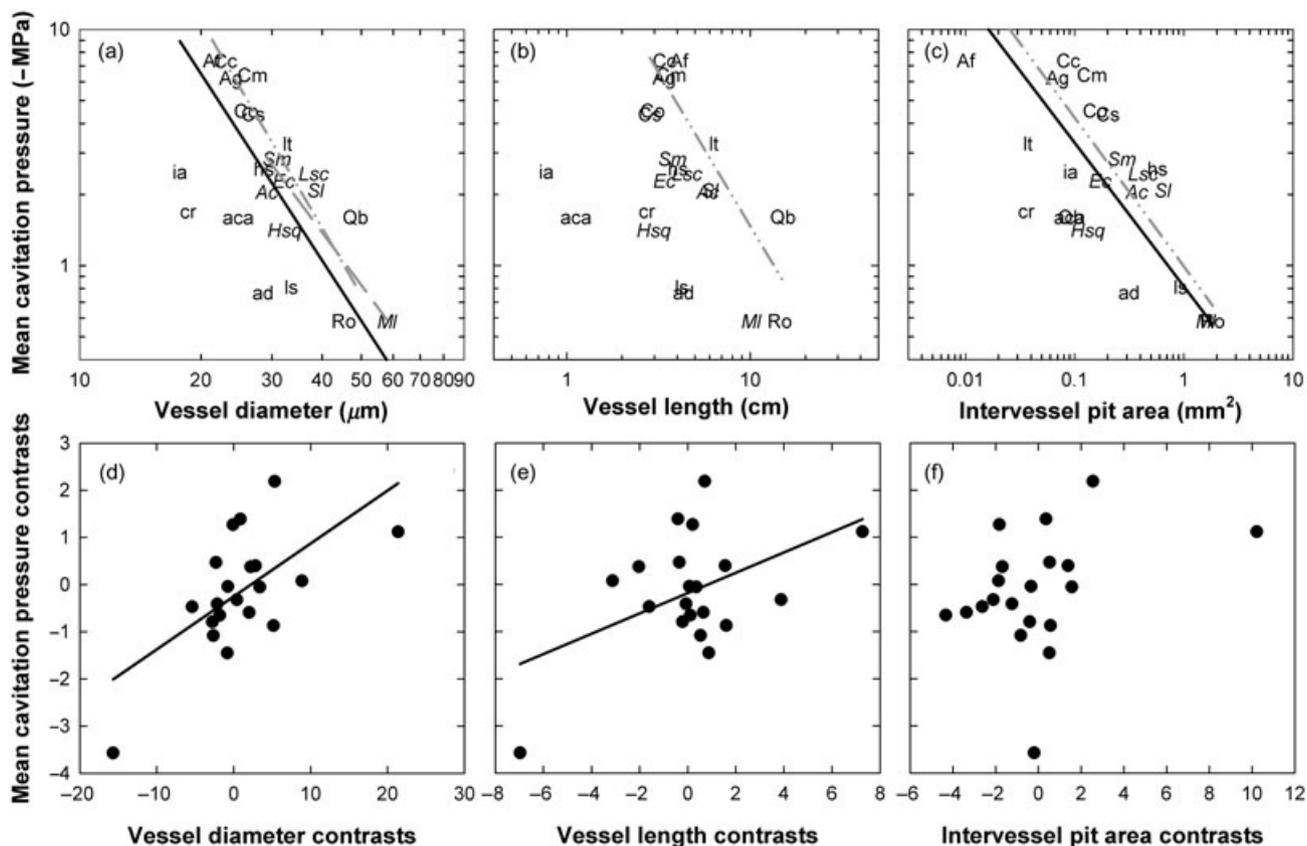


Figure 1. (a–c) Relations between the mean cavitation pressure (shown as absolute value for log scaling) of stems versus (a) vessel diameter ($r^2 = 0.34$, $P = 0.005$ for combined data; $r^2 = 0.85$, $P = 0.001$ for chaparral; $r^2 = 0.65$, $P = 0.028$ for coastal sage; $r^2 = 0.06$, $P = 0.60$ for Mojave); (b) vessel length ($r^2 = 0.12$, $P = 0.11$ for combined data; $r^2 = 0.77$, $P = 0.004$ for chaparral; $r^2 = 0.42$, $P = 0.11$ for coastal sage; $r^2 = 0.02$, $P = 0.74$ for Mojave); and (c) total inter-vessel pit area per vessel ($r^2 = 0.42$, $P = 0.001$ for combined data; $r^2 = 0.56$, $P = 0.032$ for chaparral; $r^2 = 0.35$, $P = 0.16$ for coastal sage; $r^2 = 0.31$, $P = 0.20$ for Mojave). Note the \log_{10} scale. Regression lines are standardized major axis (SMA) regressions and are shown for significant relationships only (continuous line for combined data; dash-dotted line for chaparral; long dash for coastal sage; short dash for Mojave Desert). Data points are mean values of species. Individual species are labelled with abbreviations from Table 1. Lowercase letters refer to Mojave Desert species; italic letters refer to coastal sage species; combination of upper and lowercase letters and regular font represent chaparral species. (d–f) Relationships between phylogenetic independent contrasts of these traits after excluding an outlier (*Coleogyne ramosissima*, see text). Significant correlations with mean cavitation pressure contrasts existed with (d) vessel diameter contrasts ($r^2 = 0.40$, $P = 0.003$), and (e) vessel length contrasts ($r^2 = 0.23$, $P = 0.033$), but not with (f) pit area contrasts ($r^2 = 0.11$, $P = 0.16$).

using PICs and all species were included in analyses. However, one species, *Coleogyne ramosissima* (an outlier), strongly influenced PIC analyses, potentially because it has both true tracheids within its xylem and reaches very high levels of native embolism seasonally. Because of the strong influence of this data point, these analyses were also run excluding *C. ramosissima* (Fig. 1d–f). Removing this species from these analyses resulted in a significant correlation among PICs between mean cavitation pressure and D and L ($P = 0.003$ and 0.033 , respectively); however, the relationship between mean cavitation pressure and pit area remained uncorrelated (Fig. 1f, $P = 0.16$). For clarity, only analyses which removed this highly influential data point (*C. ramosissima*) are included in Fig. 1d–f and in the discussion. In the following PIC analyses, *C. ramosissima* was not an outlier and was included.

Xylem specific hydraulic conductivity increased with increasing vessel diameter (Fig. 2a), a trend seen across all

communities as well as within the coastal sage community. In the chaparral community, K_s and D were marginally correlated ($P = 0.06$). Specific conductivity was also correlated with vessel length (L) (Fig. 2b) and A_P (Fig. 2c) across all communities and within the coastal sage community. Within the Mojave Desert community, K_s was marginally correlated with L and A_P ($P = 0.07$ for both L and A_P). Phylogenetic contrasts indicated that K_s was correlated with D and A_P (Fig. 2d,f), but not with L (Fig. 2e, $P = 0.14$). Across all species K_s was negatively correlated with wood density (Fig. 3a). Species with dense wood like *A. canescens* tended to be less hydraulically efficient than species with light wood such as *M. laurina* and *S. leucophylla*. This trend existed across all species and these traits were also correlated when analysed using PICs (Fig. 3b).

The mean vessel length of species differed by more than an order of magnitude and ranged from 0.8 to 15.1 cm (Fig. 4a). The shortest vessels were found in *I. arborea*, *A.*

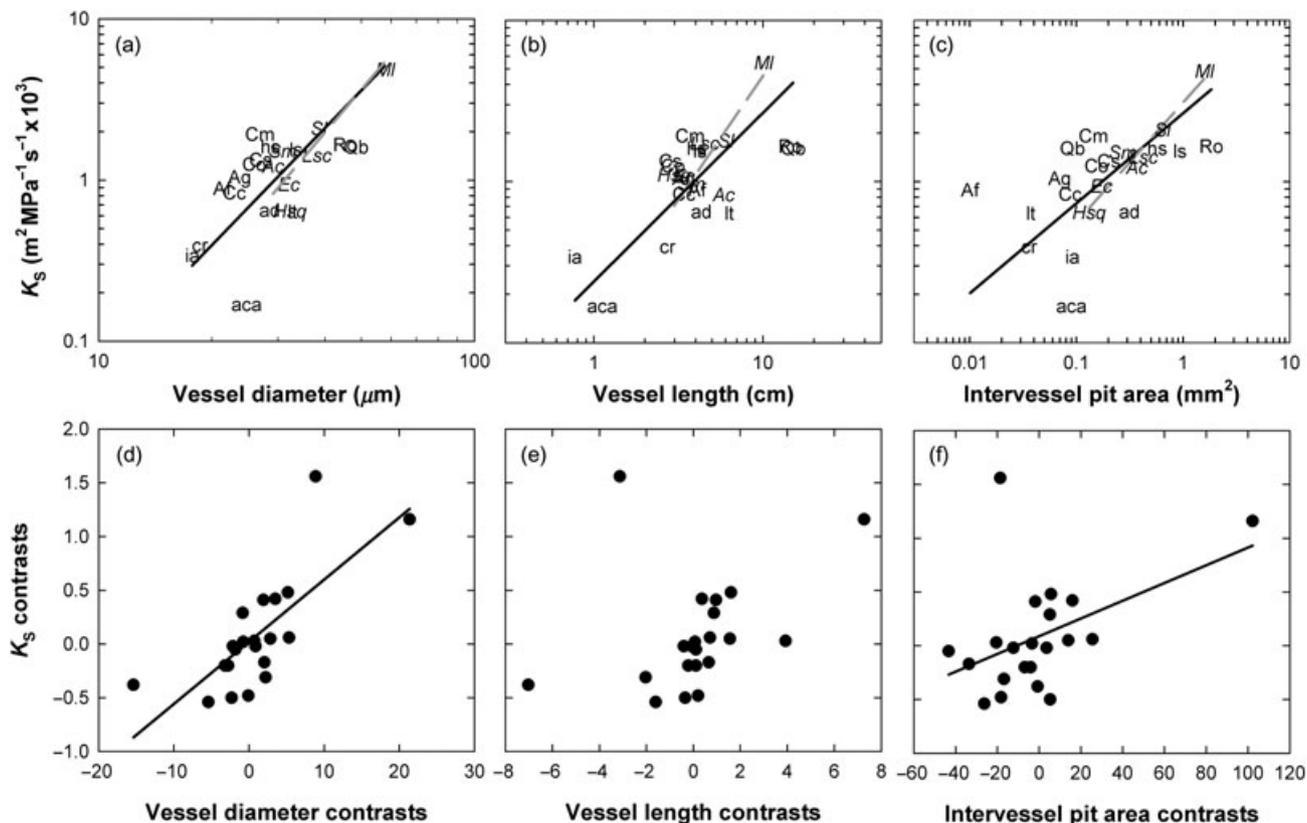


Figure 2. (a–c) Relations between the xylem area-specific conductivity (K_s) of stems versus (a) vessel diameter ($r^2 = 0.47$, $P < 0.001$ for combined data; $r^2 = 0.47$, $P = 0.06$ for chaparral; $r^2 = 0.73$, $P = 0.015$ for coastal sage; $r^2 = 0.38$, $P = 0.14$ for Mojave); (b) vessel length ($r^2 = 0.51$, $P < 0.001$ for combined data; $r^2 = 0.23$, $P = 0.23$ for chaparral; $r^2 = 0.83$, $P = 0.004$ for coastal sage; $r^2 = 0.51$, $P = 0.07$ for Mojave); and (c) total inter-vessel pit area per vessel ($r^2 = 0.36$, $P = 0.003$ for combined data; $r^2 = 0.39$, $P = 0.10$ for chaparral; $r^2 = 0.94$, $P < 0.001$ for coastal sage; $r^2 = 0.51$, $P = 0.07$ for Mojave). Note the \log_{10} scale. Regression lines are standardized major axis regressions. Lines and symbols as in Fig. 1. (d–f) Relationships between phylogenetic independent contrasts of these traits. Significant correlations with K_s contrasts existed with (d) vessel diameter contrasts ($r^2 = 0.56$, $P < 0.001$) and (f) pit area contrasts ($r^2 = 0.21$, $P = 0.036$), but not with (e) vessel length contrasts ($r^2 = 0.11$, $P = 0.14$).

canescens and *C. ramosissima*, all of which occur in the Mojave Desert. The longest vessels occurred in *R. ovata*, *Q. berberidifolia* and *M. laurina*. Across and within all three plant communities, D increased with L (Fig. 4a). The slope of the regression line for the combined data was 0.43. When analysed using PICs, D and L were strongly correlated (Fig. 4b).

Principal component analysis indicated that communities differed in the relationships among their xylem structural and functional traits (Fig. 5). The analysis resulted in two components that described 81.7% of the variability among species ($P < 0.001$). The first component (PC1) explained 63% of the variance and was associated with K_s , D , A_p and wood density (eigenvectors of -0.308 , -0.481 , -0.508 , -0.494 , 0.414 , for MCP, K_s , A_p , D and density, respectively), whereas the second component (PC2) explained 18.7% of the variance and was associated most strongly with mean cavitation pressure (eigenvectors of 0.828 , -0.371 , 0.044 , 0.132 , 0.396 , for MCP, K_s , A_p , D and density, respectively). Communities significantly differed in their principal components (Fig. 5a; $P = 0.020$ for PC1 and $P = 0.013$ for PC2). Mojave Desert species significantly differed from both

coastal sage ($P = 0.009$) and chaparral ($P = 0.028$) in PC1, whereas for PC2 chaparral species significantly differed from both coastal sage ($P = 0.029$) and Mojave Desert ($P = 0.028$) communities. Species differing in the type of tracheids present in their xylem did not significantly differ in principal components values (Fig. 5b; $P = 0.302$ for PC1 and $P = 0.518$ for PC2); although, tracheid type analysis was limited because of few species with no tracheids, true tracheids or vascular tracheids (i.e. most of the species in the present study contained vascentric tracheids).

DISCUSSION

Xylem safety and efficiency

A trade-off was found between safety versus hydraulic efficiency as indicated by the significant negative correlation between vessel diameter and cavitation resistance. This relationship was supported both across all species and within the chaparral and coastal sage communities (Fig. 1a, dashed and dash-dotted lines). There was also support for a relationship between vessel length and cavitation resistance

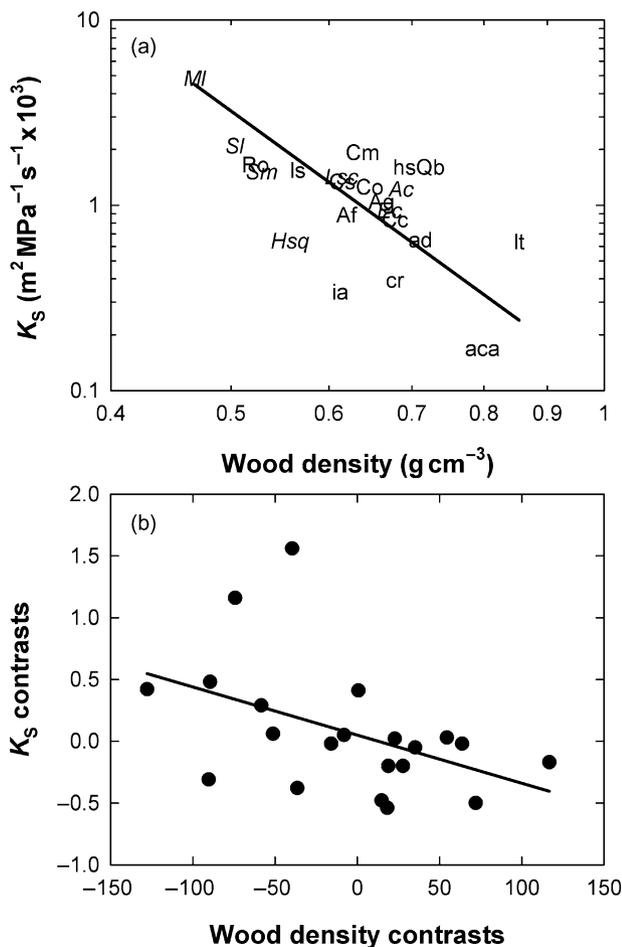


Figure 3. (a) Scaling of xylem area-specific conductivity (K_s) of stems with wood density ($r^2 = 0.37$, $P = 0.003$ for combined data). Note the \log_{10} scale. Regression lines are standardized major axis regressions. Lines and symbols as in Fig. 1. (b) Relationship between phylogenetic independent contrasts of these traits were also significant ($r^2 = 0.21$, $P = 0.036$).

within the chaparral community. Both analyses of species data as well as PICs provide support for a functional link between cavitation resistance and vessel dimensions. The pit area hypothesis provides a possible explanation for a relationship between cavitation resistance and vessel size because larger vessels tend to have more collective pit area than small ones and thus would be more vulnerable to cavitation. Moreover, cavitation of wide and long vessels will result in a greater loss of K_s than cavitation of narrow and short vessels.

There was a significant correlation between pit area and cavitation resistance when species trait values were examined; however, the relationship between these traits was not supported by PICs (Fig. 1f). These results suggest that pit area and cavitation resistance have evolved independently of one another. The lack of correlated evolution between these two traits is likely related to the nature of the relationship between pit area and cavitation resistance. Species with low pit areas (between zero and 0.2 mm^2) span nearly the full range in measured cavitation resistance (-1.4 to

-7.3 MPa) in the present study. It appears that to develop the highest levels of cavitation resistance, species must have small pit areas; however, there is apparently no limitation to having low pit area and low cavitation resistance among species in this study. The species here may have had low pit area before they evolved high cavitation resistance explaining why the traits have not evolved concurrently. Having low pit area may be an exaptation (*sensu* Gould & Vrba 1982) for evolving high cavitation resistance and may have originally evolved for reasons such as reduction of risk of pathogen spread or for mechanical advantages. Combining low pit area with low cavitation resistance results in wood that is both low in xylem efficiency and low in stress resistance. According to classical competition theory, a species with this suite of traits should be competitively excluded; however, such trait combinations may be possible in arid and semiarid environments where competitive interactions may be minimal (see more in the following discussion). The

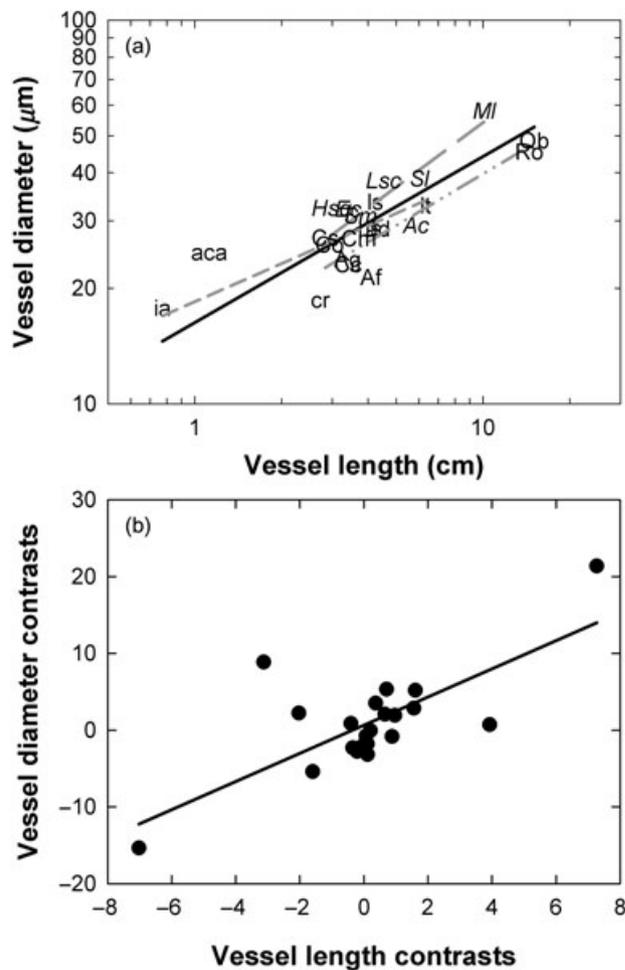


Figure 4. (a) Scaling of vessel diameter with length ($r^2 = 0.64$, $P < 0.001$ for combined data; $r^2 = 0.85$, $P = 0.001$ for chaparral; $r^2 = 0.59$, $P = 0.043$ for coastal sage; $r^2 = 0.58$, $P = 0.047$ for Mojave Desert). Note the \log_{10} scale. Regression lines are SMA regressions. Lines and symbols as in Fig. 1. (b) Relationship between phylogenetic independent contrasts of these traits were also significant ($r^2 = 0.53$, $P < 0.001$).

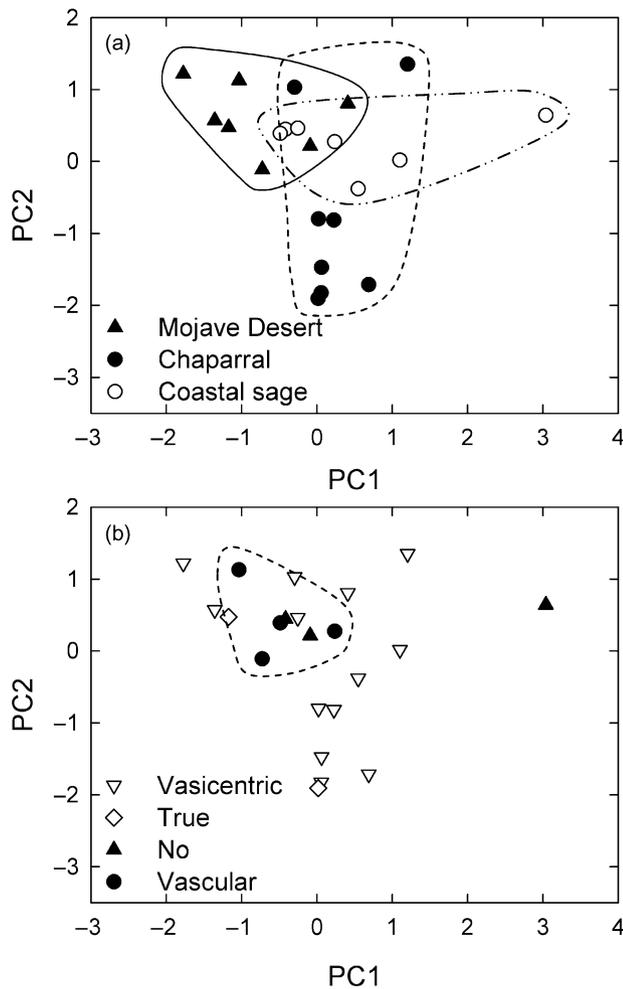


Figure 5. Principal component analysis including the xylem traits of K_s , A_p , wood density, D and mean cavitation pressure. The first component (PC1) was associated with K_s , A_p and density, whereas the second component (PC2) was associated most strongly with mean cavitation pressure. Communities significantly differed in their principal components ($P = 0.020$ for PC1 and $P = 0.013$ for PC2). (a) Mojave Desert species (triangles, enclosed by solid line) significantly differed from both coastal sage (open circles, dash-dotted line, $P = 0.009$) and chaparral (closed circles, dashed line, $P = 0.028$) communities in PC1, whereas chaparral species significantly differed from both coastal sage ($P = 0.029$) and Mojave Desert ($P = 0.028$) communities in PC2. (b) Species differing in the type of tracheids present in their xylem did not significantly differ in principal component values ($P = 0.302$ for PC1 and $P = 0.518$ for PC2). Closed circles enclosed in dashed line, vascular tracheids; open triangles, vasicentric tracheids; diamonds, true tracheids; closed triangles, no tracheids.

lack of correlated evolution between pit area and cavitation resistance suggests that these two traits are likely not functionally related in arid and semi-arid shrubs, save for the case of species with high levels of pit area which appear to be excluded from developing high levels of cavitation resistance.

Other factors besides inter-vessel pit area may explain the safety versus efficiency trade-off.

A recent study (Christman, Sperry & Adler 2009) suggests that differences in species' vulnerability are primarily because of differences in the frequency of rare, leaky pits, rather than A_p . It seems that the frequency of large pores will depend on both tissue and pit-level properties (Choat & Pittermann 2009). Pit membrane properties vary considerably among species in conductance, thickness and porosity (Sano 2005; Wheeler *et al.* 2005; Hacke *et al.* 2006; Choat, Cobb & Jansen 2008; Jansen, Choat & Pletsers 2009). Species with small pit areas do not necessarily have resistant xylem (e.g. *H. squarrosa* in Fig. 1c), which according to the air seeding hypothesis, predicts that they have thinner and more porous pit membranes than species that are much more resistant at a similar A_p (e.g. *C. megacarpus*). This prediction is testable, and a study of pit membrane characteristics of species that are matched in pit area, but different in cavitation resistance could be valuable.

The influence of tracheids on xylem air-seeding potential

The woody flora of southern California has a high number of species with vasicentric and/or vascular tracheids (Carlquist & Hoekman 1985). Most chaparral shrub species have vasicentric tracheids. Tracheids are also present in many species from the coastal sage and Mojave Desert scrub communities. However, the type of tracheids (vasicentric, vascular or true tracheids) and the extent of the vessel-tracheid contact vary among species.

Tracheids could potentially alter the relationship among xylem structural and functional traits through both alteration of xylem connectivity and through changes in vessel air-seeding potential. The connectivity of vessels refers to the number of unique vessels (or other tracheary elements) that share pits, and greater connectivity means more paths for water transport and greater hydraulic efficiency. However, greater connectivity may also mean greater probability of connecting to a gas-filled vessel, tracheid or intercellular space and thus reduced safety (Loepfe *et al.* 2007; Schenk *et al.* 2008). It is possible that some of the woods with low A_p and vulnerable xylem may have a greater degree of vessel connectivity and thus greater potential for air-seeding.

We predicted that the presence of tracheids or tracheid type may alter xylem structure-function relations; however, differences in tracheid type did not lead to consistent differences among species trait relationships (Fig. 5b). Although vascular tracheid-bearing species tended to cluster, the tracheid type did not explain significant inter-specific variability among xylem structural and functional traits. Our measures of A_p describe the collective area of vessel-to-vessel pits and do not consider vessel-tracheid pits. If air-seeding also occurs between vessels and tracheids, our data will underestimate the collective pit area of a vessel where air-seeding may occur. Studying the type and extent of vessel to tracheid contact in these species may be important in furthering understanding of the functional role of tracheids in the xylem of arid-land shrubs.

Community ecology and xylem function

Species from different communities significantly differed in their suites of xylem structural and functional traits (Fig. 5a). This is consistent with the differences in functional water use traits reported for these communities (Jacobsen *et al.* 2008) and suggests that xylem traits may reflect specific environmental or ecologically selected relationships. Chaparral species have relatively maximized efficiency for their high resistance to cavitation that likely reflects that this community is both abiotically stressful (because of protracted rainless periods) and relatively competitive (chaparral shrub densities are greater than in Mojave Desert and coastal sage scrub communities). In contrast, plants from the Mojave Desert are both vulnerable to cavitation and inefficient in water transport. This trait combination may occur in the Mojave Desert environment where species traits are more strongly shaped by stress tolerance than competition (Grime's trait strategy model; Grime 1979). This trait combination is analogous to the situation in conifers, where wood of the Podocarpaceae and Araucariaceae is hydraulically inefficient, regardless of embolism resistance (Pittermann *et al.* 2006). This is also evident in the weakened or altered trait relationships found among basal vessel-bearing angiosperms (Sperry *et al.* 2007), which occur largely in isolated refugia.

Numerous studies have found that woody plants display levels of cavitation resistance that correspond to the pressures they experience in the field and that plants maximize their hydraulic conductivity for a given cavitation resistance (Pockman & Sperry 2000; Hacke *et al.* 2006; Pratt *et al.* 2007). Yet, the assumption that safety and efficiency are 'optimized' is not supported here. In some species (notably many of the Mojave Desert species), extensive levels of cavitation may be unavoidable and may even be beneficial as a mechanism of moderating use of soil water in response to extreme water stress, particularly if cavitation is associated with seasonal patterns of leaf loss. Moreover, selection for increased hydraulic efficiency may be less intense. Thus, the nature of a species' environment has the potential to significantly alter xylem trait relationships.

'Universal' plant structure–function relationships

Certain trait relationships seem robust regardless of community, species functional strategy, or evolutionary history. A functional correlation between xylem hydraulic conductivity (K_s) and vessel diameter (D) is consistent with the Hagen–Poiseuille equation and the fact that D increased in concert with vessel length (L). The observed scaling between the diameter and length of average-sized vessels (Fig. 4) was also similar to previous findings (Wheeler *et al.* 2005; Hacke *et al.* 2006). The slope and intercept among these traits were no different than those found in the data set of Hacke *et al.* (2006). This, and the fact that these trends were observed in each of the three plant communities (Fig. 4a) suggests that D and L are likely predictive of one

another across a broad range of species, communities and plant functional types. The negative correlation of K_s and wood density agrees with findings on tropical trees (Bucci *et al.* 2004; Meinzer *et al.* 2008) and underlines the role of wood density as a key predictor of functional traits (Hacke *et al.* 2001a; Bucci *et al.* 2004; Jacobsen *et al.* 2008; Meinzer *et al.* 2008). These trait relationships are likely to be the most valuable in predictive models that seek to examine anatomical and functional trait relationships among extant and fossil woods.

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