

# Evolution of hydraulic traits in closely related species pairs from mediterranean and nonmediterranean environments of North America

Radika Bhaskar<sup>1,4</sup>, Alfonso Valiente-Banuet<sup>2</sup> and David D. Ackerly<sup>3</sup>

<sup>1</sup>Department of Biological Sciences, Stanford University, Stanford, CA 94305, USA; <sup>2</sup>Instituto de Ecología, Universidad Nacional Autónoma de México, Apartado Postal 70-275, UNAM, 04510 México, D.F.; <sup>3</sup>Department of Integrative Biology, University of California Berkeley, Berkeley, CA 94720, USA;

<sup>4</sup>(Present address) Department of Integrative Biology, University of California Berkeley, Berkeley, CA 94720, USA

## Summary

Author for correspondence:

Radika Bhaskar

Tel: +1 510 6421054

Email: radika@gmail.com

Received: 5 April 2007

Accepted: 30 June 2007

- Chaparral shrubs in California experience cool, wet winters and hot, dry summers characteristic of mediterranean-type climates; by contrast, morphologically similar close relatives in central Mexico experience summer rainfall. A comparison of closely related species pairs was conducted to examine whether evolutionary divergences in plant hydraulic conductivity were associated with contrasting seasonality of precipitation.
- Six species pairs in Santa Barbara, California and Tehuacan, Mexico were chosen to test for repeated directional divergences across the habitat contrast. Additionally, evolutionary correlations were examined using phylogenetically independent contrasts (PICs) among a suite of hydraulic traits, including stem- and leaf-specific conductivity, resistance to embolism, wood density, inverse Huber value, and minimum seasonal water potential.
- Leaf-specific conductivity was generally higher in California, but for most hydraulic traits the species pairs exhibited varied evolutionary trajectories across the climate contrast. A significant correlation was found between divergences in xylem resistance to embolism and minimum seasonal water potential, but no evolutionary trade-off was found between resistance and stem conductivity.
- Higher leaf-specific conductivity may be adaptive in California, where soil and atmospheric droughts coincide during summer months. This response is consistent with a hydraulic strategy of high leaf water supply under high evaporative demand to prevent excessive drops in water potential.

**Key words:** chaparral, drought, evolution, hydraulic conductivity, mexican, phylogenetic independent contrasts (PICs), species pairs.

*New Phytologist* (2007) **176**: 718–726

© The Authors (2007). Journal compilation © *New Phytologist* (2007)

doi: 10.1111/j.1469-8137.2007.02208.x

## Introduction

One of the fundamental problems all plants face is acquiring and transporting water from the soil to the leaves, to replace water lost to the atmosphere during photosynthesis. This continuous column of water can come under tension as a result of low water availability at the roots or high evaporative demand at the leaf; thus dry soil or dry air compounds the

challenge of water transport. Our understanding of the hydraulic traits that contribute to plant water relations has been greatly enhanced by research in mediterranean-type environments (MTEs). Because of the annual period of low rainfall characteristic of the MTE climate, the role of soil drought in particular has been the focus of many studies. A suite of covarying physiological and anatomical traits have been identified that describe the diversity of hydraulic

strategies within these communities (e.g. Martinez-Vilalta *et al.*, 2002; Filella & Penuelas, 2003; Ackerly, 2004b; Jacobsen *et al.*, 2007). Much of this variation is associated with differences in minimum seasonal water potential; species that experience greater water deficit, as a result of shallow rooting or other factors, tend to have higher wood density, more drought-resistant xylem, and associated traits.

The occurrence of morphologically similar shrub vegetation in semiarid but nonMTE climates provides an opportunity to examine more closely the adaptive significance of traits governing plant water use. In California, chaparral shrubs experience a MTE of high summer temperatures and high vapor pressure deficit (VPD) during the annual period of soil water deficit. By contrast, vegetation in central Mexico experiences a summer rain regime; accordingly, the annual period of soil water deficit coincides with cooler, winter months (Valiente-Banuet *et al.*, 1998). Comparisons between these two communities provide an opportunity to examine the consequences of the seasonal timing of soil drought. Those traits that are shared between these communities may be adaptive in seasonally dry environments in general, and not specific to the unique MTE climate type (Valiente-Banuet *et al.*, 1998); those that differ provide insight into how the timing of soil drought, and specifically its interaction with atmospheric drought, influences plant hydraulic function.

A comparison of dry-season hydraulic strategies between a chaparral community in Santa Barbara, California, and a Mexican shrub community ('mexical') in Tehuacan revealed a strong correlation between species hydraulic capacity and the extent of drought experienced: within each site, species with lower whole-plant hydraulic conductance experienced more negative minimum seasonal water potential. However, while the slope of this functional relationship was similar, there was a significant shift in the elevation of the relationship between sites: whole-plant hydraulic conductance was higher in Santa Barbara relative to Tehuacan at a given minimum seasonal water potential (Bhaskar, 2006). Higher whole-plant conductance in Santa Barbara may be part of a hydraulic strategy to allow maintenance of gas exchange under the confluence of both high VPD and high soil water deficit. Under higher evaporative demand, greater water transport to the leaves may be required to prevent excessive diurnal and seasonal declines in leaf water potential (Maherali & DeLucia, 2000, 2001; Addington *et al.*, 2006).

If the MTE climate, with simultaneous atmospheric and soil droughts, imposes a selective pressure for higher leaf hydraulic supply, the differences observed at the community level should also be reflected in evolutionary divergences between related species across this climate contrast. Here we studied patterns of hydraulic trait evolution in all available pairs of closely related species between these taxonomically similar shrub communities (see Valiente-Banuet *et al.*, 1998). Each pair represents an independent divergence into the contrasting environments, and thus we can compare the

magnitude and direction of differences in a particular trait across all species pairs in relation to climate as well as to other traits (Felsenstein, 1985).

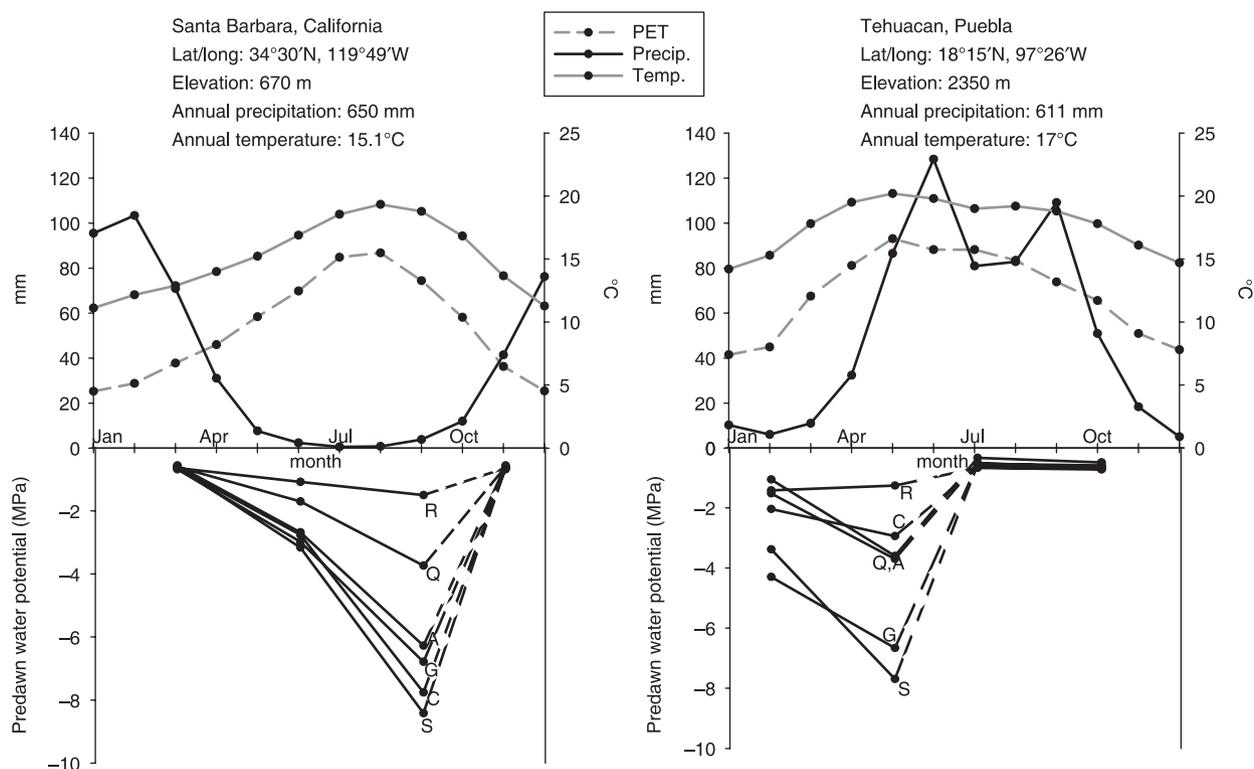
This paper addresses two objectives. Our first goal was to test for repeated evolutionary divergences in hydraulic traits associated with the timing of atmospheric drought. The community-level pattern focused on variation in whole-plant hydraulic conductance, which integrates the entire conducting pathway from soil through the xylem to the leaves; in this study we examined in greater detail the components of the pathway, specifically leaf-specific and stem-specific conductivity. We predicted that higher conductivity would be adaptive under higher VPD, and thus greater in species in Santa Barbara compared with their relatives in Tehuacan.

Our second objective was to examine evolutionary associations between hydraulic traits that represent broadly observed interspecific functional relationships. Trait correlations observed across taxonomically diverse species may imply an adaptive relationship; however, they do not necessarily reflect coordinated evolutionary change in those traits. For example, when the cross-species correlation between leaf size and leaf life-span among extant taxa was analyzed using phylogenetically independent contrasts (PICs), the trait association was seen to be driven largely by one instance of coordinated change during the split between angiosperms and conifers (Ackerly & Reich, 1999); thus, within lineages, those leaf traits do not appear to be coevolving. Similarly, there is evidence that shared ancestry can also have a large influence on cross-species hydraulic trait relationships (Maherali *et al.*, 2006). Therefore, we used PICs to test for correlated evolutionary change between traits, as evidence of an adaptive association. We tested whether higher leaf-specific conductivity was achieved through coordinated evolutionary shifts in stem-specific conductivity and the leaf area to sapwood area ratio. We also tested whether the evolution of increased conductivity was associated with decreased resistance to embolism, and finally whether divergences in resistance to embolism were correlated with divergences in wood density and minimum seasonal water potentials.

## Materials and Methods

### Site and species selection

The nonMTE site was located in the Tehuacan valley, on the border of the states of Puebla and Oaxaca, Mexico on the Cerro Zotaltepec mountain (18°15'N, 97°26'W), in the rain shadow of the Eastern Sierra Madre. The field site is a previously established research site with a recorded description of species distribution and abundance (Valiente-Banuet *et al.*, 1998). A comparable MTE site was chosen in the Santa Ynez Mountains above Santa Barbara, California (34°30'N, 119°49'W), to match mean annual temperature and precipitation (see Fig. 1 for details), as well as to maximize similarity in taxonomic composition (see Table 1 for species list). Precipitation data



**Fig. 1** Climate diagram and species seasonal dry-down curves for each site. Upper panels: monthly precipitation data from nearby climate stations, temperature data from dataloggers at each site, and potential evapotranspiration (PET) values calculated. Lower panels: solid lines connect the predawn water potentials of species during a period of soil water deficit (2003–2004); lines connecting end of dry season and rainy season measurements are dashed because the shape of rehydration is not known. A, *Arctostaphylos glandulosa* and *Comarostaphylis polifolia*; C, *Ceanothus*; G, *Garrya*; Q, *Quercus*; R, *Rhus*; S, *Salvia*.

were taken from nearby climate stations: for Tehuacan the closest station, Tecamachalco, reports 40-yr averages of monthly precipitation and for Santa Barbara precipitation data are from San Marcos Pass, with 20-yr averages. Monthly temperature was recorded over the 2 yr of this study using HOBO dataloggers placed at each site (Onset Computer Corporation, Bourne, MA, USA).

All six available closely related species pairs were included in this study (see species list in Table 1), representing many of the dominant families of chaparral shrubs (ex. Rhamnaceae and Ericaceae). Five of the six pairs were congeners, and the sixth (*Arctostaphylos glandulosa* vs *Comarostaphylis polifolia*) was based on closely related genera within the Arbutoideae (Hileman *et al.*, 2001). Noticeably absent were representatives of the Rosaceae; both areas have several species, but no closely related pairs were available across the study sites.

Five individuals were randomly sampled for each species; replicates were reproductively mature and of similar stature. Species in Santa Barbara put on new growth in early spring, while species in Tehuacan flush more continuously through the summer rainy season. Therefore, in both sites measurements made after the rainy season were from new but fully mature leaves and stems exposed to full sun.

## Water potentials

Predawn ( $\Psi_{\text{predawn}}$ ) and midday water potentials were measured using a pressure chamber (Plant Moisture Stress, Albany, OR, USA) at four time-points throughout the season (2003–2004) at each site (Fig. 1). Midday water potentials at the end of the dry season (end of September in Santa Barbara and early May in Tehuacan) were measured as an estimate of minimum seasonal water potential ( $\Psi_{\text{min}}$ ), which has been widely found to covary with other hydraulic traits (Bhaskar & Ackerly, 2006; Jacobsen *et al.*, 2007).

## Maximum stem conductivity

Maximum xylem hydraulic conductivity, both per unit sapwood area and per unit leaf area, was measured on excised stem segments at the conclusion of the rainy season, using a portable low-pressure flow meter (Feild *et al.*, 2002). Branches from each individual were collected in the early morning, placed in moist plastic bags, and brought back to the laboratory for measurements. Stem segments were re-cut underwater using a razor blade (usually to a length of between 20 and 40 cm) and all leaves and petioles were removed; cut nodes were glued

**Table 1** Species and sites sampled in this study, and end of wet season and end of dry season predawn water potentials ( $\Psi_{\text{predawn}}$ ), minimum seasonal water potential ( $\Psi_{\text{min}}$ ), sapwood-specific hydraulic conductivity ( $K_S$ ), leaf-specific hydraulic conductivity ( $K_L$ ) and stem vulnerability to embolism (the xylem tension inducing a 50% loss of stem conductance ( $\text{PLC}_{50}$ ))

Family	Species	Site	$\Psi_{\text{predawn}}$ (MPa)		$\Psi_{\text{min}}$ (MPa)	$K_S$ ( $\text{kg m}^{-1} \text{MPa}^{-1} \text{s}^{-1}$ )	$K_L$ ( $\times 10^{-4}$ ) ( $\text{kg m}^{-1} \text{MPa}^{-1} \text{s}^{-1}$ )	$\text{PLC}_{50}$ (MPa)
			Wet season	Dry season				
Ericaceae	<i>Arctostaphylos glandulosa</i>	SB	-0.56 ± 0.09	-6.28 ± 0.50	-6.36 ± 0.40	0.76 ± 0.15	1.91 ± 0.25	-4.7
	<i>Comarostaphylys polifolia</i>	Teh	-0.51 ± 0.05	-3.70 ± 0.28	-4.56 ± 0.27	0.67 ± 0.07	1.53 ± 0.10	-4.0
Rhamnaceae	<i>Ceanothus crassifolius</i>	SB	-0.64 ± 0.04	-7.76 ± 0.11	-8.20 ± 0.23	0.53 ± 0.06	5.31 ± 0.11	-8.2
	<i>Ceanothus greggii</i>	Teh	-0.66 ± 0.03	-2.94 ± 0.20	-5.06 ± 0.46	0.63 ± 0.06	4.88 ± 0.02	-6.0
Garryaceae	<i>Garrya elliptica</i>	SB	-0.68 ± 0.05	-6.79 ± 0.30	-6.98 ± 0.33	1.58 ± 0.30	7.47 ± 0.96	-6.5
	<i>Garrya ovata</i>	Teh	-0.54 ± 0.02	-6.66 ± 0.27	-7.24 ± 0.15	2.75 ± 0.70	5.92 ± 1.23	-6.6
Fagaceae	<i>Quercus berberidifolia</i>	SB	-0.60 ± 0.06	-3.74 ± 0.10	-4.46 ± 0.11	0.72 ± 0.19	4.77 ± 0.53	-2.6
	<i>Quercus sebifera</i>	Teh	-0.34 ± 0.02	-3.60 ± 0.30	-5.58 ± 0.24	0.71 ± 0.07	1.84 ± 0.26	-5.5
Anacardiaceae	<i>Rhus ovata</i>	SB	-0.64 ± 0.16	-1.50 ± 0.23	-3.34 ± 0.22	1.74 ± 0.69	5.01 ± 0.83	-2.7
	<i>Rhus standleyi</i>	Teh	-0.67 ± 0.03	-1.30 ± 0.29	-2.60 ± 0.22	2.49 ± 0.85	3.36 ± 0.16	-2.7
Lamiaceae	<i>Salvia mellifera</i>	SB	-0.62 ± 0.04	-8.42 ± 0.29	-10.24 ± 0.28	1.19 ± 0.17	4.11 ± 0.79	-8.3
	<i>Salvia candicans</i>	Teh	-0.56 ± 0.04	-7.70 ± 0.29	-8.28 ± 0.28	0.73 ± 0.13	6.05 ± 0.89	-7.3

All values are species means ± standard errors for both Santa Barbara (SB) and Tehuacan (Teh).  $\text{PLC}_{50}$  was estimated from vulnerability curves for each species, so no standard errors could be calculated.

using Super Bonder, Instant Adhesive glue (McMaster-Carr, Los Angeles, CA, USA). A flow solution of 10 mmol KCl dissolved in unfiltered Evian bottled water was used to maximize flow through pit membranes (Zwieniecki *et al.*, 2001) and passed from an elevated reservoir (4–5 kPa) through polyetheretherketone (PEEK) tubing of known conductance in series with the stem segment. Before measurements of maximum conductivity, stems were flushed with flow solution at 150 kPa for 30 min to remove embolism and both ends were re-shaved with a fresh razor blade. The flow rate through the stem segment ( $\text{kg s}^{-1}$ ) was calculated by multiplying the conductance of the PEEK tubing ( $\text{kg MPa}^{-1} \text{s}^{-1}$ ) by the drop in pressure across the tubing (MPa), measured with a pressure transducer (PX136; Omega Engineering, Stamford, CT, USA) that output to a voltmeter. Maximum xylem conductance ( $\text{kg MPa}^{-1} \text{s}^{-1}$ ) was calculated as the flow rate divided by the drop in pressure across the stem segment. After measurement, stems were injected with pressurized air to ensure that the length was longer than that of the longest vessel (Zimmermann & Jeje, 1981). Stem cross-sections were photographed and sapwood area analyzed using IMAGEJ (<http://rsb.info.nih.gov/ij/>). Sapwood-specific conductivity ( $K_S$ ;  $\text{kg m}^{-1} \text{MPa}^{-1} \text{s}^{-1}$ ) was determined by multiplying maximum xylem conductance by stem length and dividing by sapwood area. Leaf-specific conductivity ( $K_L$ ;  $\text{kg m}^{-1} \text{MPa}^{-1} \text{s}^{-1}$ ) was calculated as conductance multiplied by stem length and divided by total leaf area distal to the cut stem segment. For each individual, the average of two measurements of maximum stem and leaf conductivity was used. Leaf area to sapwood area ratio was calculated by dividing distal leaf area by the sapwood area of each hydraulic stem segment.

### Xylem resistance to embolism ( $\text{PLC}_{50}$ )

The resistance of stem xylem to water-stress induced embolism was assessed using vulnerability curves. In both sites, four to six branches were collected from each individual late in the dry season (September in Santa Barbara and April/May in Tehuacan) and dried on a bench for different durations to generate a range of xylem water potentials. Each stem was bagged at least 1 h before measurement to allow water potential to equilibrate throughout the shoot. Water potentials were measured on a side shoot, and a paired branch was used for measurement of stem conductance. A short segment of approx. 2–3 cm was cut underwater and stem conductance was measured using a portable low-pressure flow meter (as already described) under low delivery pressures (> 2 kPa; see Brodribb *et al.*, 2003). Conductance was re-measured after a high-pressure flush (175 kPa) lasting 5–10 min; the percentage loss of stem conductance (PLC) was calculated as the conductance of the flushed stem minus the conductance of the preflushed stem divided by the conductance of the flushed stem. A vulnerability curve was generated for each species by plotting the loss of stem conductance against increasing xylem tension. Data

were fit with a Weibull curve and the xylem tension that induced a 50% loss of stem conductance ( $PLC_{50}$ ) was recorded.

### Wood density

Wood density was measured on stems used for maximum conductivity measurements. After removal of the pith and bark, fresh volume was measured by water displacement. Sample mass was determined after drying for at least 2 d at 70°C. Reported values are oven-dried mass per unit fresh volume ( $g\ cm^{-3}$ ).

### Statistical analyses

Data were log-transformed when necessary to meet the assumption of normality. Two-way mixed model ANOVA was used to test for the influence of both site (fixed factor) and species pair (random factor; referred to as genus, for convenience) on variation in a number of hydraulic traits. The significance of the site effect was tested over the site–genus interaction. Correlated evolution between hydraulic traits was tested using PICs (Felsenstein, 1985). PICs were calculated for specific pairs of hydraulic traits, and contrast correlations were calculated using the formula described in Garland *et al.* (1992). Comparable phylogenetic branch lengths are not available across all species pairs, so independent contrasts were not standardized for branch lengths. Simulations using paired terminal taxa have shown that, in the absence of branch length information, treating branch lengths as equal may provide a good approximation (Ackerly, 2000). Because there is no site contrast at deeper phylogenetic levels, this analysis is based on the contrasts between the six closely related taxa and not on deeper nodes, limiting the power of the tests.

## Results

### Trait divergences across sites

At the conclusion of the dry season, variation in  $\Psi_{predawn}$  and  $\Psi_{min}$  was larger within than across sites (Table 2). The magnitude and direction of divergences across the site contrast varied, resulting in a significant genus by site interaction. Four pairs diverged in  $\Psi_{min}$  across sites, three with more negative water potentials in Santa Barbara and the fourth, *Quercus*, with the reverse pattern. The remaining two pairs, *Rhus* and *Garrya*, exhibited similar water potentials in both sites. The relative rank of  $\Psi_{min}$  was generally consistent with  $\Psi_{predawn}$  (Table 1).

Variation in hydraulic conductivity was explained largely by genus differences, and not by site differences (Table 2). Genera approximately maintained their relative ranks in maximum sapwood-specific conductivity,  $K_S$ , across sites (Fig. 2a). The differences in magnitude and direction across sites in the ratio of leaf area to sapwood area (LA:SA) varied among genera, which together with  $K_S$  influenced their leaf-

**Table 2** Significance values from mixed model ANOVA testing the effects of site (fixed factor) and genus (random factor) on hydraulic traits

Trait	Site	Genus	Site × genus
$\Psi_{predawn}$ , dry season	0.14	< 0.0001	< 0.0001
$\Psi_{predawn}$ , wet season	0.15	0.05	0.24
Wood density	0.16	0.03	0.24
$\Psi_{min}$	0.17	< 0.0001	< 0.0001
$PLC_{50}$	0.85	0.04	
Hydraulic conductivity			
Sapwood-specific (log)	0.73	0.0047	0.69
Leaf-specific (log)	0.27	< 0.0001	0.07
LA:SA	0.30	< 0.0001	< 0.0001

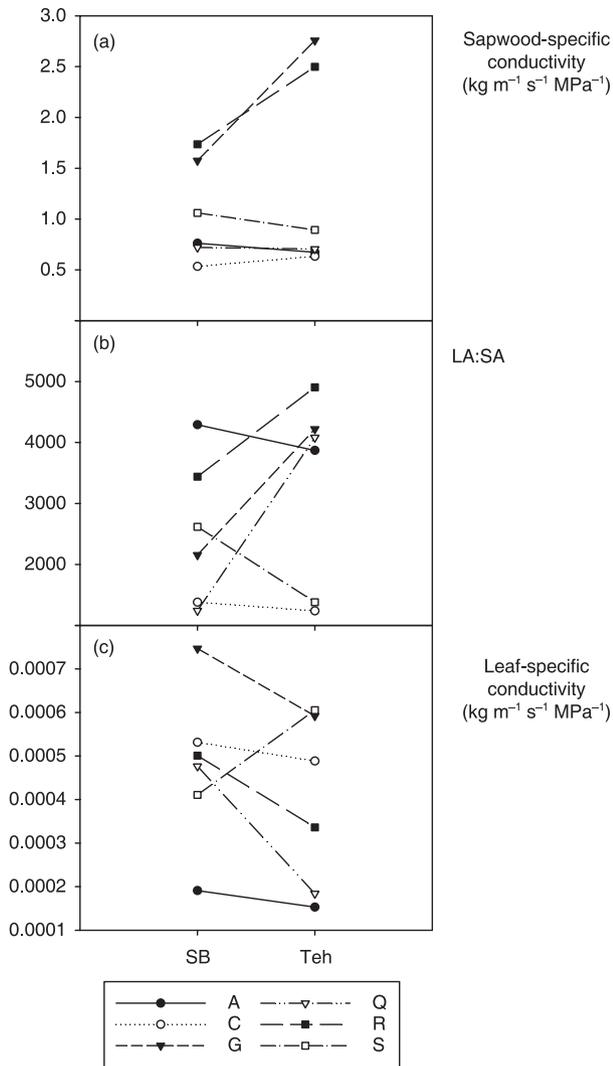
LA:SA, the ratio of leaf area to sapwood area;  $PLC_{50}$ , the xylem tension that induces a 50% loss of stem conductance;  $\Psi_{min}$ , minimum seasonal water potential;  $\Psi_{predawn}$ , predawn water potential.

specific conductivity ( $K_L$ ) (Fig. 2b,c). There was a trend towards reduced  $K_L$  in Tehuacan in all species pairs, with the exception of *Salvia*. Stem resistance to embolism varied significantly among species, but was not associated with site (Table 2). Because individual replicates were pooled to generate one vulnerability curve for species, from which  $PLC_{50}$  was calculated, the interaction between genus and site for stem resistance could not be tested.

### Pairwise correlations

Evolutionary associations between hydraulic traits were examined using independent contrast correlations. We focused on trait divergences rather than cross-species trait relationships in order to test for patterns of correlated evolution between pairs of hydraulic traits. Variation in trait values presumably reflects both heritable divergence and phenotypic plasticity in response to the contrasting environment, but their relative effects cannot be partitioned in this study.

LA:SA and  $K_S$  had a marginally significant ( $P=0.10$ ) positive association between divergences (Fig. 3a). Evolutionary shifts in  $K_S$  were not correlated with shifts in  $PLC_{50}$ ; for example, the two largest shifts in sapwood-specific conductivity, exhibited by the *Rhus* and *Garrya* pairs, corresponded with little change in  $PLC_{50}$  (Fig. 3b).  $PLC_{50}$  was significantly correlated with  $\Psi_{min}$ , using both cross-species analyses (Pearson correlation: pooled  $r=0.92$ , Santa Barbara  $r=0.95$ , Tehuacan  $r=0.94$ ) and PICs (Fig. 3c). Because  $\Psi_{min}$  was generally more negative than  $PLC_{50}$ , it is not surprising that species in both sites experienced a high degree of native embolism at the end of the dry season. The loss of stem conductivity ranged from 40 to 75%, with most species experiencing > 50% loss, and no differences between sites.  $PLC_{50}$  contrasts were positively but not significantly associated with wood density contrasts (Fig. 3d).



**Fig. 2** Species means of (a) sapwood-specific conductivity ( $K_S$ ), (b) leaf area to sapwood area ratio (LA:SA), and (c) leaf-specific conductivity ( $K_L$ ) in each site. Lines connect the six genus pairs across sites. A, *Arctostaphylos glandulosa* and *Comarostaphylis polifolia*; C, *Ceanothus*; G, *Garrya*; Q, *Quercus*; R, *Rhus*; S, *Salvia*.

## Discussion

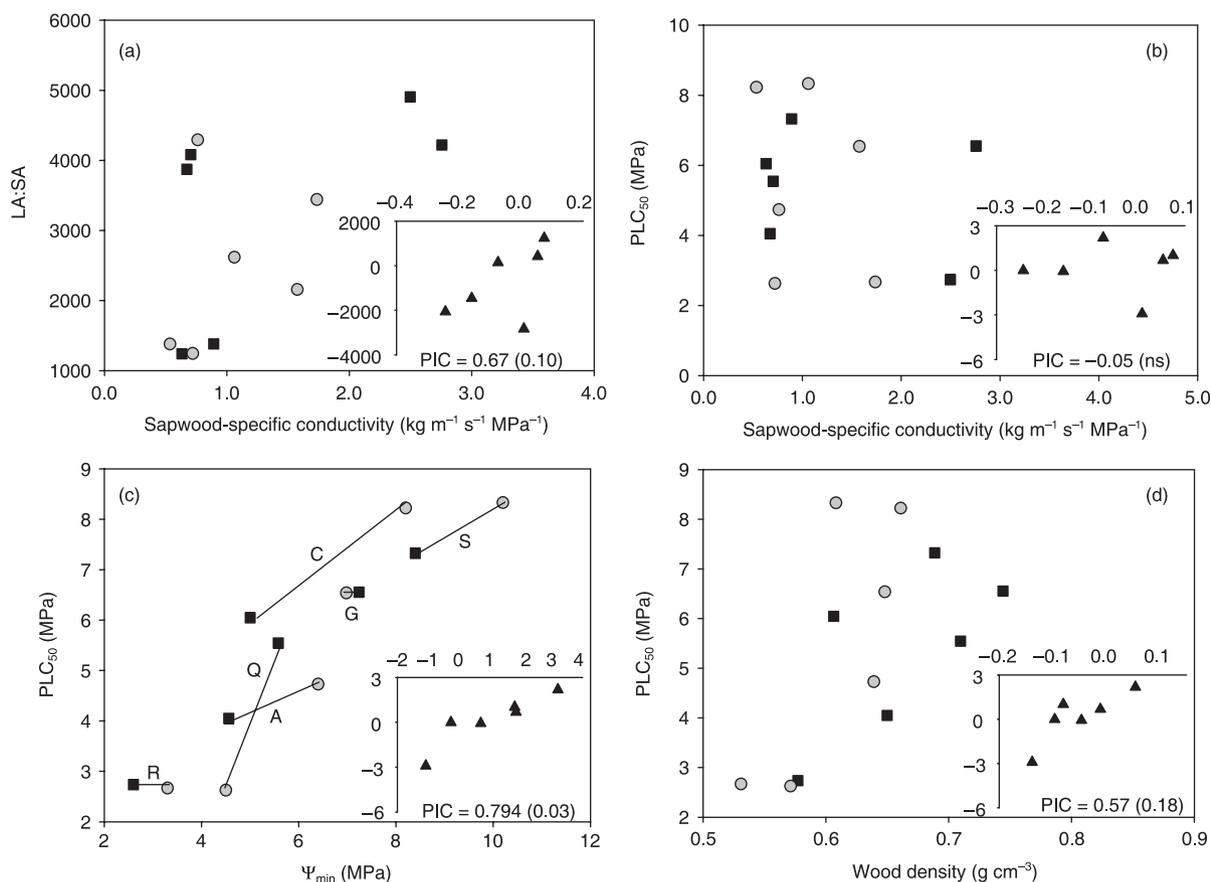
Despite the difference in rainfall seasonality between Santa Barbara and Tehuacan (Fig. 1), the extent of soil dry-down experienced by species, as indicated by water potentials, did not differ consistently between sites (Table 2). By the end of the dry season, species were more varied within than between sites, spanning a large range of  $\Psi_{\text{predawn}}$  values, presumably reflecting differences in rooting depths (Fig. 1, Table 2). While disequilibrium between soil and leaf predawn water potentials has been found in some desert shrub species (Donovan *et al.*, 2003), for four of the six California species, the relative ranking of rooting depth established by Hellmers *et al.* (1955) mirrors the ranking of  $\Psi_{\text{predawn}}$  in this study.

Evolutionary divergences in hydraulic traits among the six species pairs in this study were expected to be correlated with divergences in precipitation patterns between Santa Barbara and Tehuacan (Fig. 1). However, divergences in  $K_S$ , a measure of water transport efficiency, were not associated with site. Within both sites there was fourfold variation in  $K_S$  among genera and, more strikingly, genera generally maintained their relative ranks across the site contrast (Fig. 2a).  $K_S$  may exhibit conservatism with respect to lineage because of a number of factors, such as stabilizing selection or perhaps lack of selection. Other studies have reported low rates of evolutionary change in wood characteristics and vessel density, which should have consequences for hydraulic transport capacity (Maherali *et al.*, 2006; Preston *et al.*, 2006).

The relative stasis in  $K_S$  across sites with contrasting seasonality of rainfall raises the general question of what climate variables impose selective pressure on  $K_S$ . A global meta-analysis by Maherali *et al.* (2004) revealed a complex relationship between  $K_S$  and climate, related to life history. In deciduous angiosperms, evolution of increasing  $K_S$  was correlated with decreasing precipitation, but water availability did not explain variation in  $K_S$  in evergreen angiosperms or conifers. In fact, evolution of  $K_S$  within the evergreen angiosperms was not associated with any of the climate parameters included in their study, including atmospheric demand and temperature.

At smaller scales, studies within one or a few lineages comparing congeners of differing water status have generally found no correlation between evolutionary change in  $K_S$  and site or species water availability (Preston & Ackerly, 2003; Edwards, 2006; Choat *et al.*, 2007; but see Cavender-Bares *et al.*, 2004). Few studies have examined contrast correlations between  $K_S$  and atmospheric demand (VPD), but within *Pereskia* no correlation was found (E. J. Edwards, pers. comm.). In this study, we were able to compare a system in which high VPD coincided with maximum soil water deficit vs one in which they were separated seasonally, but by design mean annual rainfall was similar between sites. Consistent with a growing number of studies, we found no pattern of divergence in  $K_S$  associated with our climate contrast. Thus, the adaptive significance of  $K_S$  for evergreen angiosperms is unresolved with respect to climate variables.

Under contrasting humidity conditions, intraspecific comparisons have found greater leaf-specific hydraulic conductivity ( $K_L$ ) in tree populations experiencing higher VPD (Maherali & DeLucia, 2001; Cornwell *et al.*, in press). Thus, the coincidence of high atmospheric demand with soil drought in Santa Barbara was expected to result in selective pressure for higher  $K_L$  relative to Tehuacan. Consistent with this hypothesis, there was a repeated evolutionary shift towards higher  $K_L$  in Santa Barbara for five out of six pairs. Higher  $K_L$  in Santa Barbara may be part of a hydraulic strategy to balance leaf water supply with the high evaporative demand during the dry season, when predawn water potential is



**Fig. 3** Species means of (a) leaf area to sapwood area ratio (LA:SA) vs sapwood-specific conductivity, (b) the xylem tension inducing a 50% loss of stem conductance ( $PLC_{50}$ ) vs sapwood-specific conductivity, (c)  $PLC_{50}$  vs minimum seasonal water potential ( $\Psi_{min}$ ), and (d)  $PLC_{50}$  vs wood density. Lines connect genus pairs when phylogenetic independent contrasts (PICs) are significant. Plots of PICs are shown in the inset, with the corresponding correlation coefficient and significance value in parentheses. Squares, Tehuacan; circles, Santa Barbara. A, *Arctostaphylos glandulosa* and *Comarostaphylys polifolia*; C, *Ceanothus*; G, *Garrya*; Q, *Quercus*; R, *Rhus*; S, *Salvia*.

extremely negative. A highly conductive soil–leaf transport pathway can then prevent excessive drops in end of season leaf water potentials and allow continued carbon gain under high-VPD conditions (see Addington *et al.*, 2006).

The one pair that showed a reversal was *Salvia*, with higher  $K_L$  in Tehuacan compared with Santa Barbara. However, *Salvia mellifera*, the Santa Barbara species, has a distinct leaf phenology which may contribute to the observed reversal. Immediately after the rainy season, *S. mellifera* produces a cohort of drought-deciduous leaves which are dropped with the onset of the dry season, while side-shoot leaves are maintained (Gill & Mahall, 1986). A seasonal shift towards higher *in situ*  $K_L$  has been previously documented in *S. mellifera* (Kolb & Davis, 1994), which suggests that the reduction in LA:SA during the dry season may be enough to ensure higher leaf water supply to the remaining leaves, even with embolism-induced drops in  $K_S$ . Hydraulic measurements in this study were conducted under favorable water conditions in spring, and thus by the end of the dry season *Salvia* may have also had higher leaf conductivity in Santa Barbara, similar to the other pairs.

As the case of *Salvia* illustrates,  $K_L$  is determined by the interaction between  $K_S$  and LA:SA; changes in  $K_L$  can thus be achieved through various combinations of change in the other two traits. Under high VPD, one particular pattern that has been observed in intraspecific studies is higher  $K_L$  through a combination of higher  $K_S$  and lower LA:SA (Maherali & DeLucia, 2000, 2001). Of the two traits, LA:SA in particular has been hypothesized to decrease under conditions of increasing evaporative demand, as a means of increasing leaf water supply (Mencuccini & Grace, 1995). If the evolutionary pattern is similar we would expect divergences in  $K_S$  and LA:SA to be associated with site, as well as negatively correlated with each other. However, in our study, genera varied in how LA:SA differed across sites, and higher  $K_L$  in Santa Barbara was achieved in different ways (Fig. 2b,c). Additionally, contrast correlations revealed a trend in the opposite direction from that found in intraspecific studies; divergences in  $K_S$  were generally positively correlated with divergences in LA:SA (Fig. 3a). The outlier, *Quercus*, has large differences in LA:SA, with no corresponding divergences in  $K_S$ . The positive association between  $K_S$  and LA:SA has been found interspecifically

(Vander Willigen *et al.*, 2000) and between divergences (Preston & Ackerly, 2003; but see Edwards, 2006). The complicated interplay among the three traits may explain the varying direction of correlation: in some cases higher transport efficiency ( $K_S$ ) may allow maintenance of greater leaf deployment (higher LA:SA), but the resulting  $K_L$  is dependent on their relative changes.

One widely tested hydraulic relationship is the trade-off between safety and transport efficiency. Interspecific correlations across diverse taxa provide evidence of a weak negative relationship between measures of transport efficiency (conduit diameter or  $K_S$ ) and measures of stem resistance to embolism (PLC<sub>50</sub>) (Tyree *et al.*, 1994; Maherali *et al.*, 2004; Hacke *et al.*, 2006; Maherali *et al.*, 2006), although at the leaf level conductivity and drought tolerance may be decoupled (Sack *et al.*, 2003). However, evolutionary analyses reveal no significant correlations among trait divergences (Maherali *et al.*, 2004; Maherali *et al.*, 2006; Jacobsen *et al.*, 2007). Similarly, among the six pairs in this study, it appears that evolution of hydraulic transport efficiency ( $K_S$ ) is independent of the evolution of hydraulic resistance (PLC<sub>50</sub>) (Fig. 3b). Recent work on the mechanism of stem resistance has focused on the role of conduit pit structure and suggests that the anatomical traits that confer increased resistance to embolism may be somewhat decoupled from those that contribute to transport efficiency (Sperry *et al.*, 2005; Wheeler *et al.*, 2005). Because of the number of functionally linked traits that may collectively determine safety and efficiency, selective pressure can operate on distinct aspects of hydraulic architecture, and may explain the weak evolutionary correlation between stem conductivity and embolism resistance.

A tight evolutionary correlation was found between the extent of drought stress experienced in the field ( $\Psi_{\min}$ ) and xylem resistance to embolism (Fig. 3c, inset). Cross-species, this relationship has been found widely (Davis *et al.*, 1999; Pockman & Sperry, 2000), including among the species in this study (Fig. 3c) – evidence of an evolutionary correlation in this study suggests that the balance between  $\Psi_{\min}$  and resistance is adaptive. This may be consistent with an evolutionary correlation found more broadly across evergreen angiosperms between resistance to embolism and mean annual precipitation – at global scales,  $\Psi_{\min}$  may scale with mean annual precipitation (Maherali *et al.*, 2004). The cost of low resistance to embolism relative to  $\Psi_{\min}$  is apparent: high rates of embolism, stomatal shut-down and at the extreme shoot death (Davis *et al.*, 2002); however, the cost of overly resistant xylem is less clear, particularly as no trade-off is seen with conductivity. The construction costs associated with high resistance may partially explain the evolutionary match between  $\Psi_{\min}$  and PLC<sub>50</sub>. Across a large number of diverse taxa, greater stem resistance to embolism is associated with higher wood density (Hacke *et al.*, 2001). Similarly, in our study divergences between wood density and PLC<sub>50</sub> were positively, although not significantly, associated (Fig. 3d).

In conclusion, we found that the contrast in timing of rainfall had selective consequences for leaf but not sapwood specific conductivity. Variation in  $K_S$  was largely explained by differences among genera, which were maintained across sites. The majority of genera had higher  $K_L$  in Santa Barbara, which was achieved through different combinations of change in LA:SA and  $K_S$ . Interestingly, divergences in  $K_S$  and LA:SA may be interrelated; a weak positive correlation was found using contrasts. Thus, while the target of selection may be higher  $K_L$ , there may be underlying coordination between higher transport efficiency and greater deployment of leaf area relative to sapwood area. No evidence was found for an evolutionary trade-off between resistance to embolism and sapwood-specific conductivity, consistent with recent global analyses. Evidence of correlated evolution between  $\Psi_{\min}$  and PLC<sub>50</sub> suggests that the extent of drought experienced in the field has selective consequences for resistance to embolism.

The inclusion of all available pairs in this study revealed a diverse array of hydraulic strategies in both sites, as well as varied evolutionary trajectories across the climatic contrast. Studies of trait evolution in response to the mediterranean-type climate have found similarly heterogeneous responses. In California, some chaparral lineages that predate the origin of the mediterranean climate already possessed traits considered characteristic of MTE, related to fire persistence and leaf morphology, which allowed them to persist once the climate changed (Axelrod, 1989; Herrera, 1992; Valiente-Banuet *et al.*, 1998; Verdu *et al.*, 2003; Ackerly, 2004a; Pausas *et al.*, 2006). Instances where trait evolution does correlate with a change in climate have been found to reflect the biogeographic history of the lineages (Ackerly, 2004a). Thus, both trait evolution and ecological sorting contribute to the observed functional diversity of these communities.

## Acknowledgements

We would like to thank N. Flores, J. P. Castillo, A. Vital, M. Morales, L. Rios, L. Longley, and B. Holm for their invaluable assistance in the field. Many thanks to M. Martinez-Ramos, R. Dirzo, S. Davis, B. Mahall, A. Flinck, E. Mendoza, and L. Sevillano for their logistic support. K. Preston, W. Cornwell, R. Tirado, and E. Edwards provided valuable comments that greatly improved the manuscript.

## References

- Ackerly DD. 2000. Taxon sampling, correlated evolution, and independent contrasts. *Evolution* 54: 1480–1492.
- Ackerly DD. 2004a. Adaptation, niche conservatism, and convergence: Comparative studies of leaf evolution in the California chaparral. *American Naturalist* 163: 654–671.
- Ackerly DD. 2004b. Functional strategies of chaparral shrubs in relation to seasonal water deficit and disturbance. *Ecological Monographs* 74: 25–44.
- Ackerly DD, Reich PB. 1999. Convergence and correlations among leaf size and function in seed plants: A comparative test using independent contrasts. *American Journal of Botany* 86: 1272–1281.

- Addington RN, Donovan LA, Mitchell RJ, Vose JM, Pecot SD, Jack SB, Hacke UG, Sperry JS, Oren R. 2006. Adjustments in hydraulic architecture of *Pinus Palustris* maintain similar stomatal conductance in xeric and mesic habitats. *Plant, Cell & Environment* 29: 535–545.
- Axelrod DI. 1989. Age and origin of chaparral. In: Keeley S, ed. *The California chaparral: paradigms revisited*. Los Angeles, CA, USA: Natural History Museum of Los Angeles County, 7–19.
- Bhaskar R. 2006. Plant hydraulic strategies of evergreen woody plants in Mediterranean and non Mediterranean environments: the role of atmospheric versus soil drought. PhD thesis, Stanford University, Stanford, CA, USA.
- Bhaskar R, Ackerly DD. 2006. Ecological relevance of minimum seasonal water potentials. *Physiologia Plantarum* 127: 353–359.
- Brodrribb TJ, Holbrook NM, Edwards EJ, Gutierrez MV. 2003. Relations between stomatal closure, leaf turgor and xylem vulnerability in eight tropical dry forest trees. *Plant, Cell & Environment* 26: 443–450.
- Cavender-Bares J, Kitajima K, Bazzaz FA. 2004. Multiple trait associations in relation to habitat differentiation among 17 Floridian oak species. *Ecological Monographs* 74: 635–662.
- Choat B, Sack L, Holbrook NM. (2007). Diversity of hydraulic traits in nine *Cordia* species growing in tropical forests with contrasting precipitation. *New Phytologist* 175: 686–698.
- Cornwell WK, Bhaskar R, Sack L, Cordell S, Lurch C. (in press). Adjustment of structure and function of Hawaiian *Metrosideros polymorpha* at high versus low precipitation. *Functional Ecology*. doi: 10.1111/j.1365-2435.2007.01323.x
- Davis SD, Ewers FW, Sperry JS, Portwood KA, Crocker MC, Adams GC. 2002. Shoot dieback during prolonged drought in *Ceanothus* (Rhamnaceae) chaparral of California: a possible case of hydraulic failure. *American Journal of Botany* 89: 820–828.
- Davis SD, Ewers FW, Wood J, Reeves JJ, Kolb KJ. 1999. Differential susceptibility to xylem cavitation among three pairs of *Ceanothus* species in the transverse mountain ranges of southern California. *Ecoscience* 6: 180–186.
- Donovan LA, Richards JH, Linton MJ. 2003. Magnitude and mechanisms of disequilibrium between predawn plant and soil water potentials. *Ecology* 84: 463–470.
- Edwards EJ. 2006. Correlated evolution of stem and leaf hydraulic traits in *Pereskia* (Cactaceae). *New Phytologist* 172: 479–789.
- Feild TS, Brodrribb T, Holbrook M. 2002. Hardly a relict: Freezing and the evolution of vesselless wood in Winteraceae. *Evolution* 56: 464–478.
- Felsenstein J. 1985. Phylogenies and the comparative method. *American Naturalist* 125: 1–15.
- Filella I, Penuelas J. 2003. Partitioning of water and nitrogen in co-occurring Mediterranean woody shrub species of different evolutionary history. *Oecologia* 137: 51–61.
- Garland T, Harvey PH, Ives AR. 1992. Procedures for the analysis of comparative data using phylogenetically independent contrasts. *Systematic Biology* 41: 18–32.
- Gill DS, Mahall BE. 1986. Quantitative phenology and water relations of an evergreen and a deciduous chaparral shrub. *Ecological Monographs* 56: 127–143.
- Hacke UG, Sperry JS, Pockman WT, Davis SD, McCulloch KA. 2001. Trends in wood density and structure are linked to prevention of xylem implosion by negative pressure. *Oecologia* 126: 457–461.
- Hacke UG, Sperry JS, Wheeler JK, Castro L. 2006. Scaling of angiosperm xylem structure with safety and efficiency. *Tree Physiology* 26: 689–701.
- Hellmers H, Horton JS, Juhren G, Okeefe J. 1955. Root systems of some chaparral plants in southern California. *Ecology* 36: 667–678.
- Herrera CM. 1992. Historical effects and sorting processes as explanations for contemporary ecological patterns – character syndromes in Mediterranean woody-plants. *American Naturalist* 140: 421–446.
- Hileman LC, Vasey MC, Parker VT. 2001. Phylogeny and biogeography of the Arbutioideae (Ericaceae): Implications for the Madrean-Tethyan hypothesis. *Systematic Botany* 26: 131–143.
- Jacobsen AL, Agenbag L, Esler KJ, Pratt RB, Ewers FW, Davis SD. 2007. Xylem density, biomechanics and anatomical traits correlate with water stress in 17 evergreen shrub species of the Mediterranean-type climate region of South Africa. *Journal of Ecology* 95: 171–183.
- Kolb KJ, Davis SD. 1994. Drought tolerance and xylem embolism in co-occurring species of coastal sage and chaparral. *Ecology* 75: 648–659.
- Maherali H, DeLucia EH. 2000. Xylem conductivity and vulnerability to cavitation of ponderosa pine growing in contrasting climates. *Tree Physiology* 20: 859–867.
- Maherali H, DeLucia EH. 2001. Influence of climate-driven shifts in biomass allocation on water transport and storage in ponderosa pine. *Oecologia* 129: 481–491.
- Maherali H, Moura CF, Caldeira MC, Willson CJ, Jackson RB. 2006. Functional coordination between leaf gas exchange and vulnerability to xylem cavitation in temperate forest trees. *Plant, Cell & Environment* 29: 571–583.
- Maherali H, Pockman WT, Jackson RB. 2004. Adaptive variation in the vulnerability of woody plants to xylem cavitation. *Ecology* 85: 2184–2199.
- Martinez-Vilalta J, Prat E, Oliveras I, Pinol J. 2002. Xylem hydraulic properties of roots and stems of nine Mediterranean woody species. *Oecologia* 133: 19–29.
- Mencuccini M, Grace J. 1995. Climate influences the leaf-area sapwood area ratio in Scots pine. *Tree Physiology* 15: 1–10.
- Pausas JG, Keeley JE, Verdu M. 2006. Inferring differential evolutionary processes of plant persistence traits in northern hemisphere Mediterranean fire-prone ecosystems. *Journal of Ecology* 94: 31–39.
- Pockman WT, Sperry JS. 2000. Vulnerability to xylem cavitation and the distribution of Sonoran desert vegetation. *American Journal of Botany* 87: 1287–1299.
- Preston KA, Ackerly DD. 2003. Hydraulic architecture and the evolution of shoot allometry in contrasting climates. *American Journal of Botany* 90: 1502–1512.
- Preston K, Cornwell W, DeNoyer J. 2006. Wood density and vessel traits as distinct correlates of ecological strategy in 51 California coast range angiosperms. *New Phytologist* 170: 807–818.
- Sack L, Cowan PD, Jaikummar N, Holbrook NM. 2003. The ‘hydrology’ of leaves: Co-ordination of structure and function in temperate woody species. *Plant, Cell & Environment* 26: 1343–1356.
- Sperry JS, Hacke UG, Wheeler JK. 2005. Comparative analysis of end wall resistivity in xylem conduits. *Plant, Cell & Environment* 28: 456–465.
- Tyree MT, Davis SD, Cochard H. 1994. Biophysical perspectives of xylem evolution – is there a tradeoff of hydraulic efficiency for vulnerability to dysfunction? *LAWA Journal* 15: 335–360.
- Valiente-Banuet A, Flores-Hernandez N, Verdu M, Davila P. 1998. The chaparral vegetation in Mexico under non-Mediterranean climate: The convergence and madrean-tethyan hypotheses reconsidered. *American Journal of Botany* 85: 1398–1408.
- Vander Willigen C, Sherwin HW, Pammenter NW. 2000. Xylem hydraulic characteristics of subtropical trees from contrasting habitats grown under identical environmental conditions. *New Phytologist* 145: 51–59.
- Verdu M, Davila P, Garcia-Fayos P, Flores-Hernandez N, Valiente-Banuet A. 2003. ‘Convergent’ traits of mediterranean woody plants belong to pre-mediterranean lineages. *Biological Journal of the Linnean Society* 78: 415–427.
- 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 & Environment* 28: 800–812.
- Zimmermann MH, Jeje AA. 1981. Vessel-length distribution in stems of some American woody-plants. *Canadian Journal of Botany–Revue Canadienne de Botanique* 59: 1882–1892.
- Zwieniecki MA, Melcher PJ, Holbrook NM. 2001. Hydrogel control of xylem hydraulic resistance in plants. *Science* 291: 1059–1062.