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Research paper

Sex determines xylem anatomy in a dioecious conifer: hydraulic consequences in a drier world

José M. Olano[1,7], Noelia González-Muñoz[2], Alberto Arzac[3], Vicente Rozas[1], Georg von Arx[4,5], Sylvain Delzon[2] and Ana I. García-Cervigón[6]

[1]Área de Botánica, Departamento de Ciencias Agroforestales, EU de Ingenierías Agrarias, iuFOR-Universidad de Valladolid, Campus Duques de Soria, 42004 Soria, Spain; [2]BIOGECO, INRA, University of Bordeaux, 33615 Pessac, France; [3]Institute of Ecology and Geography, Siberian Federal University, 79 Svobodny pr., 660041 Krasnoyarsk, Russia; [4]Swiss Federal Institute for Forest, Snow and Landscape Research WSL, Birmensdorf 8903, Switzerland; [5]Climatic Change and Climate Impacts, Institute for Environmental Sciences, 66 Blvd Carl Vogt, CH-1205 Geneva, Switzerland; [6]CASEM – Facultad de Ciencias del Mar y Ambientales, Campus Universitario de Puerto Real, 11510 Puerto Real (Cádiz), Spain; [7]Corresponding author (jmolano@agro.uva.es)

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Increased drought frequency and severity may reshape tree species distribution in arid environments. Dioecious tree species may be more sensitive to climate warming if sex-related vulnerability to drought occurs, since lower performance of one sex may drive differential stress tolerance, sex-related mortality rates and biased sex ratios. We explored the effect of sex and environment on branch hydraulic (hydraulic conductivity and vulnerability to embolism) and trunk anatomical traits in both sexes of the dioecious conifer *Juniperus thurifera* L. at two sites with contrasting water availability. Additionally, we tested for a trade-off between hydraulic safety (vulnerability to embolism) and efficiency (hydraulic conductivity). Vulnerability to embolism and hydraulic conductivity were unaffected by sex or site at branch level. In contrast, sex played a significant role in xylem anatomy. We found a trade-off between hydraulic safety and efficiency, with larger conductivities related to higher vulnerabilities to embolism. At the anatomical level, females' trunk showed xylem anatomical traits related to greater hydraulic efficiency (higher theoretical hydraulic conductivity) over safety (thinner tracheid walls, lower Mork's Index), whereas males' trunk anatomy followed a more conservative strategy, especially in the drier site. Reconciling the discrepancy between branch hydraulic function and trunk xylem anatomy would require a thorough and integrated understanding of the tree structure–function relationship at the whole-plant level. Nevertheless, lower construction costs and higher efficiency in females' xylem anatomy at trunk level might explain the previously observed higher growth rates in mesic habitats. However, prioritizing efficiency over safety in trunk construction might make females more sensitive to drought, endangering the species' persistence in a drier world.

Keywords: dioecy, drought, hydraulic efficiency, hydraulic safety, *Juniperus thurifera*, xylem anatomy.

Introduction

The increase in drought intensity and frequency predicted by climate change scenarios (IPCC 2014) may challenge forest persistence in many water-limited environments (Allen and Breshears 1998, Gustafson and Sturtevant 2013). Drought impacts tree populations at multiple vital levels, potentially leading to abrupt changes in forest cover and composition (Benito-Garzón et al. 2013). Thus, climate warming is expected to reshape tree species distribution, i.e., taxa with higher drought tolerance will likely replace taxa architecturally and functionally adjusted to more mesic conditions (Benito-Garzón et al. 2013). In Mediterranean environments, where summer droughts are recurrent, increases in dryness may be dramatic, as water availability is the leading factor driving forest production and demography (Mitrakos 1980, Pasho et al. 2011). In this context, identifying functional traits involved in plant drought tolerance can help to anticipate and mitigate the potential impacts of climate change on Mediterranean species.

The design of the plant hydraulic system determines how plants cope with water scarcity (Tyree and Zimmermann 2002). The hydraulic efficiency of the xylem is generally measured as the hydraulic conductivity, i.e., the volume of sap that can be moved through the xylem per unit of time per unit pressure gradient, whereas hydraulic safety is inferred through xylem resistance to embolism, i.e., the ability of maintaining xylem conductivity at decreasing water potential levels (Tyree and Zimmermann 2002). Efficiency and safety have been considered to represent a trade-off in hydraulic system functionality (Sperry et al. 2003). However, evidences for a safety–efficiency trade-off at an interspecific level are not conclusive, as numerous species showed traits related to a low hydraulic efficiency combined with a reduced safety (Maherali et al. 2004, Gleason et al. 2016).

Tree species show large interspecific variation in vulnerability to embolism, which influences their ability to cope with drought (Maherali et al. 2004, Delzon et al. 2010, Choat et al. 2012, Costa-Saura et al. 2016). Intraspecific variations in vulnerability to embolism are overall weak, contrary to what has been observed in other traits such as intrinsic water-use efficiency (e.g., García-Cervigón et al. 2015). No clear pattern has emerged so far about intraspecific variation in hydraulic traits. Some studies showed low variations in resistance to embolism across the species distribution range (Martínez-Vilalta et al. 2009, Wortemann et al. 2011, Lamy et al. 2014). In contrast, other studies showed significant intraspecific phenotypic and genotypic variations in hydraulic conductivity along environmental gradients, particularly at their dry range edges (Corcuera et al. 2011, David-Schwartz et al. 2016, López et al. 2016), supporting the coexistence of different functional strategies to deal with drought (Anderegg and HilleRisLambers 2016).

Adjustments of the hydraulic system to changing environmental conditions can also be assessed through xylem anatomy (Fonti et al. 2010). Although the full mechanistic understanding of the relationship between xylem anatomy and hydraulic function still needs further investigation, xylem anatomical traits can predict hydraulic function successfully (Sperry et al. 2006, Hacke et al. 2015). In conifers, the hydraulic function depends heavily on torus-margo characteristics (Pittermann et al. 2005), which seem to scale with other tracheid dimensional traits (Lazzarin et al. 2016) that may thus be used as proxies for hydraulic function. Tracheid lumen size is highly correlated to conductivity through the Hagen–Poiseuille equation, whereas wall thickness, and the relationship between wall thickness and lumen diameter, are linked to resistance to cell implosion (Hacke et al. 2001). Altogether the xylem anatomical traits reflect the adaptation of the hydraulic system to different environments (Bittencourt et al. 2016). Thus, xylem anatomy can be scaled up to explore the interactions between hydraulic functionality and different adult tree characteristics, such as growth and productivity (Fan et al. 2012). In fact, adaptation to local environmental conditions configures spatially structured intraspecific variation of xylem anatomy along species distribution ranges (Esteban et al. 2012).

Intraspecific genotypic and/or phenotypic variation confers upon species a higher potential to adjust to environmental changes. Nevertheless, this assertion may not be valid for dioecious species if intraspecific variation is associated to sex. Under these circumstances, increased intraspecific variation may cause the opposite effect, leading to diverging ecological optima for each sex (Freeman et al. 1976, Shine 1989). Evidence of contrasting water-use strategies between sexes suggests that females may be more efficient than males at compensating for higher reproductive costs, whereas males' functionality is focused on safety-related strategies favoring survival (Tognetti 2012, Hultine et al. 2016). Thus, a drier environment may increase the differential performance between sexes (Hultine et al. 2008, 2013, 2016), promoting sex-related mortality rates and skewed sex ratios. In fact, a biased sex ratio has been routinely described in dioecious plants, with a larger proportion of males under higher levels of environmental stress (Krischik and Denno 1990, Vasiliauskas and Aarssen 1992, Ortiz et al. 2002). Thereby, understanding differences in tolerance to drought between sexes may be critical to foresee the responses of dioecious tree species to ongoing climate warming.

We explored potentially divergent drought resistance strategies in two populations of *Juniperus thurifera* L. occurring in contrasting climatic conditions. Also, we assessed how these strategies may be mediated by sex. *Juniperus thurifera* is an evergreen anisohydric conifer particularly suitable for investigating these topics because it inhabits areas covering a wide range of drought intensity under Mediterranean climate (DeSoto et al. 2012), and males show stronger tolerance to drought than females (Rozas et al. 2009, Montesinos et al. 2012*b*, Olano et al. 2015). Specifically, we aimed to evaluate the relative importance of sex and water availability as drivers of intraspecific variation of branch hydraulic function and trunk xylem anatomy. Both elements represent complementary estimates of hydraulic structure: branch hydraulic function provides a robust estimate of tolerance to drought near the end of the hydraulic pathway, whereas trunk xylem anatomy informs on the configuration of the largest component of the hydraulic system with implications for the costs associated to xylem construction. We posed two main questions: (i) is there any trade-off between traits related to hydraulic safety and efficiency at the intraspecific level, i.e., does an increased hydraulic safety imply a reduction in hydraulic efficiency, and (ii) do branch hydraulic and trunk anatomical traits differ between sexes and sites with contrasting aridity?

Materials and methods

Study sites

We selected two populations occurring in two contrasting sites representing the extremes of *J. thurifera* climatic range at the

Iberian Peninsula (Figure 1). The mesic site was in the Duero basin near Cabrejas del Pinar, Soria province, in north-central Spain (41°46′N, 2°50′W), at 1140 m altitude under relatively mesic conditions. The climate is subhumid Mediterranean continental with average annual rainfall of 518 mm. Annual mean temperature is 10.7 °C, the coldest month being January with a mean daily minimum temperature of −1.4 °C, and the warmest month is July with a mean daily maximum temperature of 28 °C. Summer drought usually occurs during a 2- to 2.5-month period, typically from mid-June to the end of August (Figure 1). At this site, *J. thurifera* forms an open monospecific woodland on calcium-rich and shallow soils with a parent rock of Cretaceous limestone.

The xeric site was located in Retuerta de Pina, near Bujaraloz, Ebro basin, Zaragoza province, northeastern Spain (41°29′N, 0°15′W), at 325 m altitude, and at the driest edge of the *J. thurifera* distribution range. Here, climate is semiarid Mediterranean continental with average annual rainfall of 317 mm, and a 3- to 4-month long summer drought period typically extending from mid-May to mid-September (Figure 1). Annual mean temperature is 15.1 °C, the coldest month being January with a mean daily minimum temperature of 2.6 °C, and the warmest is July

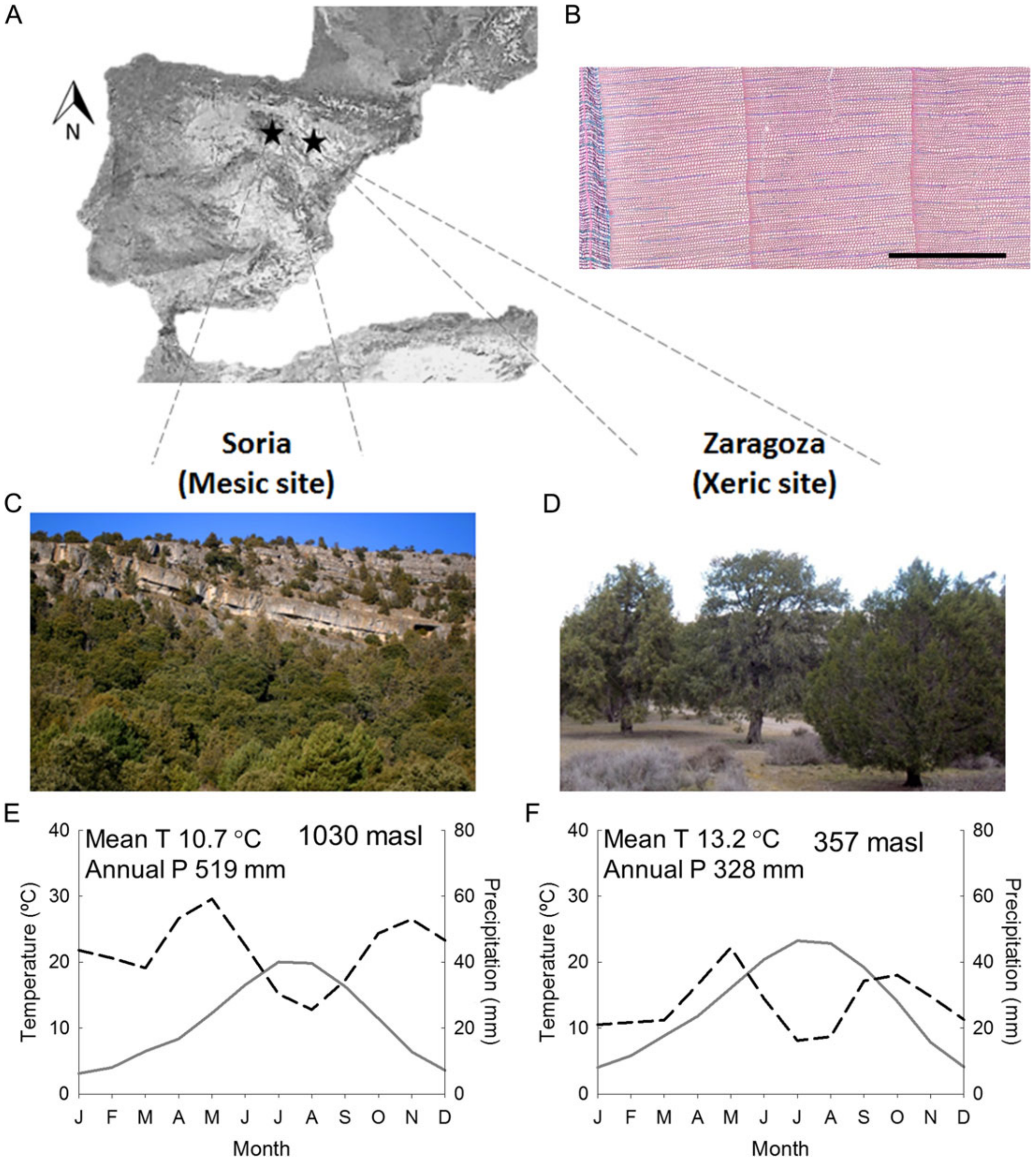

Figure 1. Location of sampling plots (A), anatomical image of *Juniperus thurifera* xylem, black line indicates 1 mm (B), view (C) and climatic diagram (E) of the mesic site in Soria province, and view (D) and climatic diagram (F) of the xeric site in Zaragoza province. Solid gray lines in climatic diagrams indicate monthly mean temperature (T) and dashed black lines indicate mean monthly precipitation (P). Meteorological stations with available climatic records for the period 1961–2015: (E) Soria located 30 km away from the mesic site; (F) Bujaraloz (precipitation) and Candasnos (temperature) located 10 and 28 km away from the xeric site, respectively. masl, m above sea level.

with a mean daily maximum temperature of 31.6 °C. Parent rock here is Miocenic gypsum and *J. thurifera* dominates open mixed woodland with intermingled *Pinus halepensis* Mill. trees and *Juniperus phoenicea* L. shrubs.

Study species

Juniperus thurifera is a long-lived evergreen conifer, endemic to the Western Mediterranean Basin, associated with continental Mediterranean climates with moderate to intense summer drought and low winter temperatures. Like other species of the genus *Juniperus*, it is an anisohydric species with the ability to tolerate very low water potentials and withstand intense drought (McDowell 2011). *Juniperus thurifera* usually shows low radial growth, usually below 1 mm per year (DeSoto et al. 2012, Granda et al. 2013, Olano et al. 2014), and its xylem anatomy is characterized by tracheids with small lumen area (Olano et al. 2012). Females show higher nutrient-use efficiency, more opportunistic water use and higher cambial sensitivity to environmental variation than males (Rozas et al. 2009, Montesinos et al. 2012*b*, Olano et al. 2015). Moreover, despite higher reproductive costs (Montesinos et al. 2012*a*), higher rates of secondary growth are found for females in moister locations, with this trend being reversed in drier sites (Montesinos et al. 2006, Rozas et al. 2009, Olano et al. 2015, DeSoto et al. 2016). Although the onset of xylogenesis resumption is controlled by spring temperatures, precipitation is by far the main climatic factor driving *J. thurifera* secondary growth (DeSoto et al. 2012, Olano et al. 2012, 2014, Granda et al. 2013).

Sampling design

In January 2013, 40 trees (20 males and 20 females) were randomly selected among dominant mature trees at each study site. Each tree was geo-referenced, and its stem diameter at breast height and total height were measured. Xylem anatomical characteristics in the trunk were quantified in 20 trees per site (10 males and 10 females) by extracting one 5-mm diameter wood core per tree at 1.3 m above ground using an increment borer. In January 2016, we sampled one branch of 1 cm of diameter in 20 trees (10 males and 10 females) per site to assess hydraulic function. Branches were cut in the morning to minimize native embolism. Immediately, leaves and side branches were removed to avoid transpiration, and the branches were covered with wet paper, placed in sealed bags, and kept in cool conditions (3–5 °C) until embolism resistance was measured within a maximum of 3 weeks after collection.

Anatomical measurements

Xylem anatomical analyses followed the protocol proposed by von Arx et al. (2016). In brief, anatomical cross-sections of 10 μm thickness were produced from the 5-mm diameter wood cores with a sledge microtome (Gärtner et al. 2015), placed on a slide and stained with alcian blue (1% solution in acetic acid) and safranin (1% solution in ethanol). Afterwards, the cross-sections were dehydrated using a series of ethanol solutions of increasing concentrations, washed with xylol, and then permanently preserved by embedding them into Eukitt glue (Kindler GmbH, Freiburg, Germany). Overlapping images covering the last five annual rings (2008–12) were captured with a Nikon D90 digital camera mounted on a Nikon Eclipse 50i optical microscope with 100× magnification and merged to a single image of 1.684 pixels μm^{-1} using PTGUI v8.3.10 Pro (New House Internet Services B.V., Rotterdam, The Netherlands).

We measured five anatomical traits associated with hydraulic function per annual ring (2008–12). Three of them were directly measured from images, and the other two were derived from the direct measurements. Directly measured traits were: (i) tracheid lumen area (LA), a trait positively associated with conductivity, (ii) tangential tracheid wall thickness, which is closely related to xylem construction costs, and (iii) percentage of ray parenchyma surface, a surrogate for ray parenchyma volume (von Arx et al. 2015), which is associated with capacitance (Borchert and Pockman 2005). From these data, we calculated the two derived traits (iv) Mork's Index (Denne 1988), using the ratio between twice the double cell wall thickness and cell lumen diameter, related to resistance to cell implosion (Hacke et al. 2001), and (v) an anatomy-based estimate of xylem-specific hydraulic conductivity (Ks_a), which is the hydraulic conductivity per unit area, where the hydraulic conductance of each cell is calculated following Nonweiler (1975) and considering ovality of cells. See Appendix 1 available as Supplementary Data at *Tree Physiology* Online for detailed information on the equations involved in this calculation.

Tracheid anatomical features were measured for the last 5 years (2008–12) using ROXAS v3.0 (von Arx and Dietz 2005, von Arx and Carrer 2014), a specific image analysis tool based on Image-Pro Plus (Media Cybernetics, Silver Spring, MD, USA). We first adjusted ROXAS settings to create different configurations of parameters used for automatic tracheid extraction, i.e., to automatically identify tracheid lumina. After a visual exploration of preliminary automatic results, we selected the two best configurations and used them to analyze all samples automatically. These two configurations were selected to adjust automatic tracheid extraction as much as possible for all images, as the accuracy of automatic tracheid extraction may vary depending on the quality of the anatomical sample. After automatic analysis, we visually compared results given by both configurations and selected the best one per image. The automatic output was manually edited by drawing ring boundaries, deleting erroneously detected tracheids (e.g., in rays), and rectifying additional misidentifications. Exclusion areas were used to factor out parts of the image with lower quality (e.g., tracheids broken during sample preparation). Tracheid lumen area and tangential tracheid wall thickness were automatically calculated for each tracheid and averaged at annual ring level (Prendin et al. 2017).

Mork's Index and Ks_a were obtained from tracheid data quantified at an individual level. The percentage of xylem rays surface was manually quantified (Olano et al. 2013) for every tree ring in the same cross-sectional images using a tailored version of ROXAS v1.6 (von Arx et al. 2015).

Hydraulic measurements

The bark was removed from conifer branches before measurement, to prevent resin contamination, and all branches were recut with a razor blade, under water, to a standard length of 27 cm. Vulnerability to embolism was measured on lateral branches in the high-throughput phenotyping platform for hydraulic traits (CavitPlace, University of Bordeaux, Talence, France; http://sylvain-delzon.com/caviplace), following the Cavitron technique (Cochard 2002, Cochard et al. 2005). This technique uses a centrifugal force to generate a negative xylem pressure, provoking a loss of conductance by cavitation. Samples were spun for 2 min at a given speed to decrease the xylem pressure progressively at its center from −1 to −13 MPa (those pressures correspond to centrifugation rotation from 3285 rpm to 11,844 rpm, respectively). The hydraulic conductivity was determined by measuring the flux through the sample. Conductance was measured three times for each pressure step, and the average was used to compute the percent loss of xylem conductance (PLC in%) at that pressure. As this technique enables measuring the hydraulic conductance of the samples under negative pressure, the vulnerability curves were generated by plotting the percentage loss of hydraulic conductivity (PLC) against the different target pressures applied.

We measured the hydraulic conductance at up to eight different pressures, and determined the PLC at each pressure following the equation:

$$\mathrm{PLC} = 100(1 - K_i/K_{\max}) \tag{1}$$

where $K_{\max}$ and K_i ($\mathrm{m^2\,MPa^{-1}\,s^{-1}}$) are, respectively, the maximum hydraulic conductivity and the hydraulic conductivity at a certain pressure.

Vulnerability curves were fitted using the equation of Pammenter and Willigen (1998):

$$\mathrm{PLC} = 100/\left[1 + \exp(s/25 \times (P - P_{50}))\right] \tag{2}$$

where P_{50} (MPa) is the xylem pressure inducing 50% loss of hydraulic conductivity and s (% $\mathrm{MPa^{-1}}$) is the slope of the vulnerability curve at the inflexion point. The slope shows how steeply PLC progresses at P_{50}, i.e., larger slope values represent a faster progress of cavitation at P_{50}. The xylem-specific hydraulic conductivity or Ks ($\mathrm{m^2\,MPa^{-1}s^{-1}}$) was calculated as the maximum hydraulic conductivity measured at low speed divided by the sapwood area of the sample.

Statistical analyses

Linear mixed models were used to assess the effects of site and sex on trunk anatomical traits (lumen area, tracheid wall thickness, Mork's Index, Ks_a and PERPAR). We included sex, site and its interaction term as fixed effects, and the year of annual ring formation as a random effect. Previously, we assessed the potential tapering effect of height (Anfodillo et al. 2013) on anatomical traits by establishing a correlation between height and mean tree anatomical values. Only the percentage of parenchyma (PERPAR) showed a marginal correlation with height, which was uncorrelated with the rest of the anatomical traits (see Table 1). Therefore, tree height was considered as a fixed covariate only for PERPAR models. As residuals of fitted models did not satisfy assumptions of linear models, we readjusted them using different variance structures, and log transforming the response variable Mork's Index. Among several variance structures (fixed, identity, power, exponential, constant power and combined constant power plus identity), we selected those leading to the most informative models according with the Akaike Information Criterion (AIC; Zuur et al. 2009). The selected variance structure was constant power in all cases except Mork's Index, for which a combined constant power plus identity was used. Pearson's correlations between different anatomical traits were performed to detect potential trade-offs between hydraulic efficiency and safety.

Differences in hydraulic traits (Ks, P_{50}, slope) were evaluated with a two-way analysis of variance (ANOVA) considering sex, site and its interaction as fixed components. Pearson's correlations were performed between the different hydraulic parameters to assess for their joint variation. Statistical analyses were performed in R environment 3.2.2 (R Development Core Team 2015), using the *nlme* package for linear mixed model adjustment (Pinheiro et al. 2016).

Table 1. Pearson's correlation coefficients between *Juniperus thurifera* xylem anatomical traits and tree height based on 40 individuals (Ks_a: anatomy-based specific conductivity; PERPAR: percentage of parenchyma). Significant correlations are highlighted by bold characters, marginal correlations are underlined.

	Tree height	Lumen area	Wall thickness	Mork's Index	Ks_a
Lumen area	$r = -0.12$, $P = 0.50$				
Wall thickness	$r = 0.11$, $P = 0.51$	$r = -0.16$, $P = 0.31$			
Mork's Index	$r = -0.09$, $P = 0.59$	**$r = -0.74$, $P < 0.001$**	**$r = 0.68$, $P < 0.001$**		
Ks_a	$r = 0.06$, $P = 0.73$	**$r = 0.89$, $P < 0.001$**	**$r = -0.49$, $P = 0.001$**	**$r = -0.89$, $P < 0.001$**	
PERPAR	$r = 0.30$, $P = 0.06$	$r = -0.22$, $P = 0.17$	**$r = -0.32$, $P = 0.046$**	**$r = -0.25$, $P = 0.047$**	$r = -0.28$, $P = 0.08$

Results

Xylem anatomy

A total of 434,272 tracheids were measured in the 200 annual rings belonging to the 40 trees. Mean tree-ring width in the five studied years was 788 μm ± 496 (mean ± SD), a low value consistent with the species' conservative behavior. Mean cell lumen area was 191.8 μm^2 ± 43.8 and was unaffected by either sex or site (Table 2, Figure 2A). Tangential tracheid wall thickness (3.66 μm ± 0.44) was lower in females (male effect size: 0.278 μm), and marginally higher at the mesic site (mesic site effect size: 0.136 μm) (Table 2, Figure 2B). Accordingly, Mork's Index (1.20 ± 0.24) was higher for males (male effect size: 0.077), with this effect being stronger in the xeric site as shown by the highly significant interaction term (Table 2, Figure 2C). Males had lower Ks_a (male effect size: –0.000309 m^2 MPa^{-1} s^{-1}), with a highly significant interaction term, reflecting the intense decrease of Ks_a in males of the xeric site (Table 2, Figure 2D). When tree height was considered, percentage of ray parenchyma (5.85 ± 1.59%) was unaffected by either sex or site (Table 2, Figure 2E).

We found significant and positive correlations between Ks_a and lumen area, and between Mork's Index and wall thickness (Table 1). In contrast, we found significant and negative correlations between Mork's Index and both lumen area and Ks_a, as well as between wall thickness and Ks_a (Table 1). Finally, percentage of parenchyma showed negative correlations with wall thickness and Mork's Index, and marginally negative correlation with Ks_a (Table 1).

Hydraulic function

Measurements of hydraulic function were obtained in 39 of the 40 sampled trees. For each sex and site, vulnerability curves showed a similar sigmoid shape. Average P_{50} was –9.27 ± 1.20 MPa and the coefficient of variation (CV = 0.13) was relatively low. Average *Ks* was 0.00217 ± 0.000099 m^2 MPa^{-1} s^{-1}, and showed larger variability than P_{50} (CV = 0.46). Two-way ANOVAs revealed neither site nor sex effect on P_{50} and *Ks* (Table 3). However, males showed a steeper slope than females (males: 21.55 ± 7.71% MPa^{-1}; females: 16.76 ± 4.39% MPa^{-1}), with a marginally larger slope at the mesic site, but no site per sex interaction effect (Figure 3). P_{50} was positively correlated with *Ks* ($r = 0.51$, $P < 0.001$) suggesting a trade-off between safety and efficiency. Both functional traits, P_{50} and *Ks*, were uncorrelated to slope (P_{50} vs slope: $r = –0.08$, $P = 0.62$; *Ks* vs slope: $r = –0.04$, $P = 0.82$).

Discussion

The combination of high embolism resistance and high wall-to-lumen ratio confirmed that *J. thurifera* is a highly drought-tolerant species (Pittermann et al. 2010, 2012, Sáenz-Romero et al. 2017). However, at the intraspecific level, our results show a discrepancy between the hydraulic traits measured in branches and the anatomical traits measured at the trunk. Whereas branch hydraulic traits were unaffected by either site or sex (except slope), trunk xylem anatomy revealed a strong effect of sex and sex per site interaction. To some degree, this discrepancy can be explained by the fact that the anatomy-based estimation of

Table 2. Size effects and standard error estimates for fixed factors derived from the general linear mixed models (tree as random factor) on the anatomical traits of *Juniperus thurifera* main stem xylem (Ks_a: anatomy-based specific conductivity; PERPAR: percentage of parenchyma). Sex and site in parentheses indicate the reference level.

	Lumen area (μm^2)	Wall thickness (μm)	Mork's Index (ratio)	Ks_a (m^{2}MPa^{-1}s^{-1})	PERPAR (%)
Intercept	201.1***	3.573***	0.199***	$3214 \times 10^{-6***}$	4.77***
Sex (male)	-9.031^{ns}	0.278***	0.078**	$-3089 \times 10^{-7*}$	0.211^{ns}
Site (xeric)	8.841^{ns}	0.136^{m}	0.005^{ns}	441×10^{-7ns}	-0.246^{ns}
Sex × site	6.605^{ns}	-0.127^{ns}	−2.205*	$4980 \times 10^{-7**}$	0.345^{ns}
Tree height	–	–	–	–	0.059^{ns}

Superscripts indicate significance: ***$P < 0.001$, **$P < 0.01$; *$P < 0.05$; $^{m}P < 0.1$; $^{ns}P > 0.1$.

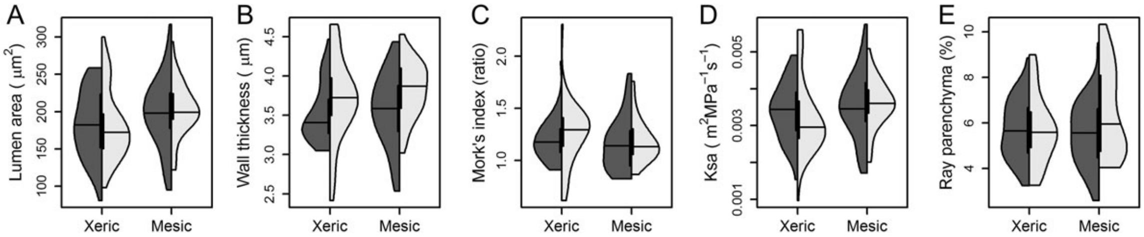

Figure 2. Split violin plots of trunk xylem anatomical traits for each sex and site combination. (A) Tracheid lumen area; (B) tangential tracheid wall thickness; (C) Mork's Index; (D) Ks_a: anatomy-based xylem-specific conductivity; (E) percentage of ray parenchyma. Females, dark gray; males, pale gray. Central horizontal lines indicate medians; the vertically oriented black polygons represent 25th and 75th percentiles; curves correspond to rotated kernel estimates of probability density functions of each trait.

Table 3. Results of two-way analysis of variance testing the effect of sex, site and their interaction on P_{50}, *K*s and slope of the vulnerability curve. Significant results are highlighted in bold characters; marginal effects are underlined.

	Sex	Site	Sex × site
P_{50}	$F = 0.67, P = 0.42$	$F = 0.08, P = 0.78$	$F = 0.29, P = 0.60$
*K*s	$F = 0.22, P = 0.64$	$F = 0.77, P = 0.39$	$F = 0.86, P = 0.36$
Slope	**$F = 6.01, P = 0.02$**	$F = 3.55, P = 0.07$	$F = 0.19, P = 0.66$

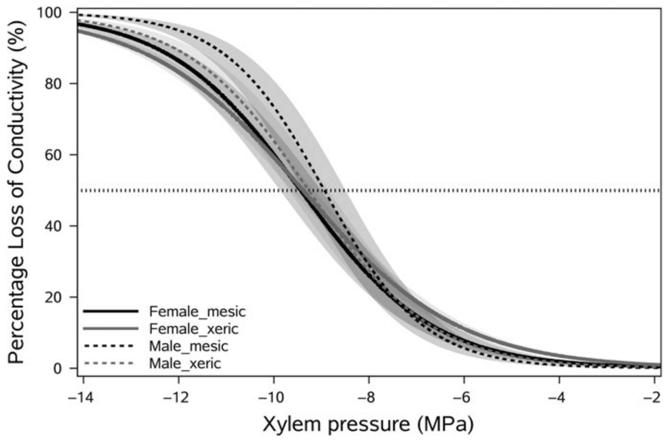

Figure 3. Mean xylem vulnerability curves for each *Juniperus thurifera* sex (males dotted lines, females solid lines) and site (mesic black line, xeric gray line). The shaded band represents the SD.

the specific hydraulic conductivity (*K*s$_a$) is based only on lumen area, whereas the xylem-specific hydraulic conductivity (*K*s) is the result of both lumen and pit resistivity (Sperry et al. 2006). Similarly, regarding xylem safety, P_{50} is largely determined by the torus-margo overlap (Bouche et al. 2014), which is not considered in the calculation of the Mork's Index. However, results by Lazzarin et al. (2016) suggest that their proportion remains relatively constant along the hydraulic pathway, i.e., also between the trunk and branch. Thus, unveiling how tracheid-level anatomical traits scale up to hydraulic function requires further investigations and highlights the need to combine different approaches to achieve a more accurate understanding of plant adaptations to drought.

The positive correlation between P_{50} and *K*s supports a trade-off between hydraulic safety and efficiency at the intraspecific level. Interestingly, trunk anatomical traits associated with safety (Mork's Index, tracheid wall thickness) were negatively correlated with traits associated with efficiency (tracheid lumen area, theoretical hydraulic conductivity). The existence of a safety–efficiency trade-off at intraspecific level contrasts with the scarce support for this trade-off at the interspecific level (Gleason et al. 2016, but see Bouche et al. 2014). Nevertheless, discrepancies between trait correlations at intra- and interspecific levels are not rare (García-Cervigón et al. 2015), and emphasize the need for extending trade-off analysis within species, where the impact of confounding factors on structure–function relationships is minimized (Martínez-Vilalta et al. 2009). Site, as a surrogate for drought intensity, did not affect hydraulic measurements of efficiency (*K*s) or safety (P_{50}). The low variation exhibited by P_{50} between populations matches with results reported for gymnosperms (Anderegg 2015), and reinforces the conservative nature of this trait (Lamy et al. 2011, 2014). Thus, adjustment of *J. thurifera* populations to the enhanced hydraulic stress in xeric environments would be explained by other components of the hydraulic system, such as leaf-to-shoot ratio or stomatal control (Martínez-Vilalta et al. 2009). Regarding *K*s, previous studies have shown a large variation among populations for different conifers and woody angiosperms (Corcuera et al. 2011, Charra-Vaskou et al. 2012). The absence of inter-population variation in hydraulic safety and efficiency may be related to the genetic homogeneity of *J. thurifera* populations across its range (Terrab et al. 2008, Teixeira et al. 2014).

Sex had a minor influence on branch hydraulic traits, affecting only the slope of the vulnerability curve. However, it had a significant effect on trunk xylem anatomy. Xylem anatomy of females' trunk showed traits associated with strategies prioritizing xylem efficiency over safety. This trade-off became more conspicuous due to differential responses of the sexes to local site conditions. Males adjusted their anatomy to increased drought stress in xeric environments by reducing their potential conductivity and increasing tracheid wall to lumen diameter ratio. Females, however, were less responsive and did not change their xylem anatomical traits depending on site conditions.

Despite the absence of differences between sexes in branch hydraulic function, the observed differences in trunk xylem anatomy may scale up to other tree functions such as growth rate. The thinner tangential tracheid walls of females (–7.34% compared with males) implies lower xylem construction costs. This would mean that, for a given amount of available resources, females would produce more xylem that would conduct more sap per unit of surface (+9.64%) than males. Thus, juniper females may outperform males in mesic environments, being able to store more resources (DeSoto et al. 2016), while maintaining higher secondary growth rates (Rozas et al. 2009, Olano et al. 2015, DeSoto et al. 2016). This hypothesis agrees with previous observations that detect a higher sensitivity of juniper females' secondary growth to drought, with higher growth rates of males in xeric environments (Montesinos et al. 2006, Iszkuło and Boratyński 2011, Olano et al. 2015, DeSoto et al. 2016).

The lower performance of females under stressful conditions has been attributed to the interaction between resource shortage and their higher reproductive costs (Montesinos et al. 2006). Our results suggest that the contrasting performances of both sexes under water stress can be related to their different investment in xylem anatomical safety and efficiency traits. Indeed, the sex-related configuration of the trunk xylem suggests that diverging evolutionary pressure may have promoted a resource-acquisitive strategy in juniper females, which also show higher photosynthetic rates, higher carbon-storage capacity, and lower water-use efficiency than males (Rozas et al. 2009, Olano et al. 2015, DeSoto et al. 2016). Prioritizing efficiency over safety in trunk construction might turn females more sensitive to drought, threatening the species persistence in a drier world. This phenomenon might be broader and extend to other dioecious species where higher sensitivity of females to drought has been reported (Retuerto et al. 2000, Hultine et al. 2008, 2016, Xu et al. 2008, Tognetti 2012), exposing dioecious species persistence to an additional risk in a warmer and drier world.

Supplementary Data

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

Abbreviations

Ks_a	anatomy-based estimate of specific conductivity
P_{50}	xylem pressure inducing 50% loss of hydraulic conductivity
PERPAR	percentage of ray parenchyma
PLC	percentage of loss of conductivity

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Conflict of interest

None declared.

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