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Polyploidy enhances the occupation of heterogeneous environments through hydraulic related trade-offs in *Atriplex canescens* (Chenopodiaceae)

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Summary

- Plant hydraulic characteristics were studied in diploid, tetraploid and hexaploid cytotypes of *Atriplex canescens* (Chenopodiaceae) to investigate the potential physiological basis underlying the intraspecific habitat differentiation among plants of different ploidy levels.
- Populations of *A. canescens* from different habitats of the Chihuahuan Desert (New Mexico, USA) were analyzed using flow cytometry to determine ploidy levels. Traits related to xylem water transport efficiency and safety against drought-induced hydraulic failure were measured in both stems and leaves.
- At the stem level, cytotypes of higher ploidy showed consistently lower leaf-specific hydraulic conductivity but greater resistance to drought-induced loss of hydraulic conductivity. At the leaf level, comparisons in hydraulics between cytotypes did not show a consistent pattern, but exhibited high plasticity to proximal environmental conditions related to soil water availability.
- The results suggest that a trade-off between stem hydraulic efficiency and safety across ploidy levels underlies niche differentiation among different cytotypes of *A. canescens*. Polyploidization may have been facilitated by environmental heterogeneity related to water availability, and variation in water-related physiology found in the present study suggests an important functional basis for the niche differentiation and coexistence of *A. canescens* cytotypes in desert environments.

Introduction

Polyploidy, the condition of organisms having more than two paired sets of chromosomes in their somatic cells, often produces significant changes in basic biology and results in strong ecological and evolutionary consequences (Shuter *et al.*, 1983; Melaragno *et al.*, 1993; Masterson, 1994; Comai, 2005; Buggs & Pannell, 2007; Soltis *et al.*, 2010; Ramsey, 2011). In plants, dramatic changes in morphology and physiology resulting from polyploidization often contribute to the obvious habitat differentiation among cytotypes (Hayman, 1960; Dunford, 1984; Senock *et al.*, 1991; Li *et al.*, 1996; Maherali *et al.*, 2009; Ramsey, 2011; Manzaneda *et al.*, 2012). Habitat differentiation increases the species' colonizing ability and amplifies divergence in reproduction, which permits cytotypes to coexist (Lumaret *et al.*, 1987). Due to the great scientific and applied implications of polyploidy, it has been extensively studied in genomics and genetics, but studies regarding its ecology and physiology are scarce (Soltis *et al.*, 2010). Moreover, the plant physiology underlying the ecological consequences of polyploidy, such as changes in plant environmental preferences, is still awaiting investigation.

Polyploidy produces significant structural and functional changes in plants (Warner & Edwards, 1989, 1993; Senock *et al.*, 1991), which apparently confer adaptive advantages under certain circumstances (e.g. Buggs & Pannell, 2007; Maherali *et al.*, 2009; Ramsey, 2011; Manzaneda *et al.*, 2012). It has long been hypothesized that polyploids have larger ecological ranges due to their ability to tolerate wider ranges of environmental stresses (Stebbins, 1950). Polyploid cytotypes often show increased resistance to drought compared with diploid cytotypes in wild plants and crops (Nassar, 1992, 2006; Pustovoitova *et al.*, 1996). Polyploids usually exhibit larger stomata in low density, a lower osmotic potential at full turgor, and a thicker epidermis, all of which tend to reduce water loss or maintain turgor in the case of lower water potentials. Some polyploids can maintain photosynthesis, although at a low level, under very negative water potentials at which the photosynthesis of diploids has completely ceased (Li *et al.*, 1996).

Polyploids generally occupy drier habitats than diploids (Baldwin, 1941; Maherali *et al.*, 2009; Manzaneda *et al.*, 2012), which is likely related to changes in xylem properties, such as resistance to drought-induced hydraulic failure. Polyploids often show altered xylem properties, such as in lignin content and cell wall thickness (Nassar *et al.*, 2008; Maherali *et al.*, 2009). Regardless of whether these changes are due to the polyploidy itself or to adaptive changes after the formation of polyploidy, they may significantly affect hydraulic function of xylem conduits, especially in drought-stressed environments. It is surprising that few studies have compared xylem hydraulic characteristics between polyploids and their diploid progenitors (but see Maherali *et al.*, 2009).

Drought can cause cavitation, the rupture of the water column in the xylem conduits under tension, which leads to embolism of xylem conduits and blockage of xylem water transport (Jarbeau *et al.*, 1995). Hydraulic transport efficiency and safety against embolism are often inversely related in stems (Piñol & Sala, 2000; Martinez-Vilalta *et al.*, 2002). This trade-off is thought to be related to the fact that larger and more porous xylem conduits are more likely to contain larger maximum pit membrane pores, through which air seeding occurs under high xylem tension (Wheeler *et al.*, 2005). The existence of a compromise between ability to cope with water stress, and ability to grow at high rates under more favorable water conditions, can partially explain why drought-tolerant plant species are displaced from mesic and humid habitats (Orians & Solbrig, 1977). The high growth rates of more mesic plants may, in part, be explained by the higher efficiency of xylem hydraulic conductivity that affects the uptake of water from the soil, and of stomatal conductance (g_s) and thus CO_2 assimilation (Brodribb *et al.*, 2002; Santiago *et al.*, 2004; Hao *et al.*, 2011). The nonrandom association between ploidy level and environmental water availability at the landscape scale may be underlain by a trade-off between xylem water transport efficiency and safety against xylem embolism.

More recent studies based on a variety of trees, from temperate to tropical, indicate that the leaf is a major bottleneck in the whole-plant water flow pathway, and resistance in leaves to water flow accounts for 30–80% of the whole-plant resistance (Sack *et al.*, 2003, 2005). Leaf hydraulic conductance (K_{leaf}) varies by an order of magnitude across species and is tightly coordinated with essential leaf functions, such as maximum stomatal conductance and net CO_2 assimilation rate (Sack *et al.*, 2003, 2005; Brodribb & Holbrook, 2004; Brodribb *et al.*, 2005). Additional evidence shows that K_{leaf} and its dynamic response to water status are crucial traits for the success of plants in different environments (Brodribb & Holbrook, 2003; Sack *et al.*, 2005; Sack & Holbrook, 2006). Polyploidization dramatically affects leaf morphology in plants (e.g. Sanderson & Stutz, 1994), which is likely accompanied by changes in leaf physiology including hydraulics. Polyploidy would thus be expected to have substantial effects on plant water relations if differentiation in leaf morphology, mediated by polyploidization, is accompanied by changes in K_{leaf}.

Atriplex canescens (Pursh) Nutt. (four-wing saltbush, Chenopodiaceae) is a salinity and drought tolerant shrub species native to the deserts of Southwestern U.S., where it exhibits a series of chromosome races, that is, cytotypes with different ploidy levels (Sanderson & Stutz, 1994). Different cytotypes vary significantly in morphology and often show contrasting environmental preferences, with higher ploidy cytotypes commonly dominating clay habitats (Dunford, 1984; Senock *et al.*, 1991). This may be related to the more severe drought stress in clay than in sandy habitats under the desert climate (Noy-Meir, 1973; Sperry & Hacke, 2002). In the present study, we investigated xylem hydraulic conductivity and resistance to drought-induced cavitation at both the stem and leaf levels in three major identified ploidy levels, that is, diploid, tetraploid and hexaploid cytotypes. We hypothesized that increased ploidy in *A. canescens* is associated with greater resistance to drought-induced hydraulic failure, which may explain the success of the hexaploid cytotype in more drought-prone clay habitats. In addition, we hypothesized that a trade-off between hydraulic efficiency and safety against drought-induced xylem cavitation may exist across cytotypes. Higher hydraulic conductivity may thus be responsible for the success of lower ploidy cytotypes in environments of higher water availability. The objective of this study was to examine the role of physiology, from the point of view of plant hydraulics and water relations, in determining the intraspecific niche differentiation of different ploidy levels in water-limited environments.

Materials and Methods

Field sites and study species

This study was conducted at three locations in the Chihuahuan Desert of southern New Mexico, USA, at the Jornada Experimental Range and in the Rio Grande valley, both located in Doña Ana County (DAC), and at the White Sands National Monument (WSNM), located in Otero County. The study area in DAC is mainly composed of the flood plain of the Rio Grande and adjacent slopes and mesas, with elevation ranges from 1100 m in the valleys to 1300 m on the mesas. The WSNM is located *c.* 60 km northeast of the Jornada Experimental Range, in Otero County. The whole region is dominated by a hot and dry subtropical climate with maximum temperatures averaging 13°C in January and 36°C in June, and an annual precipitation of *c.* 250 mm. Rainfall has a relatively strong seasonality, with *c.* 50% of annual rainfall occurring between July 1 and September 30.

Leaf and whole plant morphology was first used for identifying populations of different ploidy levels in *Atriplex canescens* (Pursh) Nutt. Ploidy levels of the identified populations were then confirmed using flow cytometry. Field observations on the soil type associated with these populations were also recorded. Ecophysiological measurements were conducted during two seasons, once in the dry season (December 2010) and once in the wet season (July 2011). Three study sites, each having a different cytotype, that is, diploids (2*x*), tetraploids (4*x*) and hexaploids (6*x*), were established in 2010 (DAC1, DAC3 and DAC5; Table 1). In 2011, we created a second study site for each cytotype in Doña Ana County (DAC2, DAC4 and DAC6; Table 1). The four sites with diploid and tetraploid populations were located in the Jornada Experimental Range of the USDA Agricultural Research Service. Hexaploid populations in DAC are restricted to the

Table 1 Results of the flow cytometry ploidy examination for different *Atriplex canescens* populations from Doña Ana County (DAC) and the White Sands National Monuments (WSNM)

Study site	Ploidy	Soil type	Latitude	Longitude
DAC 1	2*x*	Sandy	32°38′44.4″N	106°43′51.2″W
DAC 2	2*x*	Sandy	32°40′19.4″N	106°43′29.0″W
DAC 3	4*x*	Clay	32°36′35.3″N	106°44′29.8″W
DAC 4	4*x*	Clay	32°42′15.1″N	106°43′56.6″W
DAC 5	6*x*	Clay	32°21′10.9″N	106°51′08.6″W
DAC 6	6*x*	Disturbed	32°04′18.1″N	106°41′00.6″W
WSNM 1	4*x*	Loamy	32°46′01.9″N	106°11′41.0″W
WSNM 2	4*x*	Sand dunes	32°45′59.2″N	106°11′09.6″W
WSNM 3	6*x**	Loamy	32°45′55.5″N	106°12′01.3″W
WSNM 4	6*x*	Clay	32°45′59.2″N	106°11′09.6″W
WSNM 5	6*x*	Clay	32°46′56.2″N	106°09′30.9″W
WSNM 6	6*x*	Clay	32°47′15.5″N	106°11′14.4″W

*Some 5*x* individuals were also found in this site and are likely to be the result of occasional hybridisation between 4*x* and 6*x* cytotypes.

valley of the Rio Grande. In 2011, we established six new sites in WSNM (Table 1), where the tetraploid populations were found growing on the slopes of gypsum dunes, and hexaploid populations occurring in the neighboring clay soil sites (Fig. 1). During both measurement seasons, female individuals were easily distinguished from male ones by their inflorescences or the presence of winged seeds. An equal number of male and female plants were sampled for ecophysiological measurements, but pooled data were used for further analysis since no significant differences were found between sexes.

Flow cytometry

Approximately 0.5 cm^2 of leaf matter was prepared for analysis using a total of 2 ml of the combined Partec GmbH chopping and staining buffer Cystain UV Ploidy (Partec GmbH, Münster, Germany), according to the manufacturer's instructions for fresh material, except that 300 rather than 500 μl was used in chopping. The resulting nuclear suspension was passed through a 50 μm nylon mesh filter and analyzed in a Partec PAII flow cytometer (Partec GmbH, Münster, Germany) using UV excitation by a mercury vapor light source. Leaf material from a diploid or a tetraploid *Atriplex canescens* (Pursh) Nutt. plant grown at the laboratory was used as an internal standard.

Fig. 1 *Atriplex canescens* of tetraploid cytotype (4*x*) growing on the slope of a gypsum sand dune, and the hexaploid cytotype (6*x*) growing on a neighboring clay soil habitat in the White Sands National Monument, New Mexico, USA. Photo by G.-Y. Hao.

Leaf water potential

Midday leaf water potential (Ψ_L) was measured using a Scholander pressure chamber (PMS Instrument, Corvallis, Oregon, USA) in December 2010 and July 2011, during clear weather. Small terminal shoots (*c*. 5 cm in length) rather than single leaves were used for water potential determination due to the small leaf size of the studied species. Samples were collected from eight different plants at each study site during midday (12:00–14:00 h). Immediately after excision, the shoot was sealed in a plastic bag containing a moist paper towel, placed in a cooler, and transported within a short time to the laboratory located at New Mexico State University for Ψ_L measurements. In all cases, Ψ_L was measured within 5 h of sampling.

Stem hydraulic conductivity

Sapwood specific hydraulic conductivity (K_s; kg m^{-1} s^{-1} MPa^{-1}), leaf specific hydraulic conductivity (K_l, kg m^{-1} s^{-1} MPa^{-1}), and leaf to sapwood area ratio were measured for each population listed in Table 1. Long branches with diameters between 5 and 8 mm were cut using a clipper, and segments *c*. 30 cm in length were sampled under water. Materials were kept under water in plastic bags placed in a moist container and transported to the laboratory at Harvard University for hydraulic conductivity measurements. Before hydraulic conductivity was measured, samples were kept in a cold storage room with a constant temperature of 4°C. All hydraulic conductivity measurements were made within a week from sampling, and measurements on a subset of samples did not show significant change in hydraulic conductivity within the experimental period.

Stem segments of 15 cm, cut off from the middle part of the originally sampled longer stem segments, were used for hydraulic conductivity measurement. Hydraulic conductivity (K_h, kg m^{-1} s^{-1} MPa^{-1}) of the stem segment was determined by measuring the flow rate of ultra-filtered (particle size < 0.2 μm) and de-gassed water through a branch segment under a known pressure gradient (Zwieniecki & Holbrook, 1998). Sapwood area was determined at 1 cm from both ends of the segment using the dye staining method and the two values were averaged. An image of the stained wood was taken under a dissecting microscope at a magnification of ×10, and the area of the stained portion was calculated using ImageJ software (US National Institutes of Health, Bethesda, MD, USA). The leaf mass per area, determined on six plants for each population, was used to estimate the total leaf area of a branch based on total leaf dry mass. A picture of 20 leaves randomly selected from a plant (placed next to each other on a white paper with a scale bar of known length) was taken using a digital camera and the total area of these leaves was calculated using ImageJ software. Leaves were oven dried at 60°C for at least 48 h and dry mass was measured to the nearest 0.01 mg using a digital analytical balance (model CPA225D, Sartorius, Göttingen, Germany).

Measurements were made on eight branches, each from a different individual, for each of the studied populations. For the wet season measurements, data collected on the same cytotypes were pooled for DAC and WSNM, because populations of a specific cytotype within each of the two areas are very similar in environmental conditions, plant morphology and leaf water potentials.

Stem vulnerability curves

For the dry season, vulnerability of stems to drought-induced cavitation was estimated for the three cytotypes using an air-injection method (Sperry & Saliendra, 1994). Stem segments *c.* 15 cm in length were flushed with a degassed 20 mM KCl solution at 200 kPa for 40 min for maximum K_h determination. Hydraulic conductivities were then measured after the segment was exposed to progressively increased air-injection pressures ranging from 0.5 to 8 MPa. Pressure was held constant, using a gas pressure regulator, for 10 min at each pressure level and then allowed 10–30 min for equilibration before determination of K_h. The vulnerability curve was plotted as percentage loss of hydraulic conductivity (PLC) vs air-injection pressure. For the wet season measurement of stem vulnerability, we used the centrifugal force method (Alder *et al.*, 1997). Briefly, vulnerability to drought-induced xylem cavitation was measured as the reduction in K_h in response to a stepwise increase in xylem tension generated by spinning a 14.5 cm stem segment at different speeds using a Sorvall superspeed centrifuge (Model RC-5C Plus, Du Pont Instruments, Wilmington, DE, USA).

The percentage loss of conductivity (PLC) following each spin was calculated as $\mathrm{PLC} = 100[(K_{\max} - K_h)/K_{\max}]$, where K_h is the hydraulic conductivity after each spin and $K_{\max}$ is the maximum hydraulic conductivity measured on flushed segments before spinning. No significant difference within cytotypes was observed between populations and thus data were pooled for each cytotype.

Leaf vulnerability curves

Leaf hydraulic vulnerability curves were constructed by measuring small shoot hydraulic conductance (K_{leaf}) using the partial rehydration method described by Brodribb & Holbrook (2003). Briefly, relatively large branches containing multiple small side branches were sampled pre-dawn and allowed to rehydrate in deionized water for *c.* 2 h. Branches were then allowed to dehydrate on lab benches for different lengths of time before they were sealed into double black plastic bags containing a moist paper towel for a few hours, to allow equilibration among different parts of the branch. Immediately after initial water potential measurements, two small branches were cut under water at the same time and allowed to absorb water for a certain period (20–60 s). The small shoots were then sealed into a small zip bag containing a moist paper towel for 10 min, before final water potential was determined. K_{leaf} was calculated based on values of initial and final water potentials and the rehydration time, as described by Brodribb & Holbrook (2003). The K_{leaf} values were plotted against initial water potentials and fitted using an exponential sigmoid model in Sigmaplot (SPSS Inc., Chicago, IL, USA).

Wood anatomy

For each of the three cytotypes, fresh stem samples were sectioned with a microtome to obtain cross-sections of the sapwood. Cross-sections were stained using 0.1% Toluidine Blue solution to increase visual contrast, and images were taken at 100× using a digital camera (Axiocam HRc, Carl Zeiss, Jena, Germany) mounted on a light microscope (Olympus BH-2, Olympus, Tokyo, Japan). Vessel lumen diameters were estimated using ImageJ software. The diameter of each vessel lumen cross-section was calculated based on its area, assuming a circular shape. Vessel diameter frequency classes were established at intervals of 2 μm.

Theoretical individual vessel lumen hydraulic conductance was calculated using a modified Hagen-Poiseuille equation (Tyree & Ewers, 1991):

$$K_i = \pi \rho d^4/(128\eta)$$

(d, vessel lumen diameter; ρ, water density (assumed to be 1000 kg m^{-3}); η, viscosity of water at 20°C (1×10^{-9} MPa s)). The sapwood area-normalized theoretical vessel lumen hydraulic conductivity (K_t) was then calculated as the sum K_i of all the vessels in a sapwood cross-sectional image and divided by the total image area. Maximum K_s measured on the same stem segment was used for estimating the allocation of hydraulic resistance between vessel lumens and the end walls. Resistance due to vessel end walls (R_e) was calculated using the following equation:

$$R_e = 1/K_s - 1/K_t$$

($1/K_s$, total hydraulic resistance, i.e. resistance due to both vessel lumens and end walls; $1/K_t$, lumen hydraulic resistance).

Statistical analysis

Stem and leaf vulnerability curves were fitted with an exponential sigmoid model using Sigmaplot v6.0 (Systat Software, Inc., San Jose, CA, USA). Vessel lumen diameter frequency distribution data were fitted using a three-parameter Gaussian peak function. Data were analyzed using two-way ANOVA, followed by the LSD post-hoc test for comparisons between cytotypes. Comparisons between wet and dry seasons within each of the three cytotypes were performed using Student's *t*-tests (SPSS software, SPSS Inc).

Results

The results of flow cytometry showed that the diploid (2*x*), tetraploid (4*x*) and hexaploid (6*x*) plants are the major cytotypes of *Atriplex canescens* in the two study areas (Table 1). The diploid populations are found only on sand dunes. The tetraploid populations are most common and widely distributed and occupy habitats with soil type ranging from sandy to fine textured loamy and clay soils. The hexaploids show a dwarf growth form compared to the diploids or tetraploids and are associated with clay soil in the flood plains. The three cytotypes exhibited contrasting morphologies at both the leaf and shoot levels. The diploids showed a linear leaf form and had the smallest leaf area among the three cytotypes. The tetraploid cytotype had narrowly oblanceolate leaves that were shorter but wider compared to those of diploid plants, and the hexaploid cytotype had larger, oblanceolate leaves easily distinguished from the other two cytotypes (Fig. 2a; Supporting Information Fig. S1a). Plants of higher ploidy levels also tended to have shorter internodes, which in combination with larger leaves and less stem thickening, resulted in higher leaf to sapwood area ratios (Figs 2b; S1b).

Two-way ANOVA analyses showed that both cytotype and season showed significant effect on K_s and K_{l} and the interactions between these two factors were also significant in both cases (Fig. 3a,b). During the dry season, the diploid and tetraploid cytotypes had similar mean K_s values (0.057 and 0.047 kg m^{-1} s^{-1} MPa^{-1}, respectively), which were significantly lower than that of the hexaploids (0.087 kg m^{-1} s^{-1} MPa^{-1}, Fig. 3a). The K_s values measured during the wet season were several times higher than the dry season measurements in all the three cytotypes, and were the highest in the diploid cytotype (0.246, 0.148 and 0.210 kg m^{-1} s^{-1} MPa^{-1} for the diploid, tetraploid and hexaploid cytotypes, respectively; Fig. 3a), which is different from the pattern found in the dry season. The differences in K_{l} between cytotypes were consistent during both seasons, that is, 2x > 4x > 6x (Fig. 3b). Similar to the seasonal changes in K_s, K_{l} values were also significantly higher in the wet season than in the dry season in all the three cytotypes ($P < 0.05$, t-tests). The significant interaction effects in the two-way ANOVAs seem to be driven mainly by larger degrees of seasonal changes in diploid cytotype in both K_s and K_{l} than in tetraploid and hexaploid cytotypes (Fig. 3a,b).

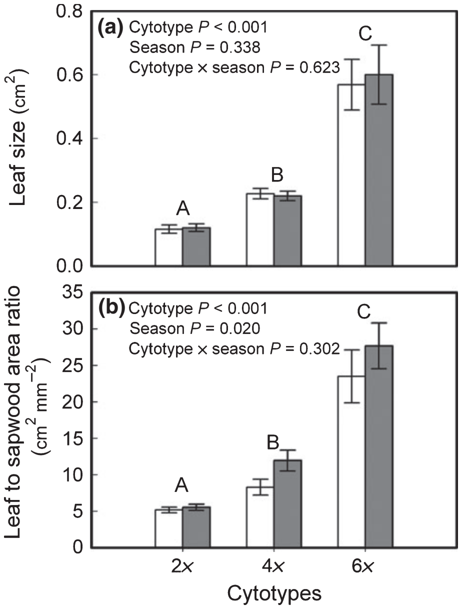

Fig. 2 (a) Leaf size and (b) leaf to sapwood area ratio in diploid (2*x*), tetraploid (4*x*) and hexaploid (6*x*) *Atriplex canescens*. Error bars show ± 1 SE ($n = 6$). Different upper case letters above bars indicate significant differences between cytotypes shown by LSD post-hoc test following the two-way ANOVA ($P < 0.05$). P-values of the main effects and their interaction are given in each panel.

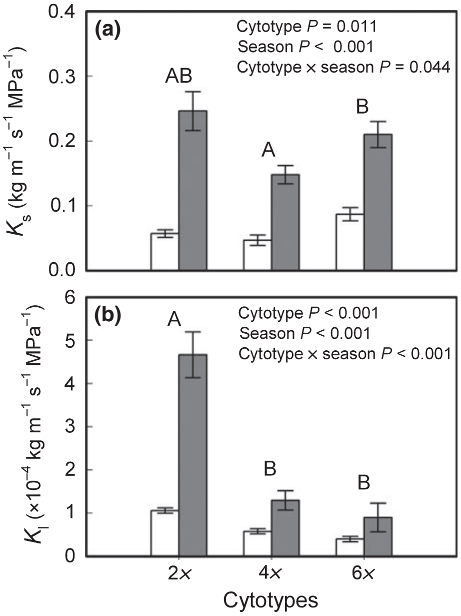

Fig. 3 (a) Sapwood specific hydraulic conductivity (K_s) and (b) leaf specific hydraulic conductivity (K_{l}) in diploid (2*x*), tetraploid (4*x*) and hexaploid (6*x*) cytotypes of *Atriplex canescens*. Error bars show ± 1 SE ($n = 8$). Different upper case letters above bars indicate significant differences between cytotypes shown by LSD post-hoc test following the two-way ANOVA ($P < 0.05$). P-values of the main effects and their interaction are given in each panel.

Stem vulnerability curves showed that across cytotypes hydraulic conductivity was negatively related to cavitation resistance, that is, the hexaploid populations had significantly greater resistance to cavitation (higher air-injection pressure or more negative tension needed to induce 50% loss of hydraulic conductivity) than the diploids and hexaploids (Fig. 4; $P < 0.05$, post-hoc test following two-way ANOVA). No significant difference in cavitation resistance was found between diploid and tetraploid cytotypes ($P > 0.05$). We did not find significant difference in stem vulnerability between the two study sites (DAC and WSNM) and thus pooled results are reported. In diploid and tetraploid cytotypes, stems were found to be more resistant to cavitation in the wet season (July) than in the dry season (December; $P < 0.05$, t-tests); no significant difference in stem xylem cavitation resistance was found between the two seasons in the hexaploid cytotype ($P > 0.05$, t-test). Because different techniques were used for the wet and dry seasons (air-injection and centrifugal force methods, respectively), caution needs to be taken in interpreting these seasonal changes in stem xylem vulnerability.

Unlike in stems, maximum K_{leaf} or leaf vulnerability to drought stress did not show an obvious pattern in comparing different ploidy levels (Fig. 5). Moreover, the tetraploids and hexaploids from DAC and WSNM showed contrasting leaf vulnerability curves within the same cytotypes (Fig. 5b–e). This suggests that

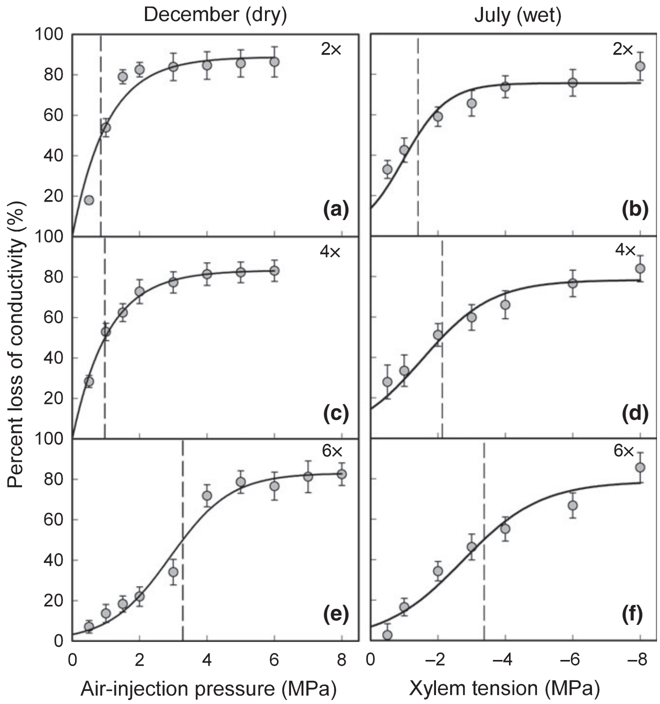

Fig. 4 Percentage loss of stem hydraulic conductivity in response to air-injection pressure (left panels), and tension generated by centrifugal force (right panels) in diploid (2*x*; a,b), tetraploid (4*x*; c,d) and hexaploid (6*x*; e, f) cytotypes of *Atriplex canescens*. Data points show mean values ± 1 SE ($n = 5–6$). Dashed vertical lines show 50% loss of conductivity.

vulnerability to xylem cavitation in leaves is relatively plastic, possibly allowing leaves to be responsive to environmental conditions, rather than being functionally constrained by intrinsic properties of specific cytotypes. In the tetraploid cytotype, populations from the WSNM gypsum dunes had significantly higher (less negative) Ψ_L than those from the DAC (Table S1) and, correspondingly, leaves were substantially more vulnerable to drought-induced cavitation in the WSNM populations (Fig. 5b,c). Hexaploids from the DAC had higher Ψ_L than those from the WSNM, and greater vulnerability to drought-induced loss of K_{leaf} was found in populations from the former study area (Fig. 5d,e).

Wood anatomical results showed that the average vessel lumen diameter tends to increase with the increase of ploidy level (Fig. 6), although the stem hydraulic conductivity tends to be lower in cytotypes of higher ploidy levels. The vessel diameters at the peaks of the frequency distribution curves are 14.5, 15.6 and 19.8 µm for the diploid, tetraploid and hexaploid cytotypes, respectively. Calculations based on xylem anatomy showed that vessel end wall resistance accounted for a much larger proportion of the total hydraulic resistance than vessel lumen, and were substantially larger in higher ploidy cytotypes, with R_e/R_l being 7.36, 13.1 and 17.2 in diploid, tetraploid and hexaploid *A. canescens*, respectively.

Discussion

Our results suggest that a trade-off between stem hydraulic conductivity and resistance to drought-induced xylem cavitation is likely to underlie the niche differentiation among cytotypes in *Atriplex canescens*. Intraspecific variation in water-related plant physiological characteristics mediated by polyploidization might be crucial for the success of a species in the drought-stressed, and yet spatially heterogeneous, desert environment.

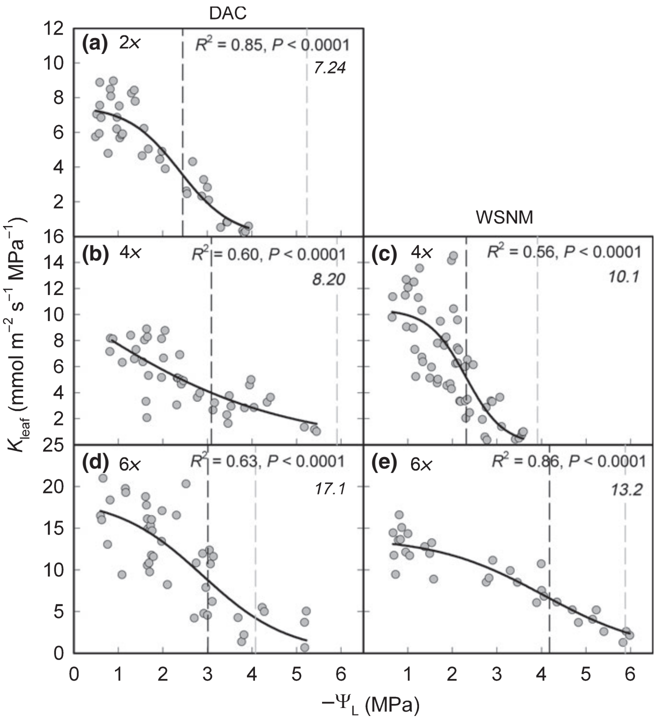

Fig. 5 Leaf hydraulic conductance (K_{leaf}) in response to the decrease of leaf water potential (Ψ_L) in diploid (2*x*; a), tetraploid (4*x*; b,c) and hexaploid (6*x*; d,e) cytotypes of *Atriplex canescens*. A three-parameter sigmoid function was fitted to the data. Black dashed lines show Ψ_L at 50% of maximum K_{leaf} and the grey dashed lines show midday Ψ_L of the wet season (July). Italic numbers in each of the panels show maximum K_{leaf} values that are extrapolated from fitted leaf vulnerability curves for an arbitrary Ψ_L of −0.6 MPa.

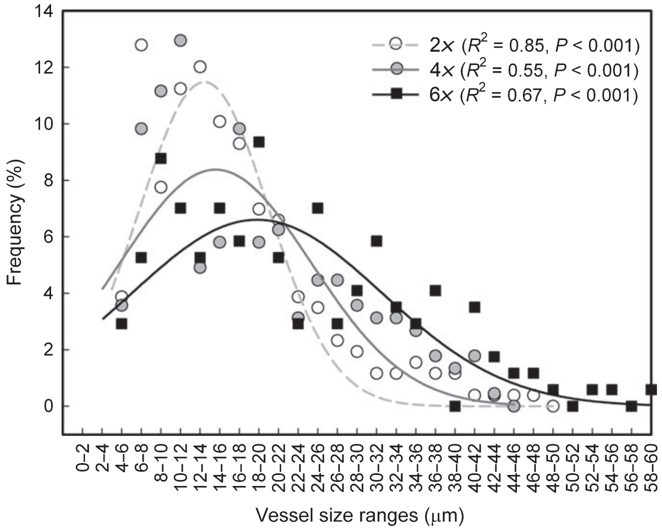

Fig. 6 Vessel lumen diameter frequency distribution in diploid (2*x*), tetraploid (4*x*) and hexaploid (6*x*) cytotypes of *Atriplex canescens*. Vessels with diameters smaller than 4 µm were excluded from analysis, due to their very small contribution to hydraulic conductivity. Data were fitted using a three-parameter Gaussian peak function.

Niche differentiation associated with soil water availability

In dry environments, such as deserts, soil texture can significantly affect plant water availability, with plants growing in sandy soil having higher water availability than those in clay soil, due to greater rainfall permeability and deeper rooting depth in sandy soil (Alizai & Hulbert, 1970; Evenari *et al.*,1971; Noy-Meir, 1973; Sperry & Hacke, 2002). Despite the fact that the clay habitats where the hexaploids occur are located in lower elevations of the landscape, due to the low permeability of the clay, water runs off the surface quickly in most cases. Extremely low soil water potential can occur in clay, according to its specific physical properties, as soil water diminishes during the long dry periods of the desert. Furthermore, plant-extractable moisture in clay is less than what the soil's physical properties alone would imply since root growth can be mechanically impeded by compact soils under dry conditions (Bengough *et al.*, 2011), which may result in lower root volume and reduced overall ability to extract soil water in plants growing in the clay habitats of the desert. The nonrandom association between population ploidy level and soil texture may suggest adaptive edaphic differences among cytotypes, which is due, at least in part, to their differences in xylem water transport efficiency and resistance to drought-induced hydraulic failure.

Stem hydraulic efficiency and safety trade-off

The previously reported K_s values measured on container-grown *A. canescens* and samples from similar environments were very close to K_s of tetraploids in the present study (Kocacinar & Sage, 2004; Jacobsen *et al.*, 2007), suggesting that sapwood conductivity is conserved across diverse environmental conditions. However, the mean K_l value reported by Kocacinar & Sage (2004) for container-grown *A. canescens* (4×10^{-5} kg m^{-1} s^{-1} MPa^{-1}) was substantially lower than that in tetraploids from the present study (1.3×10^{-4} and 6×10^{-5} kg m^{-1} s^{-1} MPa^{-1} for the wet and dry seasons, respectively), which may suggest that this species can balance water transport and demand by regulating total leaf area according to water availability.

Overall, both the present study and previous studies (e.g. Jacobsen *et al.*, 2007) showed that stems of *A. canescens* exhibited surprisingly high vulnerability, despite the very low negative water potentials the species typically experiences (Table S1). It is possible that the high vulnerability in diploids and tetraploids has been exaggerated, especially in the dry season, by the fact that they have high degrees of native cavitation, and experimentally refilled vessels show 'cavitation fatigue' and are more vulnerable than functioning vessels (Hacke *et al.*, 2001). Alternatively, low cavitation resistance may be beneficial as a mechanism of moderating water use in response to extreme drought in desert plants, particularly if leaf loss is involved in seasonal hydraulic adjustment (Hacke *et al.*, 2009).

The greater xylem cavitation resistance in cytotypes of higher ploidy levels found in the present study suggests an important physiological basis for the greater drought tolerance of polyploids (Levin, 2002; Maherali *et al.*, 2009; te Beest *et al.*, 2012; but see Buggs & Pannell, 2007). However, greater cavitation resistance does not necessarily point to superiority in environmental adaptation under all circumstances. Among other potential disadvantages related to polyploidy (Comai, 2005), lower xylem hydraulic conductivity in cytotypes of higher ploidy levels might be a major physiological constraint counteracting the beneficial effects of their stronger drought tolerance.

The less clear trend found in K_s with ploidy level change (Fig. 3a), relatively to K_l and stem xylem vulnerability (Figs 3b, 4), is likely due to the high percentages of native xylem cavitation in stems, and to contrasting behaviors by cytotypes across seasons. In the dry season, the higher K_s found in the hexaploid cytotype can be attributable to its greater resistance to xylem cavitation; thus a higher proportion of xylem remained functional. The greater K_s in lower ploidy levels is likely to be obvious only during short periods after significant rainfall events, when active growth occurs. However, this difference in hydraulic conductivity might be crucial, and likely results in substantial adaptive variation among cytotypes.

Variation in stem xylem hydraulic conductivity and resistance to cavitation amongst cytotypes thus reflects a trade-off between water transport efficiency and safety against hydraulic failure (Piñol & Sala, 2000; Martinez-Vilalta *et al.*, 2002), which provides a possible physiological basis explaining soil preferences of different cytotypes in the desert environment. A higher potential hydraulic conductivity may have endowed diploid *Atriplex* with a higher growth rate (Stutz *et al.*, 1975; Stutz & Sanderson, 1983; G.-Y. Hao, unpublished), and thus stronger competitiveness in environments with relatively high water availability, whereas greater resistance to drought-induced hydraulic failure enables hexaploids to endure the greater drought stress of clay soils in the desert climate (Sperry & Hacke, 2002). A medium xylem hydraulic conductivity and cavitation resistance, balancing hydraulic efficiency with safety in tetraploids was apparently more favorable under most environmental conditions, and thus responsible for its predominance at the landscape scale (Jones & Johnson, 1998).

Across species, the trade-off between xylem hydraulic efficiency and safety is often attributable to variation in xylem conduit diameters that is usually positively related to plant growth rates. Fast-growing species tend to produce more conductive xylem with larger conduits (Zhang & Cao, 2009; Fan *et al.*, 2012), which are often associated with reduced cavitation resistance due to the fact that larger xylem conduits are more likely to contain larger maximum conduit pit membrane pores, through which air seeding occurs under drought stress (Wheeler *et al.*, 2005). However, in the present study, higher cavitation resistances occur in higher ploidy levels with larger vessel lumen diameters, suggesting that pit size does not positively scale with vessel diameter across cytotypes. The larger vessel diameter in higher ploidy levels is likely not related to higher hydraulic requirements, but rather resulted from the strong effect of polyploidization on cell size increase (Masterson, 1994). Pit pore properties are relatively plastic in response to proximal water status, and significant changes occur even within individual plants, such as along the height gradient of tall trees (Domec *et al.*, 2008). It is possible that smaller pit pore sizes in higher ploidy cytotypes have contributed to their greater cavitation resistance despite larger vessel lumen diameters, and comparisons in pit properties deserve further investigation.

The spectacular niche differentiation between tetraploid *A. canescens* on gypsum sand dune slopes, and the hexaploid on neighboring clay sites in the WSNM (Fig. 1) particularly indicates the importance of the trade-off between water transport efficiency and safety in determining the relative advantages of cytotypes in their respective habitats. The loose texture of the gypsum sand has likely allowed tetraploid plants to access more water through deeper root systems, resulting in better water status than the neighboring hexaploids, which can be reflected by their contrastingly different leaf water potentials (−3.91 vs −5.82 MPa in tetraploids vs hexaploids; Table S1). On the other hand, to avoid sand burial by the mobile dunes, the tetraploid *A. canescens* has to have higher growth rates (G.-Y. Hao, unpublished), which requires more efficient xylem water transport.

High plasticity of leaf hydraulics

A high degree of leaf physiological plasticity can be advantageous for evergreen shrubs in a desert environment, where resources are available in a ‘pulse-and-reserve’ pattern (Noy-Meir, 1973). The relatively low construction costs and higher turnover rates of leaves, and thus the flexibility to produce morphologically and physiologically contrasting leaves under different environmental conditions, have also likely contributed to the measured high plasticity in leaf hydraulic conductance. Especially considering the high assimilation potential of C_4 plants (Sage & Pearcy, 1987), it may be more favorable to have a flush of more conductive and less drought-tolerant leaves after rainfall events, rather than maintaining stress-tolerant but less hydraulically efficient leaves throughout the year. In fact, upon high water availability, all the cytotypes can flush quickly and produce ephemeral leaves that are significantly larger in size and shorter in lifespan than regular leaves (Sanderson & Stutz, 1994). In addition, the very low Ψ_L found even in the wet season (more negative than Ψ_L that causes 50% loss of K_{leaf}; Fig. 5) suggests that K_{leaf} is kept at rates far below its capacity during most periods of the year and only reaches high values during those rainfall ‘pulses’. The divergence in environmental adaptation between cytotypes in *A. canescens* seems to depend on both the constitutive differences in stem hydraulic conductivity, and leaf-level functional coordination by means of physiological plastic responses to proximal environmental water conditions.

Our results do not support the long held hypothesis that polyploids tolerate a wider range of environmental stress and have a larger ecological range (Stebbins, 1950); rather, the physiological trade-offs associated with genome size variation suggest that polyploidy has both advantages and disadvantages (Comai, 2005). Having multiple ploidy levels, each with its own habitat preference, may thus favor a species in colonizing larger geographic ranges with heterogeneous environmental conditions (te Beest *et al.*, 2012).

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Supporting Information

Additional supporting information may be found in the online version of this article.

Fig. S1 Photos showing differences in leaf and branch morphology among diploid (upper), tetraploid (middle) and hexaploid (lower) cytotypes of *Atriplex canescens* co-occurring in the study area.

Table S1 Leaf water potentials (Ψ_L) of different *Atriplex canescens* populations measured during the dry (December) and wet (July) seasons