

# A hydraulic explanation for size-specific plant shrinkage: developmental hydraulic sectoriality

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## Summary

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**Key words:** *Cryptantha flava*, distance to xylem, dye tracking, fragmentation, Great Basin desert, hydraulic integration/sectoriality, leaf water potential.

- While great attention has been paid to the mechanisms controlling plant growth, much less is known about why and how plants shrink. The modular design of plants may facilitate the independence of modules if the xylem vasculature is hydraulically sectoried. We examined the hydraulic connectivity of modules comprising juveniles and adults of the aridland chamaephyte *Cryptantha flava* (Boraginaceae), motivated by the observation that rosette mortality is spatially aggregated in adults, but not in juveniles.
- We explored spatial patterns of leaf wilting after clipping a single lateral root, tracked physiological dyes taken up by a single root, and measured within-plant variation in leaf water potentials after watering a portion of the root system. We then measured xylem anatomical features related to hydraulic connectivity.
- Our approaches revealed hydraulic integration in juveniles but hydraulic sectoriality in adults. We attribute such developmental changes to increasing distances between xylem bundles, and larger xylem lumen and heartwood areas as plants age.
- We have demonstrated *functional* sectoriality in a desert chamaephyte, and report the mechanism by which sectoriality occurs, offering a hydraulic explanation for the death of whole plant portions resulting in shrinkage of large plants, and for the high occurrence of this design in deserts.

## Introduction

The modular design and indeterminate growth of plants allow them to display large fluctuations in size between years, both growing and shrinking. Understanding the mechanisms that control plant size is important because size is commonly used as a proxy for individual fitness (Harper, 1977). Consequently, the ecological, evolutionary and agricultural literature has paid much attention to the factors that promote and limit growth at the cellular (Wilkinson & Davis, 2008), anatomical (Hsaio, 1973; Enquist *et al.*, 2007a), physiological (Enquist *et al.*, 2007b; Niklas, 2007) and demographic levels (Caswell, 2001). However, the ability of perennial plants to decrease in size has been overlooked. This is surprising because plant shrinkage is a frequent phenomenon, occurring in published population projection matrices of over 90% of perennial plant species ( $n = 199$  species; R. Salguero-Gómez, unpublished).

In woody plants, portions of the shoot and the associated root may die during drought because the negative pressures

of the xylem water column may precipitate dissolved air, resulting in runaway embolism, which may disrupt water flow (Tyree & Dixon, 1986; Tyree & Zimmermann, 2002). It is commonly assumed that plants distribute evenly belowground resources among all modules of their architecture, a condition known as ‘hydraulic integration’ (Horwath *et al.*, 1992). However, there is increasing evidence that some species are composed of independent hydraulic units (IHUs *sensu* Schenk, 1999), and that, in them, water and nutrient transfer is limited, a condition known as ‘hydraulic sectoriality’ (Orians *et al.*, 2005).

Hydraulic sectoriality is a trait with likely adaptive value in water-limiting environments because it may allow the individual plant to avoid runaway embolism (Orians *et al.*, 2005), optimize resource usage by failing to support other, less fortunate modules foraging in low-resource soil patches, which in turn may increase the probability of whole-plant survival (Ginzburg, 1963), and suppress or slow down whole-plant senescence (Silvertown *et al.*, 2001). All of these possibilities are relevant for desert plants because they

operate close to hydraulic failure (Tyree & Dixon, 1986; Sperry *et al.*, 2008) and must forage for scarce soil resources that are heterogeneously distributed on fine scales (Jackson & Caldwell, 1993).

In the present study, we explore whether the aridland chamaephyte *Cryptantha flava* (A. Nels.) Payson (Boraginaceae; Fig. 1) exhibits hydraulic sectoriality. In this species, shrinkage during or following a particularly dry year is specific to larger individuals (Casper, 1996) and occurs through the death of spatially aggregated rosettes. Past measurements of leaf water gas exchange in this species – transpiration and photosynthesis – and leaf water potentials do not explain why larger individuals are more severely affected during drought (Casper *et al.*, 2006).

Here, we used a multidisciplinary approach to study the functional hydraulic design and underlying anatomy of *C. flava*. Specifically, we examined functional hydraulic connections between roots and aboveground modules using three complementary approaches: severing an individual root and looking for evidence of localized wilting in clusters of leaf rosettes, using dyes to track the pathway of water movement from a particular root through the shoot, and measuring within-canopy variation in leaf water potentials when only part of the root system was watered. We carried out these experiments on both juveniles and adults. Finally, we investigated whether underlying changes in xylem anatomical traits, such as the distance between neighboring xylem vessels and the area of viable xylem, occur with plant ontogeny and we argue that these traits are ultimately responsible for the shifts we find in the hydraulic architecture of *C. flava*.

## Materials and Methods

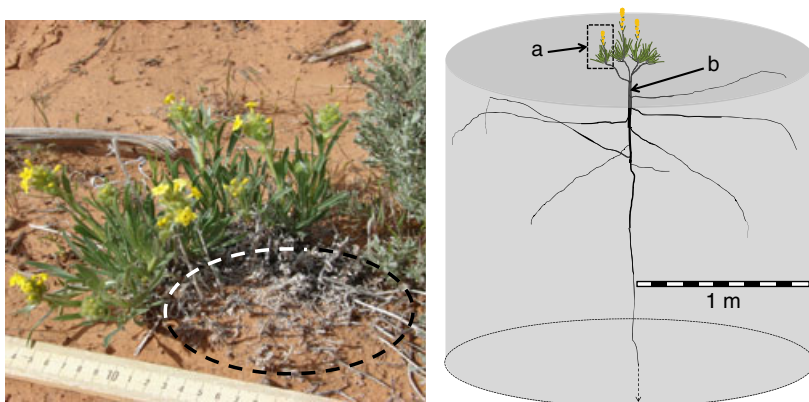
### Description of study species and field site

*Cryptantha flava* is a long-lived, aridland plant species with large scattered populations throughout the Colorado Plateau. A single plant may consist of one to > 150 basal

leaf rosettes, each with a shoot apical meristem and typically 5–13 oblanceolate leaves. Leaves first appear in mid-April, but considerable leaf turnover occurs as the growing season progresses (Casper *et al.*, 2001). New leaf rosettes arise from basal axillary buds. The species is considered a small chamaephyte (its height, not including flowering stalks, averages 10 cm) with characteristics of both herbaceous and woody species. From a demographic point of view, it is herbaceous perennial because its aboveground tissue is herbaceous and completely replaced each year as the plant enters winter dormancy by August; from a purely anatomical point of view, it is woody because it possesses secondary growth in the branched underground stem, the caudex.

We refer to a group of rosettes and the part of the branched stem to which they are connected as a ‘module’ (Fig. 1). Juveniles typically have one to three modules with one to 10 rosettes per module, while large adults may have up to approximately six modules with five to 20 rosettes in each, although wide variation in this arrangement exists. All modules are connected via the caudex to a single root system (Fig. 1), consisting of a taproot and well-defined lateral roots. The laterals emerge from the taproot at a depth of 15–40 cm and extend horizontally out to 1 m (Peek & Forseth, 2005), where they bend downward. In adults, the taproot and lateral roots have a suberized, hydrophobic covering; clusters of ephemeral white, fine roots interrupt the waxy covering every 1–3 cm along the lateral roots. Ephemeral roots grow in response to large rainfall events and their growth on different lateral roots can be induced independently by the localized application of water (R. Salguero-Gómez & B. B. Casper, unpublished).

The field site is located at the Redfleet State Park (40°30'N, 109°22'30"W, 1730 m, Utah), a cold desert habitat characterized by the woody species *Juniperus osteosperma*, *Artemisia tridentata*, and *Chrysothamnus nauseosus*, where *C. flava* is the dominant chamaephyte. The precipitation is bimodal, with peaks in May and October. Annual precipitation averages 225 mm, but variation is high (CV = 50–125%).



**Fig. 1** Spatially aggregated mortality of rosettes in an adult of *Cryptantha flava* (left), and its anatomy (right): (a) groups of rosettes branching from the same shoot are clustered into a module. Modules branch off from the caudex (b), the central, underground stem. The root system consists of a deep taproot and a discrete number of lateral roots that spread laterally c. 1 m.

## Functional sectoriality

In order to evaluate the hydraulic connectivity between roots and rosettes of *C. flava* we used the following physiological techniques on both juveniles and adults. We defined juveniles as small ( $\leq 15$  rosettes), nonreproductive individuals, and adults as flowering individuals of any size, or not currently flowering individuals of large size ( $> 15$  rosettes).

**Severing roots** With hydraulic integration, we would expect some compensation among connected modules so that no aboveground part of the plant would wilt if a single lateral root were severed. With hydraulic sectoriality, in contrast, we would expect spatially aggregated wilting/mortality of rosettes. To examine these possibilities, we severed a single lateral root in 12 randomly chosen juveniles and 24 adults in July 2007. Additionally, we severed the taproot instead of a lateral root in another three juveniles and three adults. We carefully excavated a lateral root and followed it 30–40 cm away from the caudex, where we removed a 1-cm-long portion and measured its diameter. In the case of the taproot, we exposed and cut it at a depth of 1 m, far below any lateral root. We used extreme care in excavations to prevent injury and immediately replaced the sandy soil.

To quantify the amount and spatial location of wilting, we placed a  $30 \times 30$  cm<sup>2</sup> grid on 30-cm-tall legs over the plant and photographed (Sony Cybershot DSC-H2; Sony Corporation, Tokyo, Japan) each individual from a height of 70 cm before severing the root, then 1, 24, 48 and 72 h after the treatment was applied, and again the following year, in June 2008. The high quality of the photos (1200 dpi) allowed us to count the number of wilted rosettes in the hours following the treatment. We used regressions to examine the relationship between the plant's number of rosettes the day before the treatment and the number of wilted rosettes, if any, at 72 h, both with and without the diameter of the severed root as a covariate. We also looked for abrupt changes in the number of wilted rosettes as a function of initial number of rosettes using a piece-wise regression in R (R Development Core Team, 2009). Such abrupt change, if any, would indicate a size threshold for the response of rosette wilting.

**Tracking dyes** We traced the hydraulic pathways of randomly chosen individuals in July 2007 using solutions of acid fuchsin (1% w/v; molecular weight = 585.52 g/mol), fast green FCF (0.5%; 765.89 g/mol), and toluidine blue (0.5%; 207.37 g/mol) supplied to different lateral roots of eight juveniles and 24 adults. We uncovered a root as described in the previous section (see the section entitled 'Severing roots'), cut it under water in order to avoid cavitation and immediately placed it in a 25-ml beaker containing the dye solution. We covered the beaker with plastic wrap and carefully replaced the soil. After 24 h, we quantified the number and specific location of rosettes that had taken up

the dye. We tried to implement the same procedure for taproots but we did not succeed in feeding the dye without causing almost immediate wilting of the whole plant.

To determine if dyes would redistribute within the plant if transpiration were impeded, we isolated the dyed rosettes in a water-saturated atmosphere. Such redistribution would suggest that dyes had originally followed the path of least hydraulic resistance but not the only possible pathway from a particular lateral root. We covered the stained rosettes with a plastic bag containing a piece of wet sponge and then surrounded the bag with aluminum foil. Unstained rosettes were checked 24 h later for potential dye transfer.

**Within-individual variation in water potential** We also followed the pathway of water movement more directly by measuring variation in leaf water potential within the canopy after watering only a portion of the root system. Because dye particles are much larger ( $300\text{--}1000$  g mol<sup>-1</sup>) than water molecules and other naturally occurring molecules that might be transported in the xylem, such as gibberellins (e.g. GA1: 348.4 g mol<sup>-1</sup>), cytokinins (CKs: 234 g mol<sup>-1</sup>), and abscisic acid (ABA: 264.32 g mol<sup>-1</sup>), we wanted to make sure that dye movement tracks the same pathway as water. We conducted the experiment in mid June 2008, when plants were still photosynthetically active but the soil was relatively dry (gravimetric soil water content :  $0.03 \pm 0.01$  g water per g soil; R. Salguero-Gómez & B. B. Casper, unpublished), so watering would significantly increase leaf water potentials. Lower leaf water potentials reflecting even drier soils later in the growing season would have forced us to use a psychrometer (M. Peek, pers. comm.), which is much more time-consuming than the pressure chamber we used here (Model 1001; PMS Instrument Co., Corvallis, OR, USA). All rosette modules on each individual were carefully identified and tagged at the base with thin tape. Individuals were then randomly assigned to one of three treatments: control with no water (two juveniles and three adults), partial watering (six juveniles and 12 adults) and full watering (two juveniles and three adults). In early evening on a single day (18:30 h), plants in the full watering treatment received 45 mm of water in a 1-m-radius circle centered on the plant; plants in the partial watering treatment received 45 mm of water in a 60° sector of the same sized circle. A pulse of similar intensity at the same time of year was previously shown to stimulate fine-root production (R. Salguero-Gómez & B. B. Casper, unpublished). We measured leaf water potentials in all rosette modules at 18:00 h and 22:00 h on the day on which the plants were watered at 18:30 h, and then again at 04:00 (predawn) and 12:00 h (midday) on the following day. We sampled three to six fully developed leaves per module of each individual, depending on module size.

We analyzed the effect of the watering treatment on leaf water potentials using repeated measures of variance

(MANOVA; Sokal & Rohlf, 1995), first with developmental stage as an explanatory variable, and then conducting separate MANOVAs for juveniles and adults, as developmental stage was a significant effect (Supporting Information Table S1). Watering treatment served as a fixed effect, and individuals and modules nested within individuals as random effects, with measurements repeated four times (18:00 h, 22:00 h, predawn and midday). These analyses were used to determine if partially watered plants developed greater within-individual variation in water potential over time than plants in other treatments. We also calculated variance components among individuals and among modules at each measurement time to examine their relative contributions to total variation in leaf water potential.

We used within-individual variation in leaf water potentials to cross-validate results obtained using dyes. For partially watered plants, we dug up the watered section of the soil in search of lateral roots and tracked their hydraulic connections to rosette modules using dye solutions just as described previously (see the earlier section entitled 'Tracking dyes'). We always found only one lateral root in the watered soil sector. We then used absence/presence of dye in the different modules 24 h after dye application as the response variable in a logistic regression with watering-induced changes in leaf water potential among modules at midday as the explanatory variable. We also saturated the atmosphere of the stained modules, as described above (see the earlier section entitled 'Tracking dyes'), to explore all possible hydraulic connections aboveground.

We then compared leaf water potentials in partially watered plants with those in plants either fully watered or not watered (controls). First we explored whether dyed modules of partially watered plants operated at the same water potential level as modules of fully watered plants; secondly we explored whether undyed modules of partially watered plants operated at the same water potential level as modules of control plants. We employed unpaired *t*-tests with plant treatment as the dependent variable and leaf water potential as the response variable.

### Vascular anatomy

We examined the vascular arrangement in caudexes of several juveniles and adults, including some adults from the dye-tracking experiment described above (see the earlier section entitled 'Tracking dyes'), by measuring neighboring xylem distances and lumen diameter (Dale, 1999). We fixed the caudexes in FPA (Paraformaldehyde: 30% ethanol, 5% propionic acid, 5% formaldehyde and 50% distilled H<sub>2</sub>O) with 10% glycerol to soften tissue for cross-sectioning. Discs of the caudex (0.5 cm thick) were taken at 3–4 cm below the first shoot branch and used in standard paraffin and plastic embedding procedures.

We used paraffin-mounted samples to compare general changes in vascular structure with plant size and to track internal staining with dyes used in the dye-tracking experiment. The samples underwent a dehydration gradient to 100% tert-butanol at room temperature and were then heated at 58°C for 12 h before embedding (Paraplast Plus®; McCormick Scientific, St Louis, MO, USA). We carefully sliced off paraffin until a full cross-section was exposed and soaked samples in 1% dish detergent for 24 h to further soften the tissue (J. Mauseth, pers. comm.). We obtained cross sections varying in width from 20–50 µm per sample using a rotary microtome (Reichert-Jung supercut 2065 automatic; Leica, Bannockburn, IL, USA), mounted them with poly-lysine and removed paraffin in a graded xylene series. Because we were interested in locating the dyes used in the dye-tracking experiment, we did not use any histological stain. We photographed the cross-sections through a dissecting microscope (Olympus MV Plapo 2XC; Olympus, Allentown, NJ, USA) using the image program DP CONTROLLER v. 3.1.1.267 (Olympus).

We mounted similar sections of caudexes from 10 individuals in plastic media to study xylem and heartwood characteristics in plants differing in caudex size. Samples were dehydrated in a graded ethanol series, embedded in histological resin (Technovit 7100; Heraeus Kulzer, Frankfurt, Germany) for 4 d, sectioned at 3–5 µm using the rotary microtome with a tungsten blade (Reichert-Jung, D-profile; Leica, Bannockburn, IL, USA), and stained with toluidine blue (0.5%, v/w). Cross-sections were photographed on an electronic microscope (Olympus BX51). To determine the relative spacing of xylem bundles and individual vessels, we used the point-to-target distance technique (Dale, 1999), where we chose 20 random coordinates within the nondecayed parenchyma region of each caudex cross-section and measured the distance from each point to its closest xylem vessels using the program IMAGE J (NIH, 2004). Relatively large measurements of point-to-target distance represent great distances among the targeted objects (Dale, 1999) – in this case the xylem bundles and vessels. Thus, larger values of point-to-target distance can be interpreted as spatially separated units of vertical water transport and reduced chances of lateral water transport or hydraulic integration. We also measured the lumen diameter of 50 randomly chosen xylem vessels in each individual. Because tracheids are only slightly thinner than the smallest vessel elements in *C. flava*, we are only able to tell them apart in cross-section under the electronic microscope; we did not distinguish between them in our analyses.

Because we found great variation in the area occupied by decayed heartwood in the caudexes, we also measured its area to explore whether it could explain the degree of integration-sectoriality in *C. flava*. We then correlated xylem distance, xylem lumen diameter, and decayed heartwood area (all log-transformed) with the caudex diameter to



determine whether these anatomical features change with plant size in a way that explains developmental sectoriality in *C. flava*. The regressions were carried out against caudex diameter as a measure of plant size, which correlates significantly with the number of leaf rosettes ( $t_{50} = 15.23$ ,  $P < 0.001$ ,  $R^2 = 0.23$ ), and probably better represents plant age than does canopy size because of the ability of large plants to shrink aboveground.

## Results

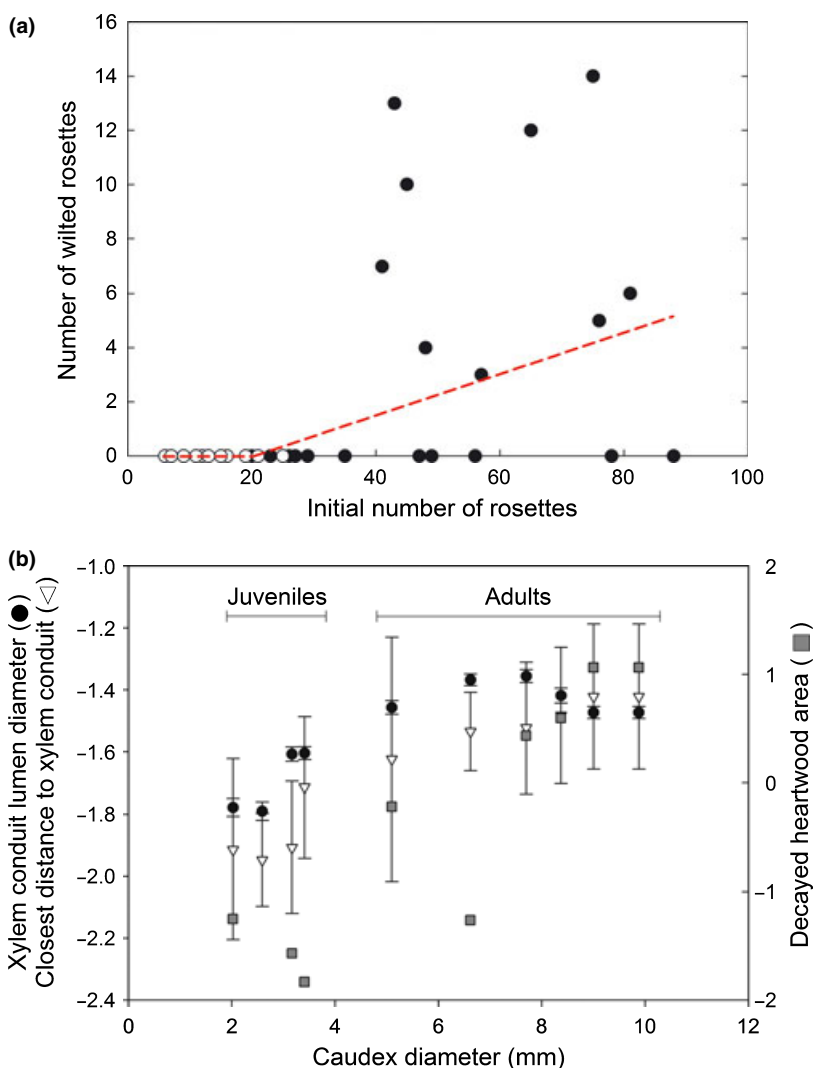
### Severing roots

Severing the taproot of either juveniles or adults caused the entire aboveground biomass to wilt in a matter of minutes, and the plants eventually died. Severing one lateral root caused spatially aggregated wilting in nine of the 24 adults but no wilting of rosettes in juveniles (Fig. 2a). Wilted tissue did not subsequently recover, and that part of the plant

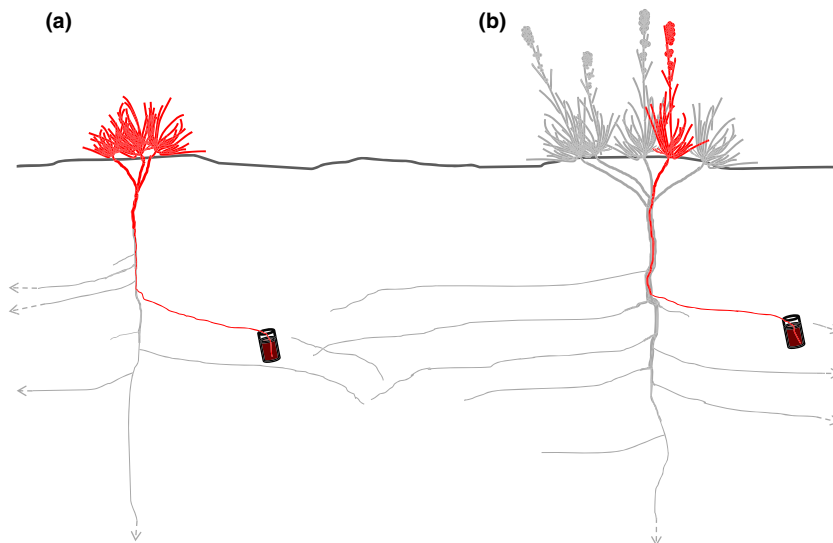
was dead the following year in all nine cases. The piece-wise regression revealed a significant size threshold in the wilting response of 20 rosettes ( $t_{34} = 11.34$ ,  $P = 0.001$ ) below which no wilting occurred and above which over 40% of the plants showed some wilting. A simple linear regression showed that the initial number of rosettes explained 25% of the variation in the number of wilted rosettes ( $t_{34} = 11.42$ ,  $P = 0.002$ ;  $R^2 = 0.25$ ), indicating more tissue loss in larger plants; adding the diameter of the clipped lateral root as a covariate did not improve the correlation (ANCOVA:  $F_{2,33} = 5.57$ ,  $P = 0.008$ , adjusted  $R^2 = 0.21$ ).

### Tracking dyes

One day after the dye solution was applied to a lateral root, the dye appeared in all rosettes of juveniles, but was aggregated in a specific group of rosettes in adults (Fig. 3). Posterior examination showed that all stained rosettes in adults belonged to the same rosette module (i.e. the dyed



**Fig. 2** Differences in individuals of *Cryptantha flava* as a function of plant size. (a) Aboveground response to the loss of hydraulic conductivity when a single lateral root was clipped per individual ( $n = 12$  juveniles and 24 adults). The dashed line represents piece-wise regression, with a breakpoint for plants of 20 rosettes. (b) Xylem vessel lumen diameter ( $\mu\text{m}$ ), distance to closest xylem vessel ( $\mu\text{m}$ ) and decayed area of heartwood ( $\text{mm}^2$ ) increase with caudex diameter and development ( $n = 4$  juveniles and 6 adults). Note different logarithm axes for the anatomical measurements.



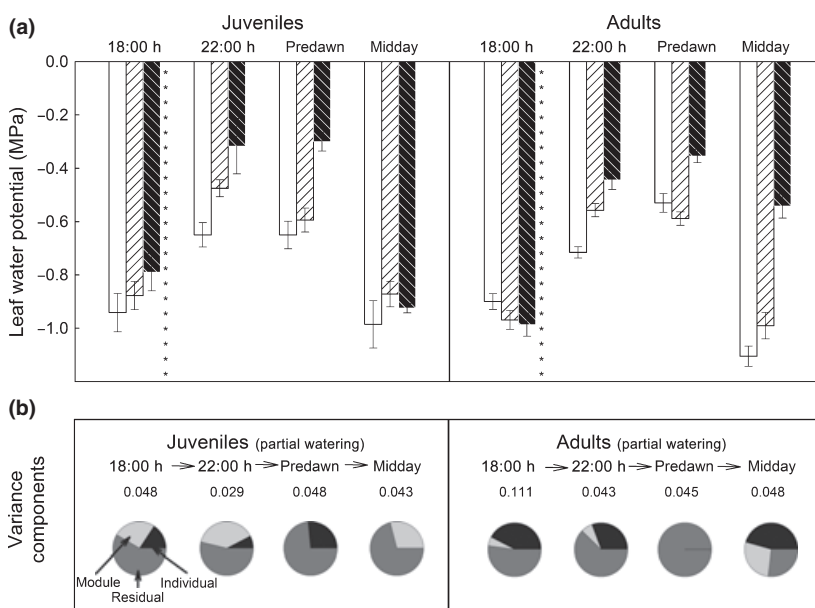
**Fig. 3** Diagram representing a juvenile (a) and an adult (b) of *Cryptantha flava* 24 h after a dye solution (acid fuchsin) was fed to one lateral root with a vial. In the case of the juvenile the dye stained all leaf rosettes, but in the adults the dye was concentrated into a specific module, as indicated by the distinctive leaf and flower red coloration. Dashed arrows indicate continuation of the root.

rosettes were all connected to the same branch of the caudex). The staining of all rosettes in juveniles was not driven by the existence of only one module, because several juveniles had two, and both were equally stained. In two large juveniles and two small adults, out of the total number of 32 plants, we detected movement of dye to another module when we saturated the atmosphere of the originally stained modules.

### Leaf water potentials

Leaf water potentials differed significantly between juveniles and adults and among watering treatments (Table S1), but not before the watering occurred. At 18:00 h, there was no significant effect of treatment ( $F_{2,131} = 0.42$ ,  $P = 0.65$ ),

developmental stage ( $t_{133} = 1.85$ ,  $P = 0.18$ ) or their interaction ( $F_{3,130} = 0.83$ ,  $P = 0.48$ ) on leaf water potential. After watering, leaf water potentials were higher – less negative – in the full watering treatment than in the partial watering treatment, which were higher than in controls ( $F_{2,532} = 8.68$ ,  $P < 0.001$ ;  $\psi_{L \text{ Full}} = -0.78 \pm 0.03$  MPa;  $\psi_{L \text{ Partial}} = -0.76 \pm 0.02$  MPa;  $\psi_{L \text{ Control}} = -0.59 \pm 0.03$  MPa; mean  $\pm$  standard error of leaf water potentials in full, partial or control watering treatments, respectively). For juveniles, partially watered plants had higher water potentials than controls only at 22:00 h, whereas partially watered adults also had significantly greater leaf water potentials at midday (Fig. 4a; Table 1). Leaf water potentials were elevated in the full watering treatment, compared with controls, at 22:00 h ( $t_{14} = 22.65$ ,  $P < 0.001$ ) and predawn ( $t_{12} = 7.22$ ,  $P < 0.02$ )



**Fig. 4** Watering either one sector of the plant or its entirety affects significantly the water status of the modules of the plant. (a) Mean ( $\pm$  standard error (SE)) leaf water potentials of whole individuals of *Cryptantha flava* before and after watering treatments were applied at 18:30 h, as indicated by the dotted vertical lines. □, control; ▨, partial watering; ▩, full watering. (b) Total contribution to leaf water potential variance components (number at the bottom of each time-point) and percentage (pie-chart) of the variation in leaf water potentials attributable to individuals, to modules within individuals and to noise for only the partial watering treatment at each time-point.

**Table 1** Separate repeated measures ANOVA, for juveniles and adults, for leaf water potentials of modules within individuals exposed to three different watering treatments and followed for four time-points

Source	Juveniles				Adults			
	df	Mean squares	F-ratio	P-value	df	Mean squares	F-ratio	P-value
Between subjects								
Treatment	2	0.330	6.074	0.004*	2	0.826	10.046	< 0.001**
Treatment (individual)	7	0.352	1.849	0.092	14	1.800	3.122	< 0.001**
Treatment (individual (module))	5	0.081	0.597	0.703	23	1.683	1.781	0.018*
Within subjects								
Time	3	3.166	38.794	< 0.001**	3	7.272	58.984	< 0.001**
Time × treatment	6	0.589	3.607	0.004*	6	1.999	8.111	< 0.001**
Time × treatment (individual)	21	1.251	2.190	0.008*	42	9.456	5.479	< 0.001**
Time × treatment (individual (module))	15	0.620	1.519	0.123	69	5.502	1.941	< 0.001**

\*,  $P < 0.05$ ; \*\*,  $P < 0.001$ .

for juveniles, and at 22:00 h ( $t_{27} = 20.90$ ,  $P < 0.001$ ), predawn ( $t_{27} = 8.70$ ,  $P = 0.005$ ) and midday for adults ( $t_{29} = 69.07$ ,  $P < 0.001$ ). Two separate repeated measures ANOVAs, one for juveniles and one for adults, revealed significant effects of watering treatment and time, as well as their interaction. However, when we considered the variation in leaf water potentials attributable to the modules nested within individuals, the model was statistically significant for adults but not for juveniles.

Watering only one sector of the plant increased variation in leaf water potential more among modules of adults than among modules of juveniles. In partially watered juveniles, the variance component attributable to modules (Fig. 4b) was 26% before watering, at 18:00 h, 38% at 22:00 h, and 29% the following midday. In contrast, variation in leaf water potential among modules within partially watered adults increased from 6% before watering and 7% at 22:00 h to 28% the following midday. Interestingly, in both partially watered juveniles and adults, the variance component of leaf water potential attributable to modules was zero before dawn when plants are assumed to be at equilibrium with soil water potential (Sperry *et al.*, 1996).

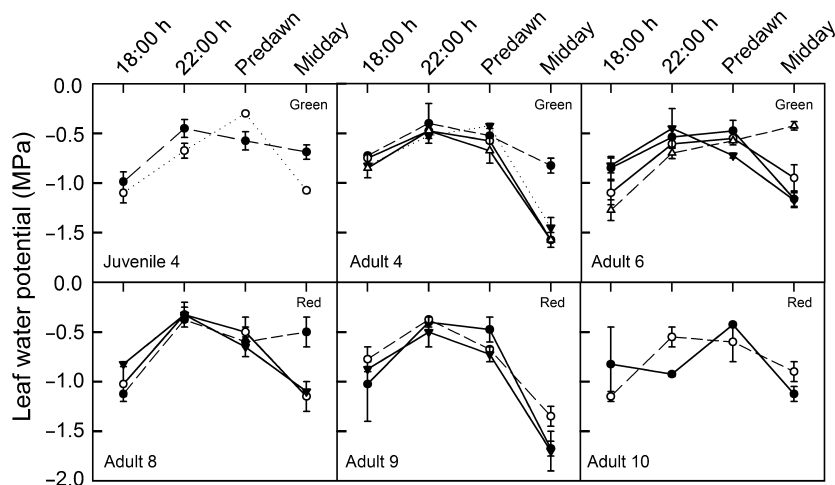
With subsequent application of dye to the root in the watered sector of partially watered plants with multiple modules, the dye always travelled to the module that had previously experienced the highest leaf water potential at midday ( $\chi^2_{135} = 46.79$ ,  $P < 0.001$ ; Fig. 6 & Fig. S1). After the surrounding atmosphere of the stained rosette modules was saturated, no dye moved to other regions of the plant, with two exceptions: in juvenile 4 (Fig. 5), the dye travelled to the only other module, staining the entire plant; in adult 4, dye travelled to one of three previously unstained modules, which had shown the second highest leaf water potential among all the modules of the plant.

Unstained modules of partially watered plants operated at similar leaf water potential as modules of control plants. For juveniles, this was true regardless of the time of measurement ( $t \geq 3.65$ ,  $P \geq 0.08$ ). In adults, unstained

modules of partially watered plants did not differ in water potential from controls ( $t \geq 3.47$ ,  $P \geq 0.07$ ) except at midday when they had higher values than controls ( $t_{53} = 13.33$ ,  $P < 0.001$ ). There was slightly less concordance between stained modules of partially watered plants and fully watered plants. In juveniles, stained modules had lower water potential than fully watered juveniles at predawn ( $t_{12} = 5.33$ ,  $P = 0.04$ ). In adults, the same was true at both predawn ( $t_{46} = 10.52$ ,  $P = 0.002$ ) and midday ( $t_{51} = 7.91$ ,  $P = 0.007$ ).

### Vascular anatomy

Xylem vasculature differed between juveniles and adults at three levels (Fig. 2b). First, distance from a random point to the closest xylem vessel increased as a function of caudex diameter ( $t_{198} = 127.59$ ,  $P < 0.0001$ ,  $R^2 = 0.39$ ; Fig. 2b), being nearly 10-fold greater for adults ( $144.87 \pm 1.74 \mu\text{m}$ ) than for juveniles ( $15.56 \pm 2.12 \mu\text{m}$ ;  $t_{119.43} = -5.77$ ,  $P < 0.0001$ ). Juveniles already exhibited some signs of sectoriality because their xylem vessels are grouped in bundles separated by the dense packs of rays of the radial system (Figs 1, 6b). This packing resulted in large distances among the xylem vasculatures of these bundles near the cambium region (Fig. 6d). Secondly, the diameter of the xylem vessels increased linearly with caudex diameter ( $t_{501} = 216.83$ ,  $P < 0.001$ ,  $R^2 = 0.30$ ), and it was almost twice as large in adults ( $40.47 \pm 0.69 \mu\text{m}$ ) as in juveniles ( $22.17 \pm 0.84 \mu\text{m}$ ;  $t_{500.11} = -17.82$ ,  $P < 0.0001$ ). Within individuals, variation in xylem diameter was very low. Thirdly, the area of decayed heartwood in the central region of the caudex increased linearly with caudex diameter ( $t_8 = 35.00$ ,  $P = 0.004$ ,  $R^2 = 0.85$ ; Figs 2b, 6a–g). Comparisons across plants of different sizes showed that heartwood decomposition took place from the pith outwards, first following the groups of rays described above (Fig. 6c,d). In very large adults, decayed heartwood extended to the surface and resulted in physical fragmentation of the live vasculature (Fig. 6e–g,i).



**Fig. 5** Average leaf water potential of modules ( $\pm$  standard error (SE)) for juveniles and adults. After all the leaf water potentials had been measured, the hydraulic conductivities were tracked using green fast FCF (green) or acid fuchsin (red). The dashed line represents the module that showed the dye after 24 h while solid lines represent modules that did not receive dye. The dotted line represents the module to which the dye transferred when the atmosphere of the previously stained module (dashed line) was saturated. Complete experimental results can be found in Supporting Information Fig. S1.

In caudex cross-sections from adults used in the dye-tracking experiment, the dye was confined to a specific portion of the xylem, demonstrating sectoriality in the distribution of the dye. Staining occurred only in the newer xylem tissue, suggesting that older xylem is not functional (Fig. 6h,i).

## Discussion

### Physiological evidence for functional hydraulic sectoriality

We have established that the transport of water is integrated throughout the caudex in juveniles of *C. flava* but becomes sectorial as plants grow larger. This was demonstrated by three complementary methodologies: in adults, but not in juveniles, severing lateral roots caused wilting and eventual death of a portion of the canopy; dyes applied to a single lateral root were distributed throughout the rosette modules of juveniles but compartmentalized in adults; and watering a lateral root of adults caused within-plant variation in leaf water potential consistent with hydraulic sectoriality. Such sectoriality was positively correlated with plant size; our study demonstrates that intermediate developmental stages can still exploit alternative hydraulic pathways to some degree, whereas fully developed adults cannot. These seem to be the first such findings for a chamaephyte. We know of only one example in a woody plant, the desert shrub *Ambrosia dumosa*, where sectorial transport of water-soluble dyes and increased variation in leaf water potential and stomatal conductance have all been linked (Espino & Schenk, 2009).

It appears that water still redistributes internally among modules when stomata are closed. We base this conclusion on the observation that leaf water potential does not vary among modules at predawn despite such variation at 22:00 h and midday. Assuming that at predawn plants reach equilibrium with the soil water potential where their connected lateral roots forage (Sperry *et al.*, 1996), partially watered adults should show high variance in leaf water potentials among

modules. The fact that significant leaf water potential variation does not exist among modules before dawn implies that small amounts of water are redistributed to other modules in the absence of high transpiration rates.

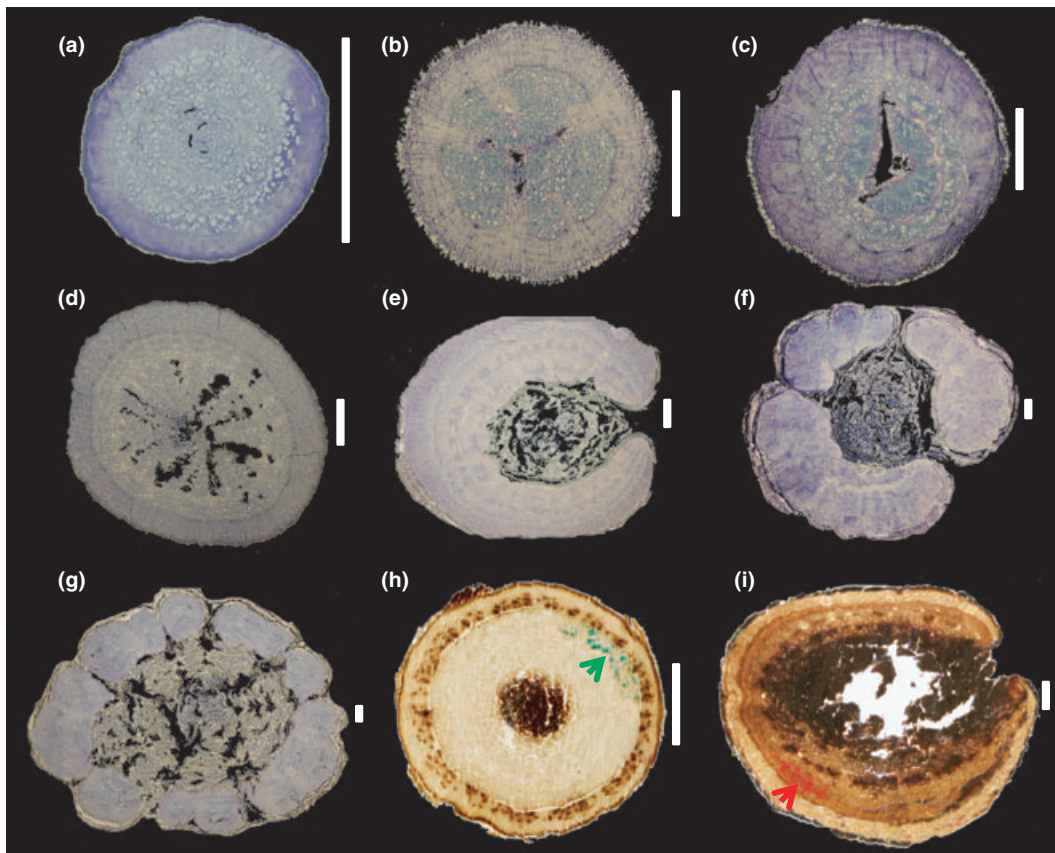
### Cross-validation of dye-tracking techniques

By coupling dye-tracking techniques with leaf water potential measurements in partially watered plants, we have proved the validity of using physiological dyes to explore the hydraulic design of *C. flava*. Dyes supplied to roots in the watered region travelled always to the aboveground sectors that experienced a significant increase in leaf water potential as a consequence of partial watering. The concern about dye-tracking experiments is that, despite their common usage to explore hydraulic pathways (Ellmore *et al.*, 2006), the molecular size of dyes may not allow for lateral movement in the xylem and so the dyes might not follow the same pathway as water and minerals (Choat *et al.*, 2003; Sano, 2004). Indeed, Hargrave *et al.* (1994) reported greater distribution of stain in the xylem vessels of the shrub *Salvia menifera* for basic fuchsin ( $337.86 \text{ g mol}^{-1}$ ) than for alcian blue, a larger molecule ( $1298.86 \text{ g mol}^{-1}$ ). Our approach with dyes is valid as long as the possibility of redistribution of dyes to nonpreferential hydraulic pathways can be ruled out. Our saturating of the atmosphere of the stained rosette module showed such redistribution in some medium-sized individuals. However, because anatomical characteristics that can strongly affect the hydraulic design (e.g. pit vessel number and area) are species-specific (Ellmore *et al.*, 2006; Jacobsen *et al.*, 2008), our success with this method may not necessarily transfer to other species.

### Anatomical bases for the switch from hydraulic integration to sectoriality

The changes we observed in three caudex anatomical features (distance among xylem vessels, xylem vessel lumen diameter,





**Fig. 6** Developmental series of cross-sections of the caudex of individuals of *Cryptantha flava*. (a–g) Plastic sections (5  $\mu\text{m}$ ) of juveniles and adults organized by increasing size. Missing heartwood tissue caused by putrefaction was photo-edited to black background. As individuals develop secondary growth, the heartwood decays and eventually discontinuities give rise to physically independent modules. (h, i) Paraffin cross-sections (40  $\mu\text{m}$ ) showing stained xylem vessels (arrows) of a small and a large juvenile and a small adult in which dyes (fast green FCF and acid fuchsin, respectively) were tracked from a lateral root. Scale bar, 1 mm.

and decayed heartwood area) with plant size probably underlie the hydraulic switch from integration to sectoriality as plants grow (Fig. 2b). Our findings support hypotheses that implicate discontinuities in the vasculature as factors contributing to functional sectoriality (Kitin *et al.*, 2004; Orians *et al.*, 2005). In at least one species that remains hydraulically integrated, these anatomical features do not change throughout development (Domec *et al.*, 2009).

In *C. flava*, anatomical sectoriality takes place relatively early in development. Primary xylem, however, lacks the reticulation imposed by the radial and ray systems. Consequently, the xylem vessels in juveniles form a single, tight cluster, which probably allows for hydraulic integration. But as secondary growth takes place in the caudex, anatomical fission of the xylem occurs through a combination of changes previously documented either in sectorial desert shrubs or in anatomically sectorial temperate herbaceous species. First, the xylem vasculature is re-organized into bundles by the formation of rays that delimit the contact of xylem vessels (Marshall, 1996; Price *et al.*, 1996). The radial growth increments then translate into greater distance among xylem bundles and a reduced probability of

lateral water movement. Secondly, the hardening and eventual decay of the heartwood (Moss, 1940) renders the more closely packed primary xylem vessels, located in the center of the caudex, nonfunctional, which means that there may be exaptive value to the formation of heartwood because it may increase the degree of sectoriality as the plant grows (Stewart, 1966). The dye-tracking experiment shows clearly that water travels preferentially through the outermost vessels (Fig. 6h,i). In some very large individuals, the decay of the heartwood in the caudex may extend, in some instances, to the epidermal cork.

The spatial aggregation of dyed vessels in *C. flava* starts to occur at about the time that heartwood decay and xylem bundle formation are first evident. In some species, the vascular system completely fragments, a condition known as axis splitting (Jones & Lord, 1982; Espino & Schenk, 2009), but even in those species, complete anatomical fragmentation may occur subsequent to functional hydraulic independence of modules (Vasilevskaya & Shokina, 1968; Zanne *et al.*, 2006; R. Dickson, pers. comm.).

Only *partial* axis splitting is evident in *C. flava*. Axis splitting is never complete because, regardless of plant size and

developmental stage, modules remain connected to a single taproot. The formation of this taproot is a large carbon expense for such a small plant, which, unlike desert trees and shrubs (Horwath *et al.*, 1992; Espino & Schenk, 2009), only possesses one such foraging structure to inspect more reliable, deep water reservoirs. Consequently, the vascular organization of its taproot deserves investigation. Our study reveals that the taproot supplies an essential quantity of water, but we have not discerned whether it is anatomically or functionally sectoried. We would like to know whether the persistence of the taproot makes more water available to the remaining modules when some modules die during drought (Casper, 1996). Such an increase in water availability would occur if the taproot is fully integrated or if sectoring of the taproot has only a small effect on total water uptake from the surrounding soil. In other words, sectoriality may not hold the same significance for the vertical taproot as it does for the caudex, whose sectors are connected to different lateral roots that forage over relatively large horizontal distances. Members of two other genera in the family Boraginaceae, *Mertensia* and *Lithospermum*, have taproots that do undergo internal fission (Moss, 1940).

#### Final remarks: plant shrinkage as a survival mechanism

Despite the fact that desert chameaphytes and fully woody species (i.e. shrubs and trees) differ a great deal in foraging strategies (Smith *et al.*, 1997), growth form (Raunkiaer, 1934) and overall size (Noy-Meir, 1973), the hydraulic sectoriality we demonstrate in *C. flava* is strikingly similar to that in desert shrubs, where it appears to have adaptive significance. The number of species with hydraulically sectoried design increases along aridity gradients (Schenk *et al.*, 2008), and sectoried desert woody species achieve longer life spans than integrated desert woody species (Schenk, 1999). It is sometimes suggested that shoot dieback in woody species increases the root : shoot ratio (Kozlowski, 1973; Davis *et al.*, 2002), but in our view this clearly depends on how much of the root system survives and on the pattern and degree of vascular integration.

The hydraulic rearrangement that occurs with development in *C. flava*, from integration in juveniles to sectoriality in adults, probably explains why drought induces the death of spatially aggregated rosettes only in larger, older individuals (Casper, 1996). Plants lacking integration may be unable to rescue a water-stressed segment. Any spatial heterogeneity the root system encounters in soil water or nutrients should be translated to variation within the shoot. Considerable spatial heterogeneity at the scale of the root system has been documented for both water (R. Salguero-Gómez, unpublished) and nitrogen (Peek & Forseth, 2009) in this habitat.

The findings of smaller vessel lumen diameter in juveniles and overall vascular reorganization with development adds

to our understanding of physiological differences between juveniles and adults in *C. flava* (Casper, 1996; Casper *et al.*, 2006). When differences occur in basic gas exchange parameters, juveniles have greater stomatal conductance ( $g_s$ ), lower carbon assimilation ( $A_{max}$ ) and lower instantaneous ( $A_{max}/g_s$ ) and integrated (carbon isotopic discrimination rate,  $\Delta$ ) water use efficiency (WUE; Casper *et al.*, 2006). Smaller plants may also show lower midday and/or pre-dawn leaf water potentials (Casper *et al.*, 2006). Their greater transpiration may help juveniles accumulate nutrients or extend carbon gain in dry soils, which could be especially important for the growth and establishment of young plants.

In a previous literature-based meta-analysis of herbaceous perennials, we found that species with the ability to decrease in size experience higher population resilience (i.e. the speed of recovery after a disturbance) and longer life spans (Salguero-Gómez & Casper, 2010). Thus, it seems likely that plant shrinkage – facilitated in *C. flava* by hydraulic sectoriality – plays a key role as a survival strategy, which may be particularly important in deserts with large temporal variation in water availability (R. Salguero-Gómez *et al.*, unpublished).

In fact, we suggest that sectoriality may help explain why the particular growth form of *Cryptantha flava* – with a combination of herbaceous and woody habit – is so common in cold deserts (McLaughlin, 1986). In general, perennials must be able to tolerate temporal fluctuations in water availability, while annual species, which are more common in warm deserts, can avoid drought through seed dormancy. The perennial growth form allows persistence of the genotype, while sectoriality enables independent foraging, death of only the weakest/less productive modules, and increased foraging efficiency, all advantages ascribed to sectoried woody species (Orians *et al.*, 2005; Espino & Schenk, 2009).

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## Supporting Information

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

**Fig. S1** Leaf water potentials of modules of control: partially and fully watered individuals.

**Table S1** Repeated measures ANOVA for leaf water potentials of modules within individuals

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