

Stage-dependent patterns of drought tolerance and gas exchange vary between sexes in the alpine willow, *Salix glauca*

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Abstract Females and males of sexually dimorphic species have distinct resource demands due to differential allocation to reproduction. Sexual allocation theory predicts that functional traits will diverge between sexes to support these demands. However, such dimorphism may be masked by the impact of current reproduction on source-sink interactions between vegetative and reproductive organs. We ask whether natural selection has led to genetic dimorphism in homologous physiological traits between sexes of the dioecious willow shrub, *Salix glauca*. In a common garden experiment we compared physiological responses to drought stress by male and female ramets in the absence of confounding demands from reproductive structures. Ramets experienced similar pre-dawn leaf water status (Ψ_1) as parental genets in flower within the natural population, indicating that experimental dry-down mirrored environmental conditions in nature. Male and female ramets achieved similar instantaneous water use efficiency, based on the ratio of carbon gain to water loss, under wet and dry conditions. However, female ramets experienced greater water stress (i.e., more negative Ψ_1) than males under dry conditions. Lower Ψ_1 for female ramets may partly reflect the maintenance of conductance under drought; males, in contrast, maintain Ψ_1 under drought by

reducing conductance. Differences between sexes in terms of conductance and leaf water status of the vegetative ramets were absent in a concomitant comparison of parental flowering plants. Our results show (1) genetic divergence in physiology between sexes of *S. glauca* occurs in the absence of gender-specific reproductive sinks, (2) males are the more physiologically plastic sex with respect to water use, and (3) paradoxically, divergence in water relations between sexes is not detectable at sexual maturity under natural conditions.

Keywords Dioecy · Drought tolerance · Genetic variation · Phenotypic plasticity · *Salix glauca* · Water relations

Introduction

Dioecy refers to the division of male and female reproductive functions between separate individuals. Due to this separation in reproductive functions, members of each sex produce reproductive structures different from those of the other (i.e., pollen-bearing vs. ovule-bearing flowers). Pollen and ovules differ in mass, nutrient composition, and water content, thereby generating distinct resource demands for male and female plants of dioecious species (reviewed by Case and Ashman 2005). Divergent selection pressures associated with intrasexual selection act on correlated life history traits to fulfill these distinct resource demands (Charnov 1982; Cox 1981; Darwin 1877; Freeman et al. 1976; Geber 1999; Lloyd and Webb 1977; Meagher 1984; Vitale and Freeman 1986). For example, Delph et al. (2005) demonstrated a genetic correlation between ecophysiological traits and sex-specific floral traits in dioecious *Silene latifolia*. Here, we explore

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whether intrasexual selection has led to genetic dimorphism in homologous physiological traits between two sexes of a dioecious alpine willow, *Salix glauca*.

Because plants face a fundamental physiological trade-off between carbon gain and water loss, inequality in the energy cost of reproduction between sexes implies that males and females should exhibit alternate water use strategies (Arntz and Delph 2001). Specifically, if females are subject to selection for increased resource uptake to support their greater carbon allocation to reproduction, they should maintain high-energy assimilation at the expense of water loss via transpiration (Dawson and Ehleringer 1993; Dawson and Geber 1999). These trends are illustrated by stream-side boxelder (*Acer negundo*): males exhibit a greater stomatal sensitivity to drought than females, closing stomates and avoiding desiccation at the expense of carbon gain (Dawson and Ehleringer 1993). Conversely, in the dioecious arctic willow, *Salix arctica* (Dawson and Bliss 1989a, b), males exhibit higher leaf elasticity and osmotic adjustment than females, and females, perhaps because they lack these drought avoidance mechanisms, persist mainly in wetter microsites. These studies indicate evolutionary dimorphism in water relations of males and females, but any generalization about sexual dimorphism in terms of water use is likely premature (Dawson and Geber 1999). Differences in conductance of males and females in *Salix polaris* vary over time (Crawford and Balfour 1983). In *Silene latifolia*, gas exchange rates in males and females are similar despite a greater biomass allocation to reproduction by females (Gehring and Monson 1994). Some of this ambiguity may reflect the challenges of interpreting physiological comparisons in natural populations. In nature, it is unclear whether discrepancies in water use between sexes reflect habitat segregation, genetic divergence, or both.

Several other factors may also impede the detection of genetic differences in secondary characters between sexes, especially with regard to traits involved in resource acquisition or allocation. Genetic differentiation may be confounded and masked by demands of concurrent sexual function in reproductive plants (Delph and Meagher 1995; Laporte and Delph 1996). For example, because of a source-sink relationship between fruits and photosynthesis, female plants may have a higher photosynthetic rate due to fruit maturation but still have no overall enhancement of photosynthesis relative to males (Dawson and Bliss 1993; Dawson and Ehleringer 1993; Laporte and Delph 1996). This problem can be avoided by making measurements during non-flowering life stages. Second, for woody or other long-lived plants, reproductive individuals may have reached a sufficient size that physiological functions (photosynthesis, water use) are buffered from environmental stress in both sexes (Bond 2000).

Consequently, only a narrow range of extreme conditions may reveal sex differences in drought tolerance. Third, because females of dioecious species characteristically grow more slowly than males and are smaller in vegetative size at any given age, they may have access to different and lower resource supplies in nature (Lloyd and Webb 1977; Putwain and Harper 1972; Wheelwright and Logan 2004; Zimmerman and Lechowicz 1982). Such discrepancies can bring about environmentally based differences in male and female physiology that mimic and potentially mask genetically based divergence. We address these problems by using a common garden experiment to measure physiological responses to water stress in experimentally propagated vegetative daughter plants of *S. glauca* (L.), a long-lived woody shrub native to alpine environments.

Salix glauca provides an excellent model for addressing genetic differentiation in water relations between sexes. In natural populations, male and female plants of *S. glauca* exhibit classic features of habitat specialization, including spatial segregation and habitat-specific divergence in annual growth rates. Growth in females is enhanced in wet conditions, while growth in males is less sensitive to aridity (Dudley 2006b). Here, we take advantage of the propensity of willows to propagate vegetatively to test for a genetic basis to physiological specialization between the sexes and to address whether such differentiation is most apparent in less buffered, small individuals.

The experiments reported in this paper address the following specific questions:

1. Do females tolerate greater water stress than males consistent with the maintenance of gas-exchange rates under dry conditions to support the high cost of fruit production?
2. Do males avoid water stress at the expense of carbon gain, exhibiting greater plasticity in gas exchange and responding to water deficit by reducing photosynthesis and/or conductance?
3. Are differences between sexes in drought responses and carbon assimilation apparent during both the vegetative and flowering life stage?

Materials and methods

Study system

Salix glauca is a shrubby dioecious willow with a circumboreal distribution (Argus 1973). Plants flower repeatedly from year to year, and during the 6 years of this study, we have not observed a single episode of sex switching in more than 100 individuals of each gender.

Terminal bud scars indicate that plants in the study population are at least 15 years old and, in many cases, two- to threefold older. As with other *Salix* species, *S. glauca* readily propagates via woody rhizomes, allowing genets to be cloned repeatedly. For this experiment, genets were delineated in the field as unisexual clumps of centrally radiating shoots separated from other clumps by at least 1 m. The study population forms the krummholz transition zone between the tree line and the true alpine zone at 3620–3890 m a.s.l. on Pennsylvania Mountain (Park County, N 39:15, W 106:07), in the Park Range (Pike National Forest) of the Colorado Rocky Mountains (USA). The krummholz habitat is highly heterogeneous, with standing water and dry, silty soils often within meters of one another. Males and females of *S. glauca* occur across the krummholz mosaic, but segregate spatially on Pennsylvania Mountain and elsewhere in the central Rocky Mountains (Dudley 2006a).

Source material

Cuttings were taken in January 2002 from randomly sampled and permanently marked genets in mixed stands (14 female genets and 15 male genets) and brought back to the greenhouse, University of Missouri (Columbia, Mo.). Each was rooted in a $7.6 \times 7.6 \times 7.6$ -cm plastic pot filled with 1:1 (v/v) mixture of Pro-mix soil-less potting medium (Pro-Mix BX Professional General Purpose Growing Medium; Premier Horticulture, Red Hill, Pa.) and sand, and the pot placed into a tub of water to keep soil moist under 14/10-h (light/dark) days to break dormancy. After dormancy was broken and the plants were well rooted, watering to saturation was reduced to watering once per day and then once every other day. Each original plant gave rise to two to three vigorous daughter ramets. Four sequential rounds of propagation (February, March, April, May) were made per original ramet to reduce maternal and environmental carryover effects and increase the number of ramets per genet. Ramets produced in the fourth round of propagation ($n = 2\text{--}4$ ramets per original genet) were combined as necessary with ramets from earlier rounds, providing a sample size of four ramets per original genet. Because ramets from earlier rounds were distributed evenly across sexes and randomly with respect to treatments, the propagation sequence was ignored in the statistical analyses. Each daughter ramet was placed into a $10.2 \times 10.2 \times 15.2$ -cm plastic pot containing a 1:1 (v/v) mixture of Pro-Mix BX and fritted clay (Hi-Dri clay absorbent; Sud-Chemie Absorbents, Meigs, Ga.). Fritted clay was used to increase the water retention capacity of the growing medium (Ehret et al. 1998). At the time of planting, 15 ml of 14–14–14 (NPK) slow-release fertilizer (Osmocote; Scotts Company, Marysville, Ohio) was added to each pot. The

ramets were approximately 20 cm in length at the start of the experiment and had at least ten leaves each. Observations at the end of the experiment confirmed that roots were not pot-bound and rarely touched the pot sides.

Dry-down experiment

Ramets were transported to the field on June 14, 2003, held in a large, screened enclosure (Weatherport) at 3111 m a.s.l. until June 28, then moved to 3583 m a.s.l. at the tree line on Pennsylvania Mountain on July 5. A total of 83 ramets survived transport and acclimation for use in the experiment. Pots were randomly arranged on tables to prevent small mammal herbivory and watered daily to soil saturation until the start of the experiment. There was no evidence of insect herbivory before or during the experiment. Because pots were placed on tables, the ramets likely experienced an elevated vapor pressure deficit relative to the intact willow krummholz. However, in other respects, (e.g., light intensity and quality, ambient temperature) the potted ramets were subject to conditions representative of the surrounding alpine environment. Two or more individuals from each original genotype were randomly assigned to each of two treatments, control and dry-down, with the position of the treatments completely randomized. Controls were watered daily with 185 ml of distilled water, which was slightly greater than soil saturation, whereas ramets in the dry-down treatment did not receive any water during the 12-day time span of the experiment. A clear sheet of plastic was placed above the individuals in both treatments during rain-storms to control for watering level.

To monitor differences in water availability and water stress between treatments, leaf water status (Ψ_1) was taken at pre-dawn (0300–0530 MST) and mid-day (1000–1300 MST) intervals before and at the end of the dry-down period using a plant water status console (3000 series; Soil Moisture Equipment Corp. Santa Barbara, Calif.). A random set of ramets was sampled repeatedly for all four Ψ_1 measurements (five female control, six male control, seven female dry-down, eight male dry-down). Only 30 ramets were measured due to time constraints, and small differences in sample size among the groups resulted from the random draw of individuals for measurement. For each measurement, the most distal leaf on the central stem was excised and placed immediately into the chamber of the console. Ψ_1 was measured 1 day prior to gas exchange rates (see below) to minimize the impact of any wound-induced respiration on net carbon gain.

Instantaneous rates of gas exchange were measured on intact leaves of all ramets ($n = 82$: 22 females and 20 males in the control group; 21 females and 19 males in the dry-down group) using a portable photosynthesis system with the 414-ml clear cuvette (Li-6200; Li-Cor, Lincoln,

Neb.). We measured the rates of photosynthesis (A) and conductance (g) on a per unit leaf area basis and calculated instantaneous water use efficiency (WUE_i) as the ratio of A/g . Conductance is an accurate estimate of transpiration, provided that the vapor pressure does not drop excessively during the measurement. Gas exchange rates were measured during the highest incident solar radiation (1000–1300 MST), when there was a median quantum flux of $1488 \mu\text{mol m}^{-2} \text{s}^{-1}$. The most distal leaves were chosen after inspection for lack of necrosis. Leaves sampled for gas exchange rates were collected and the leaf area measured using a CI-202 Area Meter (CI-A 11297; CID, Vancouver, Wash.).

Statistical analysis

Analyses were conducted using SAS statistical software ver. 9.1 by SAS Institute (2003) unless noted otherwise. Ψ_1 was analyzed using a repeated measures analysis of variance (r-ANOVA; PROC GLM). Leaf area significantly influences water potential in small plants of *S. glauca* ($R^2 = 0.08$, $P < 0.003$, $\beta = -0.09 \pm 0.03$); therefore, residuals from linear regression (PROC REG) of Ψ_1 on leaf area were used as the dependent variable in the r-ANOVA. Time of day (pre-dawn or mid-day) and phase of the experiment (pre- or post-dry-down treatment) were repeated (within subjects) effects. Watering treatment and sex were fixed between-subjects effects. A four-way treatment \times sex \times time \times phase effect would indicate that the sexes respond differently to experimental drought. In order to ascertain if the dry-down treatment was effective in reducing soil moisture, treatment means were compared using Tukey–Kramer analysis for pre-dawn measures at each phase of the experiment (LSMEANS option). Similar contrasts were used to test for drought stress by comparing mean mid-day Ψ_1 under control versus dry-down treatments at each phase. Because Ψ_1 was measured for only one daughter ramet of each original genotype per treatment, genotype could not be included in this analysis.

The impact of sex, treatment, genotype, and phase of experiment on gas exchange rates [photosynthesis (A), conductance (g), and instantaneous water use efficiency (WUE_i)] was evaluated using separate mixed-model ANOVAs for each dependent variable (PROC MIXED). All analyses were conducted on square root transformed data to meet the assumptions of ANOVA. The subject for repeated measures was genotype (random effect) nested within the sex by treatment interaction. A significant three-way interaction between treatment \times sex \times phase would suggest that the sexes respond differently to drought. Degrees of freedom were estimated using the Satterthwaite method. Several covariance structures for each model were evaluated using Akaike information criteria (AIC),

Burnham and Anderson's AIC corrected for a finite sample (AICC), and Schwarz Bayesian information criteria (BIC) (Littell et al. 1996). Based on these criteria (consistent for all analyses), a first-order autoregressive structure was used for A , a heterogeneous first-order autoregressive was used for g , and a variance component structure was used for WUE_i . A priori contrasts were conducted among post-treatment group means using Tukey–Kramer's adjustment for multiple pair-wise comparisons (LSMEANS option). The probability of significance was then adjusted for multiple comparisons by Bonferroni corrections at $\alpha = 0.05$. It was not possible to test for interactions between genotype and other fixed effects due to the limited degrees of freedom.

Ecophysiology of flowering genets

Physiological measurements were taken on flowering plants from which the vegetative ramets in the dry-down experiment were originally propagated to determine whether flowering genets experienced similar predawn leaf water potentials as control or experimentally droughted ramets and to ascertain whether differences in water relations between sexes in the experiment were detectable at large in the flowering population. Flowering genets (female $n = 14$, male $n = 15$) were not manipulated in any way. Leaf water status and gas-exchange measurements were taken on flowering genets in the parental population either immediately before or shortly after measurements of daughter ramets and at 8 days after the most recent rainfall event. Shoots of flowering genets averaged 0.6 ± 0.3 m (± 1 SD) in length, which was threefold longer than the shoots of daughter ramets. Leaf water status and gas exchange rates were measured using the methods described above.

Statistical analysis

Sources of variation in leaf water status of flowering genets were examined using a repeated measures ANOVA (JMP, v6.0) with sex as a fixed effect and time of day (pre-dawn or mid-day) as repeated. The impact of sex on leaf gas-exchange parameters (A , g and WUE_i) of parental genets was analyzed statistically using a separate one-way ANOVA for each variable.

Results

Dry-down experiment

Experimental drought reduced pre-dawn and mid-day Ψ_1 compared to controls of both sexes (treatment \times phase,

Table 1). At the onset of the experiment, males and females exhibited similar Ψ_1 at the pre-dawn and mid-day sampling intervals (Fig. 1). However, at the end of the experiment, the sexes showed a marginally significant difference in leaf water status (sex \times phase \times time, $P < 0.0572$; Fig. 1). Planned contrasts showed that females had significantly lower mid-day Ψ_1 than males in the drought treatment, but not in the control group (Fig. 1).

Drought decreased the photosynthetic rate (A) during the experiment (Table 2). Under dry-down, A was reduced from an average of 12 ± 1 to $8 \pm 1 \mu\text{mol m}^{-2} \text{s}^{-1}$. Control and dry-down ramets had similar conductance rates (g) prior to dry-down (respectively, $\bar{x} = 0.25 \pm 0.03 \text{ mol m}^{-2} \text{s}^{-1}$, $\bar{x} = 0.24 \pm 0.03 \text{ mol m}^{-2} \text{s}^{-1}$); whereas after dry-down, the conductance of control ramets was twofold that of dry-down ramets (respectively, $\bar{x} = 0.41 \pm 0.08 \text{ mol m}^{-2} \text{s}^{-1}$, $\bar{x} = 0.20 \pm 0.03 \text{ mol m}^{-2} \text{s}^{-1}$). The impact of drought on g varied between sexes; males, but not females, reduced g significantly in response to drought (Fig. 2). WUE_1 decreased in both control and dry-down plants over the time course of the experiment, but it did not vary significantly between treatments or sexes (Table 2). Genotype nested within sex accounted for a significant portion of the variance in g , but not A or WUE_1 (Table 2).

Table 1 Repeated measures ANOVA for leaf water status (Ψ_1) in ramets of *Salix glauca* under experimental drought conditions

Source	<i>df</i>	<i>F</i>	<i>P</i>
Between subjects			
Treatment	1	5.6	0.0287
Sex	1	7.63	0.0124
Treatment \times sex	1	0.49	0.4926
Error	19		
Within subjects			
Phase	1	0.14	0.7154
Phase \times treatment	1	13.04	0.0019
Phase \times sex	1	0.01	0.9273
Phase \times treatment \times sex	1	0.76	0.3951
Error (phase)	19		
Time	1	0.21	0.655
Time \times treatment	1	1.47	0.2406
Time \times sex	1	2.17	0.1568
Time \times treatment \times sex	1	0.46	0.5076
Error (time)	19		
Phase \times time	1	0	0.9487
Phase \times time \times treatment	1	2.06	0.1671
Phase \times time \times sex	1	4.1	0.0572
Phase \times time \times treatment \times sex	1	0.09	0.7645
Error (phase \times time)	19		

For flowering plants, leaf water potentials were similar between the sexes (Sex $F_{1,28} = 0.0129$; $P > 0.9$), whether at pre-dawn or mid-day (time \times sex, $F_{1,28} = 0.25$; $P > 0.86$), even though on average pre-dawn Ψ_1 ($-0.28 \pm 0.03 \text{ MPa}$) was significantly less negative than mid-day Ψ_1 (-1.2 ± 0.07) ($F_{1,28} = 277.45$, $P < 0.0001$). Flowering males and females also did not differ in any of the gas exchange parameters measured (for A : $F_{1,28} = 0.03$, $P > 0.8$; for g : $F_{1,28} = 0.14$, $P > 0.7$; and for WUE $F_{1,27} = 0.00$, $P > 0.9$; Table 3).

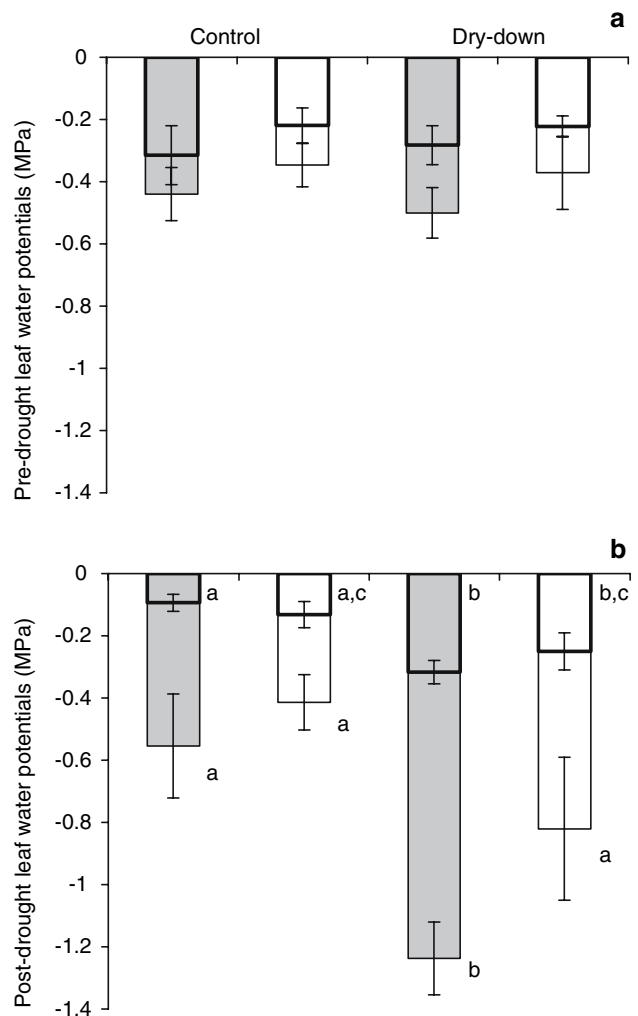


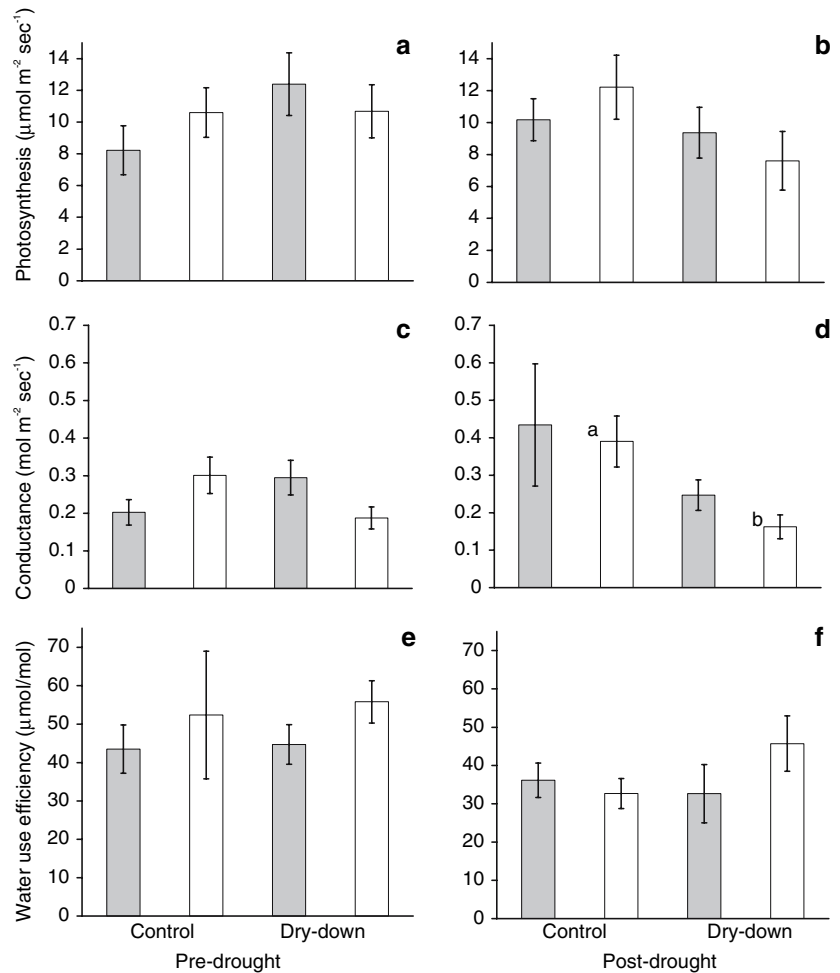
Fig. 1 Pre-dawn (thick-edge bars) and mid-day (thin-edge bars) leaf water status (Ψ_1) of female (filled bars) and male (open bars) ramets of *Salix glauca* grown in control and dry-down treatments. For pre-drought (a) and post-drought (b) conditions, bars show means ± 1 SE. Different letters indicate significant differences at $\alpha = 0.05$ in pairwise comparisons between sexes within the pre-dawn or mid-day groupings

Table 2 ANOVA of gas-exchange rates – photosynthesis (A), conductance (g), and instantaneous water-use efficiency (WUE_i) – for ramets of *S. glauca*

Effect	A			g			WUE_i		
	df (n/d) ^a	F	P	df (n/d) ^a	F	P	df (n/d) ^a	F	P
Sex	1/26.5	0.01	0.9147	1/29.8	0.22	0.6410	1/27.3	0.24	0.6265
Treatment	1/26.9	0.16	0.6928	1/29.9	7.25	0.0115	1/80.7	2.34	0.1296
Phase	1/51.9	0.74	0.3947	1/54	1.61	0.2103	1/80.7	4.38	0.0395
Sex × Treatment	1/26.9	1.91	0.1779	1/29.9	4.63	0.0397	1/80.7	0.37	0.5470
Sex × Phase	1/51.9	0.99	0.3241	1/54	0.59	0.4460	1/80.7	0.92	0.3398
Treatment × Phase	1/51.9	8.92	0.0043	1/54	7.36	0.0089	1/80.7	0.07	0.7977
Sex × Treatment × Phase	1/51.9	0	0.9935	1/54	0.74	0.3932	1/80.7	0.02	0.8755
		Z	P		Z	P		Z	P
Genotype (sex)		1.42	0.0776		2.58	0.0049		0.88	0.1889

^a Degrees of freedom based on Satterwaite approximations

Fig. 2 Photosynthesis (a, b), conductance (c, d), and water use efficiency (e, f) of female (filled bars) and male (open bars) ramets of *S. glauca* grown in the control and dry-down treatments, before (a, c, e) and after (b, d, f) the treatments. Experimental dry-down bars show means ± 1 SE. Different letters represent significant differences at $\alpha = 0.05$ in pairwise comparisons



Discussion

We found that non-flowering female ramets of *S. glauca* incur greater water stress than male ramets under dry

conditions. The lower Ψ_1 for females may partly reflect the maintenance of conductance under drought; males, in contrast, maintain Ψ_1 under drought by reducing conductance. Even though the rates of conductance and photo-

Table 3 Means \pm 1 SE (n) for physiological parameters of flowering willow genets for ramets used in the dry-down experiment

Sex ^a	Pre-dawn Ψ_1 (MPa)	Mid-day Ψ_1 (MPa)	A ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	g ($\text{mol m}^{-2} \text{s}^{-1}$)	WUE _I (umol mol^{-1})
Female	-0.27 ± 0.04 (14)	-1.25 ± 0.11 (14)	19 ± 4 (14)	0.5 ± 0.1 (14)	42 ± 5 (14)
Male	-0.29 ± 0.03 (16)	-1.21 ± 0.08 (16)	18 ± 1 (15)	0.5 ± 0.1 (15)	42 ± 4 (14)

^a Parental genets and ramets were measured on the same dates

synthesis were highly correlated in this experiment (Pearson's correlation $r = 0.75$; $P = 0.0001$), photosynthesis did not vary significantly between sexes either before or after drought. Males and females also achieved similar WUE_I. These results support the view that gas exchange rates of female plants are genetically canalized despite environmental flux to support greater costs of reproduction, whereas physiological plasticity allows male plants to occupy more arid environments (Dudley 2006a).

For both sexes, experimentally droughted ramets experienced similar pre-dawn water potentials as flowering genets, indicating that soil moisture under experimental dry-down mirrored natural soil regimes in the population at large. Flowering genets had higher rates of photosynthesis and conductance than vegetative ramets of the same genotypes (Table 3 vs. Fig. 2). Thus, differences in gas exchange rates and leaf water status were more pronounced in the common garden experiment than in surrounding flowering plants. These results suggest that physiological differentiation between sexes may be more cryptic during reproduction than at vegetative life stages, although reduced environmental variance in the dry-down experiment likely enhanced our ability to detect genetic sources of physiological differentiation.

We predicted that if male *S. glauca* are adapted to arid conditions, they would show greater drought avoidance behavior than females. In agreement with this prediction, males showed greater plasticity in physiological traits under drought than females as evidenced by the change in conductance across treatments. Box-elder males show similar trends, avoiding water stress through lower stomatal conductance and transpiration in response to increased soil water deficit and leaf evaporative demand (Dawson and Ehleringer 1993). In *Maireana pyramidata*, males (but not females) also exhibit greater plasticity in water relations, reducing transpiration and increasing water use efficiency during flowering (Leigh and Nicotra 2003). However, in *Pistacia lentiscus*, males exhibit higher rates of A and g than females under dry – but not wet – conditions (Correia and Diaz Barradas 2000). Discrepancies among species in sex-specific physiology may reflect interspecific variation in the cost of reproduction to males and females: for example, in *Silene latifolia*, males pay the higher cost of reproduction and exhibit higher rates of transpiration and conductance than females (Delph et al. 2005).

For males of *S. glauca*, plasticity in g may allow established plants to avoid drought when small in size where the cost of tissue loss or impact of low leaf water status on growth is exacerbated on a per gram basis. Increased stomatal sensitivity in vegetative male willows could reflect one or more of several underlying mechanisms. For example, hydraulic conductivity, which can lead to a decrease in stomatal conductance (Hubbard et al. 2001), may differ between sexes. In *Sabina vulgaris*, an evergreen shrub, vascular bundles of the males are twice the size of those in females (He et al. 2003). Alternatively, sexes may differ in sensitivity to abscisic acid, the major hormone involved in plant response to soil water deficit (Li et al. 2005).

Female ramets of *S. glauca* maintained gas-exchange in the face of the water deficit. If females exhibit a greater stomatal limitation to carbon assimilation, they may ensure carbon gain by maintaining conductance under conditions of a soil water deficit. However, sexes did not differ significantly in terms of photosynthesis. Transpiration, a strong correlate of conductance, may also ensure the uptake of soil nutrients by enforcing the water pressure differential between the soil and root xylem (Phillips et al. 2001). Because *S. glauca* females allocate more phosphorus and nitrogen to reproduction than males (Dudley 2006b) and alpine soils are characterized by low nutrient availability (Bowman et al. 1993), nutrient gain may have greater fitness consequences for females than instantaneous water use efficiency.

Even though flowering genets and vegetative ramets experienced similar soil moisture regimes, as indicated by the pre-dawn leaf water potentials, only vegetative males avoided leaf water stress, presumably by reducing conductance. Because small vegetative ramets originated from larger flowering plants of the same genotypes, this adjustment by males suggests stage-specific plasticity in water use. In *S. glauca*, as with other dioecious species, larger flowering plants may maintain higher rates of photosynthesis and conductance because of sink-demand from flowers and (for females) fruit (i.e. Delph and Meagher 1995; Laporte and Delph 1996) or reduced desiccation under the larger and denser leaf canopy. In *Salix*, leaves of flowering plants may also experience a greater carbon demand due to associations with microbial symbionts: in *S. viminalis*, rhizodeposition of carbon increases with plant

age (de Neergaard et al. 2002). Microbial sinks would have been eliminated in the experimental potting media used for vegetative plants.

Our dry-down experiment revealed two sources of genetic variance in conductance (g) of *S. glauca*: differentiation between sexes and genotypic variance within sexes. These results add to a small but growing number of studies showing the genetic potential for adaptive evolution of physiology within and between sexes of dioecious plants (Case and Ashman 2005). Our findings support the proposal that adaptive evolution of physiology may proceed in dioecious species despite strong intrasexual selection to support gender-specific reproductive costs.

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