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Permalink
https://escholarship.org/uc/item/0ts5b3j7

Journal
American Journal of Botany, 93(12)

ISSN
0002-9122

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Publication Date
2006-12-01

Peer reviewed

American Journal of Botany 93(12): 1775–1783. 2006.

ECOLOGICAL CORRELATES OF SECONDARY SEXUAL DIMORPHISM IN *SALIX GLAUCA* (SALICACEAE)[1]

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The ecological causation hypothesis for secondary sexual dimorphism was tested in *Salix glauca*, a dioecious willow shrub. Plants growing in a Colorado Rocky Mountain (USA) krummholz mosaic of mesic and xeric patches were monitored for four consecutive years. Ecological causation is predicated on unique resource demands associated with sexual function. In *S. glauca*, seeds have twofold higher N and P concentrations compared to pollen. P, but not N, allocation costs differed between sexes at plant and flower scales. Ecological causation also predicts spatial segregation of sexes along underlying habitat gradients. In five populations of *S. glauca*, sexes displayed significant spatial segregation. The theory also predicts that sexes differ in performance across gradients of environmental stress or resource availability. Consistent with this hypothesis, females had lower drought tolerance than males under years of extreme aridity. Furthermore, over 10 years, annual shoot growth for females was greatest in mesic habitat patches, while males grew at a consistent rate regardless of habitat aridity. Because current shoot growth is correlated with future catkin production, habitat specialization likely provides a fitness payoff in females. Overall, this long-term study provides some of the strongest evidence to date for ecological causation of secondary sexual dimorphism in plants.

Key words: Colorado Rocky Mountains; dioecy; ecological causation hypothesis; Salicaceae; *Salix*; secondary sexual dimorphism; water relations; willows.

The separation of male and female sexual functions into discrete individuals is a common animal phenomenon and occurs in a large number of plants as well. Dioecy is found in 157 flowering plant families and in 7.5% of flowering plant genera (Renner and Ricklefs, 1995). Once dioecy has arisen, this does not end natural selection on gender dimorphism (see reviews of the evolution of dioecy in Bawa, 1980; Charlesworth, 1999), but rather initiates the evolution of secondary sexual dimorphism. Secondary sexual dimorphism is the separation of male and female characters that are not related directly to gamete production (i.e., the androecium and gynoecium in plants). These characters could be morphological as in body size differences in many animal species (Darwin, 1874), physiological involving differentiation in photosynthesis or water use (Dawson and Geber, 1999; Obeso, 2002), or phenological as in the timing or age of onset for reproduction (Delph, 1999). The theory of ecological causation explains differentiation in secondary characteristics between sexes by postulating that males and females have adapted to different ecological niches (Shine, 1989). Here, I address evidence for niche divergence in accord with the ecological causation hypothesis, noting that other major hypotheses for secondary sexual divergence are not mutually exclusive and that sexual selection and fecundity selection could also act on niche constructing traits.

Ecological causation may proceed via any of three general mechanisms: dimorphic niches (Ralls, 1976; Hedrick and Temeles, 1989), bimodal niches (Slatkin, 1984; Shine, 1989), or competitive displacement (Slatkin, 1984; Eppley, 2006). Although these mechanisms may not be mutually exclusive and may piggyback on one another (Shine, 1989), this study focuses on the dimorphic niche scenario of ecological causation as the mechanism giving rise to secondary sexual divergence in *Salix glauca* (Salicaceae). The dimorphic niche mechanism is based on the assumption that there are intrinsic differences between males and females due to their reproductive roles (Ralls, 1976; Hedrick and Temeles, 1989).

A complete assessment of the dimorphic niche hypothesis requires several lines of evidence. First, the hypothesis is based on sexual allocation theory and assumes that allocation to reproduction differs between sexes (Charlesworth and Charlesworth, 1978, 1987; Charnov, 1982; Stanton and Galloway, 1990; Charlesworth, 1999). Under resource limitation, niche differentiation will be favored if resources limiting reproduction differ between males and females, reflecting the nutrient makeup of pollen vs. seeds and fruit as well as the timing of allocation to reproduction (Bullock and Bawa, 1981; Ågren, 1988; Stanton and Galloway, 1990; Ashman, 1992; Ashman and Baker, 1992). Consistent with this view, on a per flower basis, nutrient allocation to reproduction differs between the sexes in several gynodioecious and dioecious plant species (Ågren, 1988; Ashman, 1992, 1994b; Ashman and Baker, 1992; Gehring and Monson, 1994). However, it is unclear whether these differences scale up to the whole plant level or are balanced by sexual divergence in flower number (Carroll and Delph, 1996).

A second prediction of the dimorphic niche hypothesis is that spatial segregation of the sexes will accompany secondary dimorphism if resources are distributed in a heterogeneous manner across a population's range (Cox, 1981; Geber, 1999). In the dwarf willow *Salix arctica*, female plants are common in mesic, nutrient-rich habitats while males are common in xeric, nutrient-poor sites (Dawson and Bliss, 1989b). If spatial segregation is not evident in dioecious populations, it may be that the appropriate niche dimensions have not been explored. For example, sexes could possess different resource efficiencies or morphologies (e.g., rooting depth) allowing them to co-occur and subdivide seemingly homogenous habitats.

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[1] Manuscript received 16 July 2005; revision accepted 13 October 2006.

The author thanks C. Galen, D. Bowers, J. Carrell, R. Crocroft, S. Pallardy, R. Semlitsch, M. Brock, A. Dona, J. Geib, and three anonymous reviewers for helpful comments on the manuscript; and M. Carter, J. Huddle, D. Patillo, and J. Uren for assistance in the field. This project was funded by University of Missouri GAANN to L.S.D. and by the National Science Foundation grant DEB-0316110 to C. Galen.

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Third, the dimorphic niche hypothesis predicts that males and females should differ in performance across gradients of abiotic stress or resource availability. One of the most important resources to terrestrial organisms is water. Several studies support the idea that the sexes of dioecious plants differ in water relations (Dawson and Bliss, 1989a, b, 1993; Dawson and Ehleringer, 1993; Laporte and Delph, 1996; Dawson and Geber, 1999; Espirito-Santo et al., 2003). In a classic study, Dawson and Bliss (1989b) showed that males and females of *S. arctica* differ in drought tolerance and water use efficiency (WUE). Females exhibited greater stomata conductance in mesic habitats, but males had greater WUE than females under xeric conditions. These findings indicate that males and females have diverged genetically to exploit relatively dry vs. wet conditions.

Last, if ecological niches of sex morphs diverge, then growth rates of the two sexes should be maximized under unique environmental conditions. Most comparisons of performance between sexes of dioecious plants have focused on short-term physiological measurements rather than long-term growth. Comparisons of short-term measures of performance alone can be misleading because correlations of fitness and growth to physiological traits varies widely among species, environments, and life stages (Poorter, 1989; Geber and Griffen, 2003). The one study to date comparing growth rates of males and females was conducted in a common garden environment, potentially inflating the magnitude of genetically based differentiation in growth rate between sexes (Wheelwright and Logan, 2004).

Here, I comprehensively address the four predictions of the dimorphic niche hypothesis in *Salix*, a model system for the study of secondary sexual divergence. Through the use of experiments and observations in natural populations of *Salix glauca* over 4 years, I focused on the following questions: (1) Do sexes exhibit contrasting patterns of nutrient investment in reproduction? (2) Do spatial distributions of the sexes differ, as predicted under divergent selection for resource uptake? (3) Do sexes exhibit specialization in physiological traits related to resource gradients in the natural environment? (4) Do unique combinations of environmental conditions favor growth in each sex? While these questions have been addressed separately in other studies, here I attend to all four predictions in the same plant species.

MATERIALS AND METHODS

Study system**—***Salix glauca* L. (Salicaceae) is a dominant species within the alpine krummholz habitat that forms the interface between true alpine vegetation and subalpine forest in the Rocky Mountains of western North America. *Salix glauca* has a circumpolar distribution (Argus, 1973). Willows hybridize broadly and at the field site, *S. glauca* may form cryptic hybrids with the co-occurring species *S. brachycarpa* (Dorn, 1997). Hybridization has the potential to decouple genetic linkage with sex, making it more difficult to detect adaptive differences between sexes. *Salix glauca* is dioecious, and over 3 years of observations, I have not observed a single incidence of sex switching in a sample of 100 males and 100 females that I have surveyed repeatedly. Plants have a mixed mode of pollination, relying on both insects and wind for pollen transfer (P. Kevan, University of Guelph, unpublished data). For males and females, shoots produce catkins (clusters of unisexual flowers) solely on the previous year's growth (L. Dudley, personal observation). Males and females did not differ in levels of foliar N (pooled mean ± 1 SE, 2.9 ± 0.08%, $N = 55$, $P > 0.9$) and specific leaf area ($\bar{x} = 0.0117 \pm 0.0003$ g·cm^{-2}, $N = 149$, $P > 0.5$ for the difference between morphs). As with other willow species (Keoleian and Volk, 2005), *S. glauca* propagates readily through woody rhizomes. In this study, I selected individuals that could be distinguished from one another based on central axis identity and spatial discontinuities among neighboring plants.

The main study population is located at 3620–3890 m a.s.l. on Pennsylvania Mountain (Park County 39°15′ N 106°7′ W) in the Mosquito Range (Pike National Forest) of central Colorado, USA. At this site, willows are one of the first species to leaf out and flower in early June and one of the last to senesce in late August to early September. Four other sites within the Pike National Forest were used for a survey of sex ratio and spatial segregation: Boreas Pass (39°24′ N 105′58° W, 3461–3521 m a.s.l.), Hoosier Ridge (39°22′ N 106°3′ W, 3515–3624 m a.s.l.), Kite Lake (39°19′ N 106°6′ W, 3515–3610 m a.s.l.), and Weston Pass (39°8′ N 106°11′ W, 3572–3641 m a.s.l.).

The krummholz comprises a heterogeneous environment, having standing water, snow banks, rocky outcrops, and fine silty soil within meters of one another. In the present study, mesic habitat patches were designated as those having standing water during most of the growing season with hydrophilic plants such as primrose, marsh marigolds, and moss typically present. Xeric habitat patches were characterized by bare soil intermixed with lichen covered rocks, cushion plants, grasses, and drought-tolerant herbs. Soil cores show a sixfold difference in relative water content between soils of mesic sites (0.6 ± 0.1% w/w) and xeric sites in one sampling during 2002 ($\bar{x} = 0.1 \pm 0.02$% w/w; $t = 2.13$, $P < 0.01$, $N = 6$; here and elsewhere error brackets show standard errors unless otherwise noted). Over the course of this study, the average predawn leaf water potentials (Ψ_l) of willows in xeric sites ($\bar{x} = -0.32 \pm 0.01$ MPa) were more negative compared to those of plants in mesic sites ($\bar{x} = -0.26 \pm 0.01$ MPa; $F_{1,380} = 8.43$; $P < 0.004$;). Additionally, ammonium concentration is higher in soil of mesic habitat patches ($\bar{x} = 32.3 \pm 1.9$ g·g^{-1}) than soil of xeric patches ($\bar{x} = 22.9 \pm 1.9$ g·g^{-1}; $F_{1,8.49} = 11.42$; $P < 0.009$). Mesic habitat patches also tend to have more acidic soils than xeric patches (water as solvent: respectively, soil pH averages 4.30 ± 0.23, $N = 11$ and 4.79 ± 0.21, $N = 13$; $F_{1,22} = 2.49$; $P = 0.13$; with 1 M KCl as solvent, respectively, soil pH averages 3.73 ± 0.08, and 3.96 ± 0.08; $N = 7$; $F_{1,12} = 4.65$; $P < 0.05$).

This study was conducted from June to September 2001–2003. Precipitation varied dramatically over the course of the experiment with 2002 being the driest of the past 100 years. In 2001 the average precipitation trends in this region were mirrored except for an earlier snowmelt. Although not as dry as 2002, 2003 also was characterized by lower than average precipitation and earlier snowmelt (USDA, 2004).

Sex-specific resource allocation to reproduction**—**In 2000, ten 100-m transects were laid out to sample most of the krummholz area on the east slope of Pennsylvania Mountain. In each transect, 10 random points were selected, and the nearest pair of male and female plants to each point was located. Pairs were chosen such that plants of the two sexes were either touching or within 1 m of each other and of approximately the same size based on visual inspection. Plants were permanently tagged. In 2002, catkin nutrient content (N and P) was compared between paired male and female plants. Male catkins in full flower were collected before pollen dehiscence (16–18 June). Female catkins were collected after fruit maturity and before dispersal (21–24 August). Three flowering catkins from each male and five fruiting catkins from each female were randomly chosen, collected in small envelopes, and air-dried with silica gel. Fewer catkins were collected from males to minimize possible effects of clipping early in the growing season on subsequent growth. A random subset of plants from which catkins were collected was included in the analysis of nutrient content (for N, female $N = 23$, male $N = 24$; for P female $N = 15$, male $N = 18$). Male catkins were ground using a mortar and pestle. For female catkins, seeds were separated from the rest of the catkin material by forceful rubbing through a sieve. The ground and separated material was dried again at 50°C for several days, reweighed, and put into a concentrated acid (H_2SO_4) for digestion. The digestion was then diluted using DI water to 1 mL from which samples were taken for nitrogen (N) and phosphorous (P) analysis following Ashman and Baker (1992). To estimate the average whole plant nutrient budgets for reproduction in the different sexes, catkin nutrient content was scaled up to the whole plant level using mean estimates for catkin mass and shoot number per plant in the population [(g nutrient/g seed or pollen) × (g seed or pollen/catkin) × (catkins/shoot) × (shoots/plant)] with shoot number based on plant circumference (regression, $r^2 = 0.98$ for the relationship between shoot number and circumference).

One-way analysis of variance (ANOVA) with sex as the explanatory variable was used to compare reproductive allocation with respect to N and P at the catkin level (Proc GLM, Statistical Analysis System, version 9; SAS Institute, Cary, North Carolina, USA). P content was angular-transformed to meet model assumptions.

Spatial distribution of the sexes—Frequency and distribution of male and female willows were surveyed in 2004 in five populations (Boreas Pass, Hoosier Ridge, Kite Lake, Pennsylvania Mountain, and Weston Pass). The distance between populations ranged from 9.2 km to 36.2 km. Ten 50-m transects, running north–south, were laid out at 100-m intervals in each population. Ten random points were chosen on each transect, and the sex closest to each point was recorded. I also recorded the sex of the closest neighbor one or more meters away from this plant in a randomly chosen compass direction.

A χ^2 goodness of fit test was used to compare the ratio of females to males in each population with an overall expectation of 50 : 50. Sex was compared between nearest neighbors along sampling transects in a frequency analysis so that spatial segregation (S_i) of the individual sexes in each of the five populations could be tested (Dixon, 1994). S_i is similar to a log-odds ratio, based on the frequency ratio of each sex. If $S_i > 0$ for a sex, then individuals of that sex are more segregated than expected by chance. If $S_i < 0$, then individuals of the other sex occur more frequently as neighbors than expected at random. If sexes segregate according to microhabitat, then S_i should be greater than zero. A *Z*-score (significance test criterion value) was computed for each population to test whether the observed S_i value differs significantly from the value predicted with random intersex spacing.

Sex-specific divergence in physiological traits—On Pennsylvania Mountain, four sites, each consisting of a pair of adjacent mesic and xeric krummholz habitat patches, were chosen in 2001 based upon accessibility and adequate density of *S. glauca*. A transect was laid through the willows in each habitat patch, and the closest male or female shoot (branch, $N = 6$ each) to each of 12 random points was located, permanently tagged, and sampled in the summers of 2001 to 2003 (one site was dropped due to loss of permanent tags at the end of the 2001 growing season). When original branches were broken or died, I sampled adjacent branches from the same individual ($N = 5$ branches replaced).

Gas exchange measures were taken on intact leaves of each shoot using a portable photosynthesis system (Li-6200; Li-Cor, Inc., Lincoln, Nebraska, USA): photosynthesis (*A*) and conductance (*g*) were measured, and instantaneous water use efficiency (WUE_i) was calculated as the ratio of A/g. Conductance should reflect transpiration, provided that the vapor pressure gradient does not drop excessively during the measurement; to minimize this drop, the measurement was completed quickly. Measurements were taken on fully expanded healthy leaves between 0900 and 1400 MST, during the time of highest incident radiation. For 2001–2003, measurements were made respectively from 30 July to 18 August, 27 to 28 June, and 28 July to 8 August). Only four plants per sex·per patch were sampled in 2002 and 2003 due to time constraints.

Leaf water status was examined for the same plants during the growing seasons of 2001–2003. On 12 occasions, plants did not have any acceptable leaves or could not be located during predawn measurement; this resulted in a slightly reduced sample size ($N = 84$ total). Leaf water potentials (Ψ_l) were measured at predawn (0300–0600 hours MST) and mid-day (1100–1300 hours MST), using a plant water status console (3000 series; Soil Moisture Equipment Corp., Santa Barbara, California, USA). In each year, measurements were made respectively from 6 July to 20 August, 22 June to 21 August, and 25 July to 2 August. Leaves chosen for Ψ_l had petioles of sufficient length to accommodate the chamber gasket and appeared healthy. Leaves with mite domatia, other evidence of herbivory, or discoloration were not used. For 2001–2002, leaves were cut, placed into a moistened paper towel inside of a plastic sandwich bag, placed on ice in a cooler, and then transported to the water status console. In 2003, the pressure chamber was moved to each habitat patch and cut leaves were placed immediately into the chamber. The change in sampling reduced transit effort, but likely had little impact on measurements (see Fig. 2).

Photosynthesis, conductance, and WUE_i were square-root transformed to meet assumptions of ANOVA. Sources of variation in gas exchange rates were tested by mixed model ANOVA (PROC Mixed; SAS Institute). Sex, habitat type, and year (2001–2003) were fixed effects, and site (1–4) was a random blocking variable. Repeated measurements were taken on subjects (plants) nested within the site by sex by habitat type interaction. Because conductance was strongly correlated with photosynthesis and I was interested in comparing photosynthesis between sexes at a given level of conductance, evaluations of photosynthesis used residuals from a regression of photosynthesis on conductance ($r^2 = 0.62$; $P < 0.0001$). While A/g represents an instantaneous measure of water use efficiency, the residuals from the regression of photosynthesis on conductance provide an alternative and perhaps more robust index of instantaneous water use efficiency, i.e., how does photosynthesis vary at a given level of conductance. Here and elsewhere, least-squares means were used to compare means between pre-planned groups (i.e., between males and females within a given year or between habitat types within a given year). After exploring several possible covariance structures for each model, I chose the final structure based on Akaike information criteria (AIC), Burnaham and Anderson's AIC corrected for a finite-sample (AICC), and Schwarz Bayesian information criteria (BIC) (Littell et al., 1996). Based on these values (consistent for all analyses), a compound symmetry structure was used for *g*, first-order autoregressive for photosynthetic residuals, and a heterogeneous first-order autoregressive structure for WUE_i. The Satterthwaite method was used to estimate degrees of freedom. All subsequent analyses using Proc Mixed followed the same criteria.

Leaf water potentials (Ψ_l, Mpa) were also analyzed using SAS, Proc Mixed with sex, habitat type, time of day (predawn or mid-day), and year (2001–2003) as fixed effects and site as a random blocking effect. Measurements were repeated on subjects (plants) nested within the site by sex by habitat type interaction. A heterogeneous first-order autoregressive covariance matrix was found as the best model for the variance matrix based on AIC, AICC, and BIC criteria.

Sexual dimorphism in growth rate—To test whether males and females had growth-specific optima in relation to microhabitat differences, permanently tagged shoots (64 original, three deaths, four lost tags, male $N = 29$, female $N = 28$) were collected in 2003, and annual shoot growth rates for the preceding 10 years were estimated as incremental distance between terminal bud scale scars along the main axis of the shoot (Fox and Stevens, 1991). Although some shoots had scars that indicated ages of 30 or more years, most could be aged reliably only to 10 years, so measurements were confined to that interval, reducing variance due to measurement error.

The relationship of flowering catkin production per plant in 2003 to annual shoot growth increment in 2002 and shoot basal diameter in 2003 was examined to address whether factors affecting growth rate and plant size influenced reproductive success for plants of each sex. Annual shoot growth in 2002 provided the structural template for 2003 catkin production. Shoot basal diameter reflects the cumulative resource status of the shoot as influenced by its history of past growth (Ashman, 1994a); a thicker shoot should have greater access to nutrients through more xylem and phloem conduits. Measurements were made on shoots of a random subsample of the plants that were permanently tagged in 2000 and used for the catkin nutrient analysis. Shoot growth increment over the growing season, defined as the distance between the most distal pair of terminal bud scars, was measured after leaf senescence in 2003 (24 September) for 5% of the shoots per plant. Basal diameter was measured in 2003 just above the ground.

Sources of variation in annual growth rate from 1993 to 2003 were analyzed using Proc Mixed. Sex, habitat type, and year (1993–2003) were fixed effects, site was used as a random blocking effect, and plants represented repeated subjects nested within the site by sex by habitat type interaction. A first-order autoregressive covariance structure was chosen based on AIC, AICC, and BIC criteria.

To ascertain the relationship between growth and reproductive output, 2003 basal diameter, 2002 shoot growth, and sex were used in an additive linear model to explain variation in catkin production in 2003 (SAS, Proc REG). Catkin production was $\log_{10}$-transformed to meet assumptions of regression, and sex was entered as a dummy variable (female = 0, male = 1).

RESULTS

Sex-specific resource allocation to reproduction—N and P concentrations were greater in seeds than pollen. Seeds averaged 0.0020 ± 0.0002 g P·g^{-1} dry mass, and pollen averaged 0.0008 ± 0.0002 g P·g^{-1} dry mass ($F_{1,31} = 16.77$; $P < 0.0003$). Similarly, seeds averaged 0.029 ± 0.002 g N·g^{-1}, and pollen averaged 0.015 ± 0.002 g N·g^{-1} ($F_{1,45} = 18.92$; $P < 0.001$). When scaled to the whole plant, P budgets to reproduction were four times greater for females (0.20 g·plant^{-1}) than males (0.05 g·plant^{-1}). This result is consistent with a higher nutrient cost of reproduction for females vs. males. However, whole plant N budgets did not differ strongly between the sexes; N allocation to reproduction averaged 0.36 g·plant^{-1} in females and 0.33 g·plant^{-1} in males.

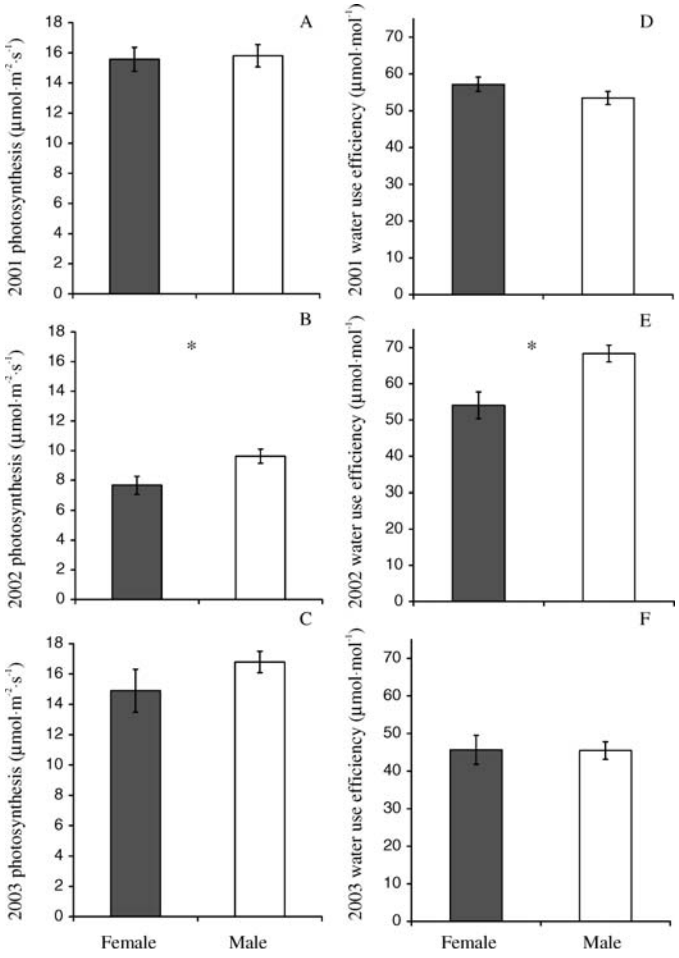

Fig. 1. Photosynthesis (A–C) and water use efficiency (D–F) of female (shaded bars) and male (open bars) *Salix glauca* during 2001–2003. Bars represent means ± 1 SE. Significant pairwise comparisons are denoted by $^*P < 0.01$.

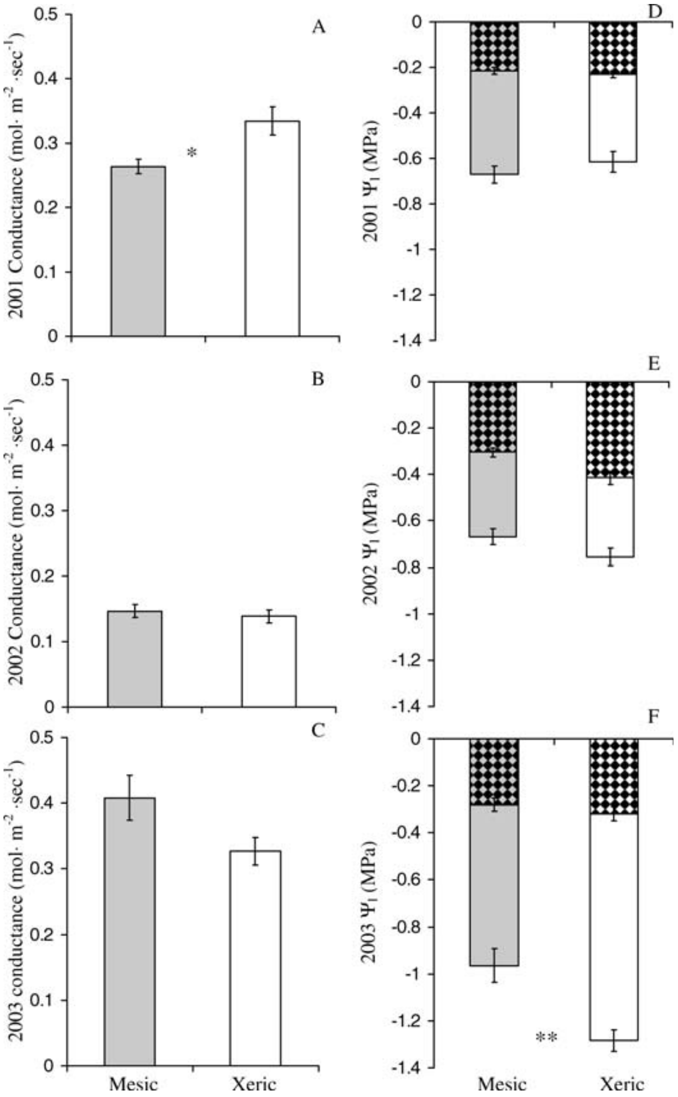

Fig. 2. Conductance during 2001–2003 (A–C) and leaf water potentials (D–F, predawn checkered bars, mid-day uncheckered bars) for *Salix glauca* in mesic (shaded bars) and xeric (open bars) habitat patches. Bars represent means ± 1 SE. Significant pairwise comparisons are denoted by $^*P < 0.05$ and $^{**}P < 0.0001$.

Spatial distribution of sex—At all five localities, willow populations were strongly female biased ($\chi^2 = 139.4$; $P < 0.001$; Boreas = 72%, Hoosier = 76%, Kite = 84%, Pennsylvania = 71%, and Weston = 79% female). Males and females had significant spatial segregation in 90% of cases (Table 1). Across populations, sexes had a similar degree of segregation with S_i averaging 0.55 ± 0.14 in females and 0.54 ± 0.49 in males. The pronounced spatial segregation of the sexes supports the prediction that the sexes are sorting out in a heterogeneous environment.

Sex-specific divergence in physiological traits—Divergence between the sexes in photosynthesis varied from year to year as evidenced by a significant two-way interaction between sex and year ($F_{2,201} = 4.75$; $P < 0.01$; Table 2). Photosynthetic rates at a given level of g were lower for females than males in mesic and xeric patches during the extremely dry year of 2002 ($P < 0.002$; Fig. 1B). In 2001 and 2003, drought was not as extreme, and both sexes had higher photosynthetic rates that did not differ significantly ($P > 0.5$). Habitat patch and all interactions involving habitat patch had nonsignificant effects on photosynthetic rate.

Sex did not explain a significant proportion of the variation observed in conductance; rather, there was significant annual and spatial variation as shown by the two-way interaction between habitat patch and year ($F_{2,198} = 4.78$; $P < 0.01$). In 2002 and 2003, plants in mesic and xeric patches had similar conductance; whereas in 2001, a marked difference in conductance characterized plants growing in xeric and mesic habitat patches ($P < 0.02$; Fig. 2A–C).

Instantaneous WUE showed a significant sex by year interaction ($F_{2,143} = 7.13$; $P < 0.001$; Table 2). In the less extreme years of 2001 and 2003, females and males had similar water use efficiencies (Fig. 1D, F). In 2001, $\mathrm{WUE_i}$ averaged 57 ± 2 $\mu\mathrm{mol{\cdot}mol^{-1}}$ for females and 53 ± 2 $\mu\mathrm{mol{\cdot}mol^{-1}}$ for males. In 2003, $\mathrm{WUE_i}$ averaged 46 ± 4 $\mu\mathrm{mol{\cdot}mol^{-1}}$ in females and 45 ± 2 $\mu\mathrm{mol{\cdot}mol^{-1}}$ in males. However in the drought year of 2002, females had significantly lower average $\mathrm{WUE_i}$ than males (respectively, 54 ± 4 $\mu\mathrm{mol{\cdot}mol^{-1}}$ and 68 ± 2

TABLE 1. Frequencies used in estimating spatial segregation (*S*) based on a nearest neighbor contingency table of willows (*Salix glauca*) growing in five populations and estimates of *S* for sexes at each site. N_{ii} is the frequency of plants with a member of the same sex as the nearest neighbor, and N_{ij} is the frequency of plants with a member of the other sex as the nearest neighbor, * $Z > 1.96$ at $\alpha = 0.05$.

	Female				Male			
Population	N_{ii}	N_{ij}	*S*	*Z*	N_{ii}	N_{ij}	*S*	*Z*
Boreas	68	7	0.52	15.57*	5	20	−0.11	1.22
Hoosier	69	6	0.59	9.80*	21	4	1.22	11.02*
Kite	76	5	0.56	9.82*	10	9	0.70	6.61*
Penn	61	11	0.34	8.67*	12	16	0.30	4.51*
Weston	73	4	0.74	10.20*	12	11	0.58	6.27*
Mean ± 1 SD			$S = 0.55 \pm 0.14$				$S = 0.54 \pm 0.49$	

μmol·mol^{-1}; $P < 0.001$) as expected given females' reduced photosynthesis (Fig. 1E).

Leaf water status (Ψ_l) varied temporally and spatially as indicated by a significant three-way interaction between habitat type, year, and time of day ($F_{2,213} = 7.89$; $P < 0.0005$; Table 3). The difference in mid-day Ψ_l between habitats grew more pronounced over time from 2001 to 2003 (Fig. 2D–F). There was a trend for plants in xeric sites to have more negative Ψ_l than plants in mesic sites in 2002 ($P = 0.09$), but a clear difference was seen in 2003 with xeric sites having a much lower mid-day Ψ_l ($P < 0.0001$). For both sexes, Ψ_l responded similarly to diurnal, spatial, and annual variation in aridity with no significant main or interaction effects for sex ($P > 0.1$; Table 3).

Sexual dimorphism in growth rate—The difference in incremental annual growth between sexes depended on habitat type (sex × habitat interaction $F_{1,49.6} = 4.62$; $P < 0.04$; Table 2). Both sexes had reduced growth in xeric patches compared to mesic patches, but the trend was more pronounced in females than in males (Fig. 3). In mesic patches, incremental growth rate for females averaged 74 ± 4 mm·yr^{-1}, while growth rate for males averaged 60 ± 3 mm·yr^{-1}. In xeric sites, the converse pattern occurred with average incremental growth of females (40 ± 2 mm·yr^{-1}) significantly lower than that of males (56 ± 3 mm·yr^{-1}). This trend suggests that growth depends more strongly on aridity in females than males. Both sexes also showed a reduction in incremental growth with shoot age that may reflect shoot senescence.

Linear regression showed that 2003 catkin production depended on 2002 shoot growth (partial $r^2 = 0.01$; $P < 0.0001$), shoot basal diameter (partial $r^2 = 0.27$; $P < 0.0001$), and sex (partial $r^2 = 0.20$; $P < 0.0001$; model $r^2 = 0.46$; $P < 0.0001$). Both 2002 shoot growth ($\beta = 0.010 \pm 0.002$ mm) and shoot basal diameter ($\beta = 0.057 \pm 0.007$ mm) had positive and similar effects on catkin production in both sexes; however, at a given growth rate, males produced more catkins than females (female intercept = −0.20 ± 0.12, male intercept = 0.26 ± 0.19).

DISCUSSION

In this study, I addressed the dimorphic niche hypothesis of ecological causation as an adaptive explanation for secondary sexual dimorphism. I did this by first examining the main assumption of sexual allocation theory—females invest more resources into reproduction than males—in a comparison of nutrient costs for male and female organs. Results show that the validity of this assumption for *Salix glauca* depends on how reproductive allocation is assessed. Females possessed greater P concentrations in their seeds than males contained in their pollen at both the catkin and whole plant level; however, N concentrations, although greater in seed than pollen, were equivalent between the sexes at the whole plant level. Because nutrients are broadly limiting in the alpine habitat of *S. glauca*, results support gender-specific reproductive costs. Second, spatial segregation of sexes in five geographically discrete populations is consistent with the prediction of the dimorphic niche hypothesis that sexes should segregate due to environmental heterogeneity. Third, an organism's fundamental niche is defined by its resource use and/or environmental conditions under which it persists in the absence of competitors and natural enemies (Silvertown, 2004). Females and males of

TABLE 2. *F*-test table for mixed-model ANOVAs of photosynthetic residuals (*A*), conductance (*g*), instantaneous water use efficiency (WUE_i), and incremental growth rate between female and male willows (*Salix glauca*) in mesic and xeric habitat patches over 3 years. NF and DF = numerator and denominator degrees of freedom, respectively; *N* = number of individuals repeatedly measured during the study. Significant *P* values are in bold.

	A			*g*			WUE_i			Incremental growth		
Effect	NF/DF	*F*	*P*	NF/DF	*F*	*P*	NF/DF	*F*	*P*	NF/DF	*F*	*P*
Sex	1/84.6	1.85	0.1777	1/62.4	2.90	0.9380	1/80.1	1.03	0.3128	1/49.6	0.00	0.9939
Habitat type	1/85	1.90	0.1713	1/62.8	0.00	0.9980	1/80.3	1.78	0.1859	1/49.6	8.05	**0.0066**
Year	2/182	7.31	**0.0009**	2/186	127.26	**0.0001**	2/135	16.95	**0.0001**	10/109	7.85	**0.0001**
Sex × habitat	1/84.3	1.22	0.2726	1/62.2	0.94	0.3364	1/79.8	0.32	0.5749	1/49.6	4.62	**0.0365**
Sex × year	2/201	4.75	**0.0096**	2/198	0.03	0.9744	2/143	7.13	**0.0011**	10/109	0.65	0.7658
Habitat × year	2/201	0.80	0.4509	2/198	4.78	**0.0093**	2/140	2.01	0.1374	10/109	2.08	**0.0317**
Sex × habitat × year	2/201	0.10	0.9076	2/198	0.43	0.6500	2/149	0.32	0.7233	10/109	0.60	0.8123
Z-test, random site effect			0.3			0.2			0.2			0.0002
N			74			74			74			57

TABLE 3. *F*-test table for a mixed-model ANOVA of leaf water potentials (Ψ_l) of male and female willows (*Salix glauca*) growing in mesic and xeric habitat patches over 2000–2003 growing seasons. Significant effects are noted in bold. NF and DF = numerator and denominator degrees of freedom, respectively.

Effect	NF/DF	*F*	*P*
Sex	1/158	0.34	0.5582
Habitat type	1/159	8.34	**0.0044**
Time of day	1/221	952.52	**0.0001**
Year	2/185	24.64	**0.0001**
Sex × habitat	1/158	2.01	0.1587
Sex × time	1/220	0.05	0.8293
Sex × year	2/188	0.97	0.3800
Habitat × time	1/220	3.99	**0.0470**
Habitat × year	2/190	2.93	0.0558
Time × year	2/213	53.36	**0.0001**
Sex × habitat × time	1/220	0.81	0.3679
Sex × time × year	2/213	0.02	0.9843
Sex × habitat × year	2/188	2.46	0.0881
Habitat × time × year	2/213	7.89	**0.0005**
Sex × habitat × time by year	2/213	0.33	0.7168
Z-test for random site effect			0.2

S. glauca have clearly diverged in their fundamental niche with respect to water use. Notably, this discrepancy was only apparent in extreme-drought years when females had reduced photosynthesis compared to males. The fourth prediction asserts that if ecological niches of sex morphs diverge, then growth rates of the two sexes should be maximized under unique environmental conditions. In accordance, there is divergence in incremental growth rates of male and female willows growing in mesic and xeric microhabitats. Female annual growth is 85% higher in mesic sites, while males have a nonsignificant 8% increase in incremental growth over the same moisture gradient. This pattern indicates that female willows have a narrower niche breadth than males over a water gradient.

Unique reproductive ecologies may arise through temporal divergence between sexes in allocation to reproduction. For example, Delph (1990) found that females of *Hebe subalpina* were able to grow as much as males despite having greater reproductive investment because females produced more leaves than males early in the growing season before fruit maturation.

In *S. glauca*, seasonal variation in allocation to reproduction between sexes may exacerbate nutrient limitation for females due to temporal flux of nutrients in alpine soils. In the alpine, the timing of nutrient availability, especially in dry habitats, is correlated with snowmelt and consequently with male, but not female reproductive allocation (Bowman et al., 1993). More severe nutrient competition between growth and reproductive organs likely characterizes females, even when, as with N, whole plant allocation to reproduction is similar between sexes. As the soil matrix dries, whether from wet to dry sites or with late summer drought, water is less available to carry nutrients into plant roots, and competition between growth and reproduction may become more severe. For females that allocate resources to maturing fruit throughout the season, nutrient trade-offs between growth and reproduction are likely to be more intense than in males that allocate resources to maturing flowers and pollen only early in the growing season. In *S. glauca*, growth discrepancies between male and female willows in wet and dry sites conform to this view.

Divergence in allocation between the sexes should be considered when predicting changes in population structure for dioecious species under global change. For example, as anthropogenic activity increases in habitats above tree line, a shift from plant growth limitation by N to P is predicted (Körner, 1999). My results suggest that this change may have a larger impact on females compared to males because of the greater P allocation to reproduction. For female-biased populations like those of *S. glauca,* as well as numerous other dioecious plant species (see compilation in Delph, 1999), such anthropogenic influences could reach beyond the individual plant level to alter population sex ratios, population stability, and even species' distribution.

Studied populations were all highly female-biased. Female dominance may in part arise from other compensatory mechanisms not directly measured in this study. For example, photosynthate demand in females may be partially offset by the photosynthetic bracts on maturing catkins (Bazzaz et al., 1979) or by sink-driven photosynthesis of maturing female catkins (Laporte and Delph, 1996). Reproductive costs may also be partly offset by translocation of resources from nonreproductive shoots to reproductive shoots within a plant (Banuelos and Obeso, 2004). If resource translocation occurs among shoots to support fruit set, then physiological, growth, or demographic costs of reproduction may be more evident in herbaceous plants

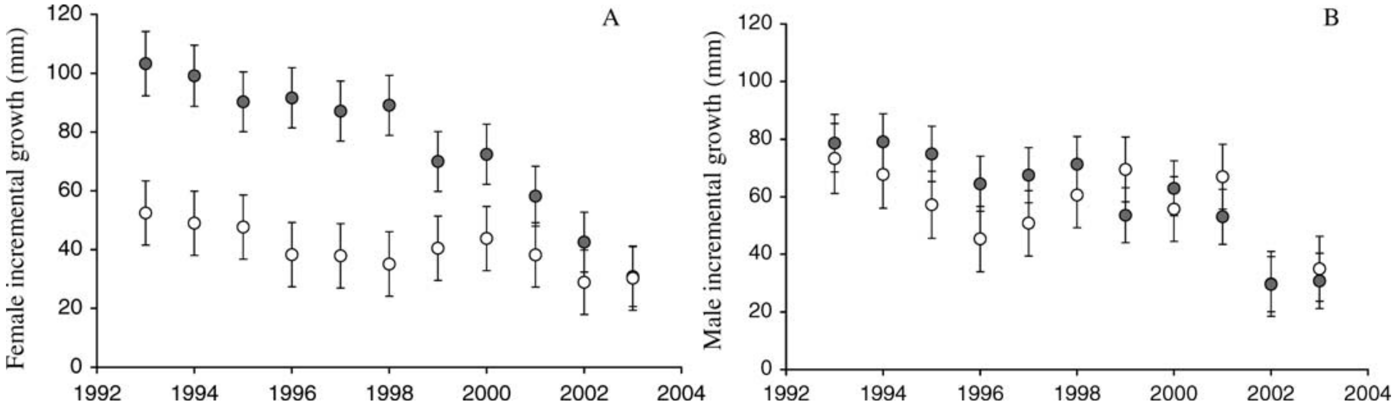

Fig. 3. Annual shoot growth of female (A) and male (B) *Salix glauca* growing in mesic (closed symbols) and xeric (open symbols) habitat patches. Points are least square means ± 1 SE.

or smaller shrubs than in the relatively large, mature individuals included in this study (e.g., Donovan and Ehleringer, 1991; Mediavilla and Escudero, 2004). Measurement of drought tolerance and carbon assimilation in small vegetative plants of *S. glauca* reveals marked differences in water relations between sexes, suggesting that some or all of these compensatory mechanisms occur at maturity, masking (or reducing) fecundity costs (Dudley, 2006).

Biotic factors such as herbivory may also play a role in determining sex frequency in dioecious species (Boecklen and Hoffman, 1993). Damage from insects and ungulate herbivores is prevalent in the alpine krummholz habitat and may favor female willows (Danell et al., 1991). However, several non-ecological mechanisms may also contribute to sex ratio bias, including prezygotic selection via gamete competition, irregular meiosis, or sibling competition (Stehlik and Barrett, 2005). From 13 crosses in *Salix viminalis*, sibships grown under optimum conditions were largely female biased (Alstrom-Rapaport et al., 1997). Sex ratio asymmetry could also be reinforced by restricted seed dispersal. Because seed dispersal is leptokurtic away from maternal plants, a clumping of offspring around seed parents is expected (Heilbuth et al., 2001). Hence most seeds should fall into microsites favoring females, promoting a female-biased sex ratio.

Male and female willows have a spatial segregation consistent with their different niche requirements (Fox and Harrison, 1981; Bierzychudek and Eckhart, 1988). While spatial segregation may alternatively be due to intermorph competition (Freeman et al., 1976), few studies have looked at this idea directly, and the two that do argue instead for the dimorphic niche hypothesis (Fox and Harrison, 1981; Waser, 1984). For clonal plants such as willows, spatial segregation may also arise from vegetative propagation. Single genets in other *Salix* species can cover hundreds of meters of stream bank in riparian habitat (Douhovnikoff et al., 2004). The extent of clonal spread in *S. glauca* is not clear, but I took precautions to insure that shrubs sampled in my survey represented discrete individuals. The consistent pattern of spatial segregation for both morphs in the five geographically isolated populations sampled in the present study is strong evidence that natural selection has acted on sexes of *S. glauca* to promote segregation in different microhabitats.

Physiological specialization between the sexes in *S. glauca* was only evident in the extreme drought environment of 2002, unlike other studies that show upregulation in carbon gain in females under benign conditions (Wheelwright and Logan, 2004). In 2002, males also had greater instantaneous WUE than females, while maintaining their photosynthetic rates. This finding suggests that males may better tolerate drier environments and extreme drought years, supporting Dawson and Bliss's (1993) conclusion that male willows have a larger fundamental niche than females. It is noteworthy that 3 years of sampling were necessary to capture the conditions under which sexes of *S. glauca* diverge in drought tolerance. Studies that report physiological trends based on only 1 year of sampling may miss extreme years, which nonetheless have significant effects on fitness and survival, especially for long-lived, perennial species such as willows.

Smaller, establishing plants are more sensitive to the environment than larger, mature individuals (De Soyza et al., 1996). Growth rates for female *S. glauca* were greatest under mesic conditions, but the trend weakened as shoots aged, indicating that selection on physiology in dioecious plants may occur at smaller sizes prior to maturity. In *Acer negundo,* biased sex ratios are thought to arise prior to the development of large root systems that access groundwater and buffer mature plants from dry periods (Dawson and Ehleringer, 1993).

Previous shoot growth and current shoot diameter were positively related to catkin production, suggesting underlying growth constraints on fitness for each sex. The influence of the previous year's growth on catkin number probably reflects architectural constraints (Diggle, 1997). Males and females limit catkin initiation to shoot length laid down in the previous year; a longer shoot has more potential bud sites than a shorter one. This architectural feature provides direct feedback between previous and current reproductive allocation (but see Fox and Stevens, 1991; Nicotra, 1999). The relationship between catkin production and shoot basal diameter may reflect the capacity of large shoots, with greater phloem inputs to sustain current reproductive investment (Banuelos and Obeso, 2004). Although I cannot exclude the hypothesis that growth rate and reproduction are spuriously correlated due to underlying environmental variation (Rausher, 1992), the finding of a positive relationship between the two processes, coupled with habitat-specific growth optima for male and female *S. glauca*, supports the idea that spatial segregation of the sexes is adaptive.

Willows are used in a variety of ecosystem management projects: as biomass crops (Volk et al., 2004), in soil stabilization (DLWC, 2005), and in riparian restoration (Rood et al., 2003). Management practices informed by morph differences in niche dimensions could be beneficial. For example, planting females in wet areas and males in drier ones could provide better yield or coverage than a haphazard distribution of the sexes. Overall, this study demonstrates that alpine *Salix glauca* possesses secondary sexual dimorphism likely as a result of natural selection for exploitation of dimorphic niches in a relatively species sparse community. Whether sexes of riparian willows in lowland habitats have reduced niche partitioning awaits further study.

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