

MECHANISMS OF SELECTION FOR DROUGHT STRESS TOLERANCE AND AVOIDANCE IN *IMPATIENS CAPENSIS* (BALSAMINACEAE)¹

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For longer lived annual plants, high water-use efficiency (WUE) and low stomatal conductance are hypothesized to confer a fitness advantage under drought stress. To directly test the adaptive significance of WUE and stomatal conductance under drought stress, inbred lines of *Impatiens capensis* were grown in two field environments (watered and not-watered), in a year of unusual early-season drought. In contrast to the results from a previous study of late-season drought in the same system, selection was detected for lower WUE, increased stomatal conductance, and early flowering time. These findings suggest that early-season drought conditions may select for drought avoidance traits such as low WUE and early reproduction, whereas later drought selects for tolerance traits such as high WUE.

Key words: Balsaminaceae; drought stress; flowering time; *Impatiens capensis*; natural selection; water-use efficiency.

Although water availability has been shown to be a significant selective agent within natural plant populations (e.g., Farris, 1987, 1988; Lechowicz and Blais, 1988; Bennington and McGraw, 1995; Dudley, 1996a, b; Silim et al., 2001; Heschel et al., 2002), the mechanisms of selection for drought tolerance or avoidance have rarely been examined directly (Dudley, 1996a, b; Arntz and Delph, 2001; Geber and Griffen, 2003). Manipulation of soil water conditions in the field allows for the direct testing of the adaptive value of traits. This, in turn, may help reveal the causes of natural selection on traits hypothesized to confer stress tolerance or avoidance (Wade and Kalisz, 1990; Schmitt et al., 1999).

Theory predicts that drought-tolerant plants maximize fitness by decreasing both leaf size and stomatal conductance to water vapor in response to limited water availability (Cohen, 1970; Givnish, 1979; Zangerl and Bazzaz, 1984; Donovan and Ehleringer, 1992; Dudley, 1996a; Nativ et al., 1999; Ares et al., 2000). Givnish (1979) hypothesized that smaller leaves should increase fitness in dry conditions as the decreased surface area to volume ratio of smaller leaves inhibits desiccation (Cohen, 1970; Larcher, 1995). Zangerl and Bazzaz (1984) hypothesized that plants could adapt physiologically to drier conditions by decreasing stomatal conductance to water vapor (g_s , hereafter referred to as g) and thereby increasing their water-use efficiency (WUE = the ratio of carbon gained per unit water lost). However, increasing WUE concomitantly decreases photosynthesis by reducing carbon dioxide intake through

partially closed stomata (Larcher, 1995). Therefore, the adaptive value of WUE may depend on growing season length. For longer lived annuals and perennials, decreasing leaf size and/or stomatal conductance (increased WUE), should result in drought tolerance and fitness gains in dry conditions (Geber and Dawson, 1990, 1997; Pimentel et al., 1999; Querejeta et al., 2003). In contrast, shorter lived annuals may maximize fitness by increasing stomatal conductance (low WUE) in order to increase net rates of carbon gain to avoid drought stress. This may allow them to grow rapidly, flower early, and increase yield prior to the onset of substantial soil drying (Cohen, 1970; Mooney et al., 1976; Geber and Dawson, 1990, 1997; McKay et al., 2003). Generally, these functional arguments predict that the adaptive value of stomatal conductance and leaf size depend on growing season length, plant lifespan and life-history class (Mooney et al., 1976; Donovan and Ehleringer, 1992; Schuster et al., 1992; Querejeta et al., 2003). When drought is experienced at later developmental stages, selection should favor decreased stomatal conductance (high WUE) and smaller leaves, whereas when plants experience drought at early developmental stages, increased stomatal conductance (low WUE) should be selected for and leaf size may be of no adaptive value. In other words, natural selection may favor a tolerance strategy of surviving drought and delaying reproduction during a longer growing season but may favor a strategy of rapid growth and reproduction to avoid stress during a short growing season.

Here, we examine the causes of selection in drought conditions in *Impatiens capensis*, a typically longer lived annual plant. *Impatiens capensis* is a fleshy species lacking cambium and a thick, waxy cuticle; thus it reacts quickly to changes in turgor pressure. Because it is vulnerable to drought (Schulz et al., 1993), both WUE and morphology may be important to increase fitness under water-limited conditions (Maliakal et al., 1999). Previous investigations (Heschel et al., 2002) demonstrated that increased instantaneous WUE and lower stomatal conductance increased reproductive fitness of *I. capensis* under unmanipulated, relatively dry field conditions. To directly test whether water limitation selects for higher WUE and decreased stomatal conductance, a manipulation of soil moisture conditions in a common garden is required (e.g., Dudley,

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1996a). Here, we directly manipulated water availability at precisely the same location of the previous study (Heschel et al., 2002), to address the following question: Do plants maximize fitness by decreasing leaf size and/or stomatal conductance under water limitation, as predicted by functional arguments?

MATERIALS AND METHODS

Study system—Jewelweed, *Impatiens capensis* Meerb. (Balsaminaceae), is a self-compatible, annual herb of North American deciduous forests and wetlands (Gleason and Cronquist, 1963; Leck, 1979, 1996). *Impatiens capensis* exhibits a mixed-mating system in which cleistogamy (selfing) is more common than chasmogamy (Waller, 1979). As seeds typically disperse less than 1.5 m from parent plants (Schmitt et al., 1985; Kelly, 1997), natural populations are essentially comprised of collections of inbred lines (Paoletti and Holsinger, 1999). Restricted dispersal and high selfing rates in *Impatiens* may contribute to the local population differentiation and substructure observed for morphological and life history traits (Schemske, 1984; Schoen et al., 1986; Schoen and Latta, 1989; Schmitt and Gamble, 1990; Argyres and Schmitt, 1991; Schmitt, 1993; Dudley and Schmitt, 1995; Bennington and McGraw, 1995; Kelly, 1997; Donohue and Schmitt, 1999; Donohue et al., 2000).

Previous studies with *I. capensis* documented genetic differentiation in WUE, stomatal conductance (Heschel et al., 2002), and abscisic acid sensitivity (Heschel and Hausmann, 2001) between populations from wet and dry sites, indicating that populations had been selected for drought-tolerance strategies. These populations are longer lived and persist from May until September during a typical growing season (M. S. Heschel, unpublished data). Based on these physiological and persistence data, we predicted that decreased stomatal conductance and increased WUE would result in increased fitness under water-limited conditions.

These two populations are separated by approximately 1 km at Haffenreffer Reserve in Bristol, Rhode Island: one is located in a sunny clearing and the other in a wooded site under an hickory-oak canopy (Dudley and Schmitt, 1995; Donohue et al., 2000). During the growing season, the sunny site is relatively wet, experiencing soil water potential values from 0 to -0.025 megapascals (MPa), whereas the wooded site is shaded and relatively dry, experiencing soil water potentials from -0.015 to -0.065 MPa (see Heschel and Hausmann, 2001, for detailed soil moisture data from 3 growing seasons). Soil water potential less than or equal to -0.030 MPa is stressful to *I. capensis* and results in wilting (M. S. Heschel, unpublished data). These populations represent the typical wet and dry conditions experienced by *I. capensis*; wet populations are usually in sunny sites and dry populations are always in shady sites (M. S. Heschel, unpublished data). We define drought tolerance in this system as the ability, through WUE adjustment, to live longer during drought conditions than would otherwise be possible (note: for this annual weed, only a few days of extended survival translates into fitness gains (Heschel and Hausmann, 2001)).

Experimental design—To determine the causes of selection for increased water-use efficiency, a common garden experiment was conducted at the wooded site within the same microsite used previously to examine late-season drought responses (Heschel et al., 2002). This microsite was chosen because it dries consistently; thus plants should experience water-limited conditions in the absence of irrigation.

Seeds were collected from 25 inbred lines derived from the wet-site population and 25 lines from the dry-site population in the Brown University greenhouse. The inbred lines were single-seed derived and selfed for six generations, creating highly homozygous individuals. Lines from both the dry- and wet-site populations were included to expand the range of variation for physiology, morphology, and fitness. In a companion study, genetic correlations between gas exchange and morphological traits were examined in detail (Heschel, 2000).

Seeds from the 50 inbred lines were stratified in distilled water in microtiter trays at 4°C for 4 mo and subsequently planted into flats in the greenhouse containing MetroMix 350 (Scotts-Sierra Horticultural Products, Marysville, Ohio, USA). After initial establishment (approximately 2 wk of growth), seed-

lings were transplanted into each of six blocks in the field over the course of two rainy days in early May 1999. Planting density was approximately 100 plants per 1 m² in a 10 by 10 m square array with plants separated by 10 cm. Two treatments were applied: watered or unwatered (drought treated). Block was nested within a watering treatment in a split-plot design such that whole blocks were either watered or not watered. Six replicates of each inbred line were represented in each water treatment for a total of 12 plants per line and 600 plants overall. Thus, each of the three drought and three watered 1-m² blocks contained two replicates from each line. Across the blocks, midday photosynthetic photon flux density ranged from 8 to 312 $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ on two clear days in early June. These light conditions were identical to those presented by Heschel et al. (2002).

Local pond water was used to irrigate the well-watered blocks to simulate natural precipitation. The three blocks in the watered treatment were sprayed with approximately 6.8 L of water every 2 d until 1 July, at which time 6.8 L were supplied daily; 6.8 L is equivalent to about 0.84 cm of rainfall, so plants received an equivalent of 2.6 cm of rain per week before July, but greater than 5.2 cm after 1 July, which is typical for the area during wet summers (U.S. Weather Station, Newport, Rhode Island, USA). A soil tensiometer (2900F1: Soilmoisture Equipment Corp., Santa Barbara, California, USA) was used to quantify water availability in each treatment. Moisture readings were taken 6 cm below the soil surface once per week for all six blocks for the duration of the experiment. This depth of measurement was appropriate because most *I. capensis* roots reach no deeper than 6–7 cm (Waller, 1984).

The initial height of plants was measured 1 wk following transplantation. Initial height is a good indicator of differential greenhouse effects on *I. capensis* seedling growth (J. Schmitt, Brown University, personal communication). Time until first flower was scored on all plants, and height was measured again in late June. Flowers, fruits, and pedicels of previously dehisced fruits were counted every week from the second week of June until the end of the experiment during the second week of July to estimate fitness. The water-limited plants all died by the first week of July and the well-watered plants died 12 d later during a heat wave ($>38^\circ\text{C}$ for 4 d). Therefore, water-limited plants died due to a severe early-season drought (all individuals in drought blocks had reached the permanent wilting point), whereas well-watered plants persisted for several days following the death of water-limited plants. Cumulative lifetime fitness was estimated by summing all flowers, fruits, and pedicels.

Physiological measurements—An ADC (ADC Bioscientific Ltd., Hoddesdon, UK) LCA 4 Infrared Gas Analyzer (IRGA) was used to measure carbon assimilation rate ($A = \mu\text{mol CO}_2 \cdot \text{m}^{-2} \cdot \text{s}^{-1}$), stomatal conductance to water vapor ($g = \text{mol H}_2\text{O} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$), and water-use efficiency ($\text{WUE} = A/g$). Gas exchange measurements were taken in the field during the second and third weeks of June, approximately 1.5 mo after the start of the experiment. Because it was impossible to enclose attached leaves in the sampling cuvette without damaging the focal plant and its neighbors, the most-recent-fully-expanded leaf of each plant was removed for gas exchange analysis. Each excised leaf was immediately placed into the PLC, gas exchange measurements were recorded when IRGA readings had stabilized, and then each leaf was traced to get an estimate of leaf size. Pilot data demonstrated equivalent rates of gas exchange between removed and attached leaves (see below). Leaf tracings were digitized and individual leaf areas calculated with NIH Image (Windows version, Scion Corp., Frederick, Maryland, USA).

All gas exchange measurements were made between the hours of 1000 and 1500. The LCA 4 was “environmentally controlled” with an adjustable light source and a Peltier-cooling unit built into the Parkinson Leaf Chamber (PLC). The measurement light level was maintained at about 800 $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$. The chamber temperature ranged between 25° and 29°C among days, but on any given day the temperature was kept within 2°C. *Impatiens capensis* exhibits maximum carbon assimilation rates at a photon flux density of approximately 800 $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, irrespective of sun-shade acclimation (Heschel et al., 2004). Thus, gas exchange measurements were made under light-saturating conditions. The light levels used were higher than average ambient levels under the tree canopy, but typical of sun flecks (M. S. Heschel, un-

published data); capitalizing on sun flecks by rapidly increasing stomatal conductance and carbon assimilation should have important fitness consequences, particularly for shade-acclimated leaves (Chazdon, 1992). Leaf temperatures did not vary among lines. PLC humidity conditions were maintained within the ambient range of relative humidity (50–60%). Effects of measurement time and date were removed by adjusting gas exchange values with residuals from linear regression models (Type I Sums of Squares) that included the effects of time and date (Farris and Lechowicz, 1990; Dudley, 1996a). Temperature and light levels were not included in the regression models, as they did not significantly explain variation among measurements. Boundary-layer conductances were estimated with moist Whatman Filter Paper leaf mimics (Parkinson, 1985). To correct for different leaf areas in the PLC, gas exchange values were adjusted for leaf area for each measurement.

No effects of leaf removal on gas exchange—To assess the effects of leaf removal on gas exchange measurements, gas exchange traits were measured on the two most-recent-fully-expanded leaves from month-old replicates of the experimental lines in watered conditions in the Brown University Greenhouse. Gas exchange traits were measured using the protocol described above on an excised leaf within 1 min of removal. An attached leaf at the same node was measured immediately thereafter (*I. capensis* has opposite leaves early in development). A paired *t* test showed that gas exchange measurements on removed leaves were statistically indistinguishable from attached leaves ($t = 0.161$, $df = 34$, $P = 0.87$). A later study using a subset of these experimental lines was performed to determine whether leaf removal affected gas exchange rates in well-watered and water-limited conditions. Lines were grown in both well-watered and water-limited conditions for 2 wk prior to gas exchange measurements. Measurement protocol was identical to the first pilot study except that an ADC LCA3 IRGA was used for these gas exchange measurements. The line replicates were similar in height and developmental stage to plants in the field experiment. Light levels were maintained at $800 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$. The gas analyzer and computation units were the same for the LCA 3 and LCA 4, using the same rate of airflow and identical equations in calculations. An ANOVA model was used to test for water treatment, leaf removal, and line differences in *A* and *g*. Carbon assimilation rate and stomatal conductance were significantly lower in drought conditions (*A*: $F = 6.04$, $df = 1$, $P = 0.02$; *g*: $F = 5.97$, $df = 1$, $P = 0.02$). Leaf removal had no effect on carbon assimilation rate ($F = 1.28$, $df = 1$, $P = 0.27$) or stomatal conductance ($F = 0.0022$, $df = 1$, $P = 0.96$). These non-significant leaf removal effects did not depend on water treatment; no genotype by environment interaction was detected for either gas exchange trait (*A*: $F = 0.019$, $df = 1$, $P = 0.89$; *g*: $F = 0.0072$, $df = 1$, $P = 0.93$).

Data analysis—Statistical analyses were performed with JMP (version 4.0, SAS, 2000).

Selection differentials—Separate regression models for the water-limited and well-watered treatments were used to estimate the strength of natural selection on *A*, *g*, leaf size, and flowering time (Lande and Arnold, 1983; Wade and Kalisz, 1990; Rausher, 1992). To control for the effect of microhabitat variation on the relationship between traits and fitness, genotypic (line) trait means were used for selection analyses (Rausher, 1992; Stinchcombe et al., 2002). Significant differences among lines were detected for *A*, leaf size, flowering time, and reproduction, and a marginally significant line effect was found for *g* (Heschel, 2000), so the use of genotypic selection analyses is justified here (Rausher, 1992). In all the linear models, relative fitness was the response variable. Population (fixed), and *A*, *g*, leaf size, or flowering time were the predictor variables. Relative fitness was calculated as the total number of flowers, fruits, and pedicels divided by the mean reproduction within each treatment (Donohue et al., 2000). Genotypic means were standardized in order to present selection differentials in standard deviation units, S' (Lande and Arnold, 1983). Population was included to control for potential fitness differences between populations due to selection on unmeasured characters (Donohue et al., 2000). Regression models with quadratic terms were used to estimate stabilizing or disruptive selection. WUE had an exponential distribution and could not be transformed to meet normality assumptions. There-

fore, to test for associations between relative fitness and WUE, nonparametric Spearman correlations were calculated in the water-limited and well-watered treatments on line means.

ANCOVA was used to test whether selection differentials differed between treatments. These models included the standardized trait, a drought treatment effect, and a trait by treatment interaction. Relative fitness was calculated over both treatments for these analyses. The interaction term tests whether the association between a particular trait and fitness differed across treatments.

Selection gradients—Multiple regression models were used to determine the strength and direction of direct natural selection on each trait while holding constant the value of other traits (Lande and Arnold, 1983). Separate models were used for each drought treatment with population (fixed), *A*, *g*, leaf size, flowering time, and trait-by-trait interactions as predictor variables. Relative fitness, which was calculated within each treatment, served as the response variable. Trait-by-trait interactions were included to test for correlative selection, i.e., combinatorial effects of trait levels on relative fitness. Quadratic terms were initially included in the models; however, no stabilizing/disruptive selection was detected so these terms were dropped to increase model power. Final plant height was not included in the selection analyses because height was collinear with other traits in the models. Standardized line means were used for each trait to control for microsite variation and to present selection gradients in standard deviation units. ANCOVA was used to test whether significant selection gradients differed between treatments, with fitness relative to the grand mean.

Population differences—Although not properly replicated at the population level, the documented differences between the two study populations in soil moisture (Heschel and Hausmann, 2001) and trait values (Heschel and Hausmann, 2001; Heschel et al., 2002) make tests of population differentiation potentially illuminating. Nested ANCOVA and ANOVA were used to test for treatment and population differences in *A*, *g*, leaf size, height, flowering time, and reproductive fitness (total number of flowers, fruits, and pedicels). Values for *A* and *g* were natural log transformed to meet normality assumptions. The few plants that died prior to flowering were scored as having zero fitness and included in the analysis. Drought treatment and population source were considered fixed factors and experimental block and inbred line were considered random factors (mixed model—Type III sums of squares). Block was nested within treatment, and inbred line was nested within population. Plant height at the start of the experiment was used as a covariate to control for greenhouse effects on genotypes, with the exception of the gas exchange models. Initial height was not used in gas exchange models because it is not clear what effect initial height might have on physiological traits (J. Schmitt, Brown University, personal communication). The effect of treatment was tested over a synthetic denominator composed of block and drought treatment by line effects, the effect of population was tested over the line term, and the population by drought treatment interaction was tested over the drought treatment by line term. Interactions with block were pooled with error variance because the purpose of these models was to determine the response of populations and lines to treatment across all blocks (Newman et al., 1997). Linear contrasts were calculated on model least-square means to determine where specific differences lay within interaction terms. *F* tests for these contrasts had the same denominator as the *F* tests for their respective population by treatment interaction terms. All models were examined for homoscedasticity and normality of residuals.

WUE could not be transformed to meet normality assumptions so parametric and nonparametric analyses were used. An ANCOVA was first used to examine population differences in *A* while holding the effect of *g* constant as a covariate. To test whether the populations differed in WUE in the drought treatments, contrasts were conducted within the population by drought treatment interaction of this model. Separate nonparametric tests were then used to determine whether populations differed in their WUE responses to treatment. WUE had an exponential distribution in a normal probability plot, so median tests were used (JMP User's Manual, SAS, 2000). Because the results of both parametric and nonparametric tests provided the same results, only the nonparametric tests are presented for conciseness.

TABLE 1. Results of genotypic selection analysis. Standardized selection differentials (S) and selection gradients (β) are shown for each drought treatment combination. Italicized selection coefficients indicate that values were significantly different across treatments ($P < 0.10$). A = carbon assimilation rate; g = stomatal conductance.

Trait	Drought		Watered	
	S	β	S	β
A	-0.034	0.024	0.019	0.0021
g	0.096*	0.0079	0.025	0.010
Leaf size	0.0068	0.00034	0.026	-0.094
Flowering time	-0.35***	-0.34***	-0.36***	-0.36***
Flowering time $\times g$	—	-0.15**	—	-0.081

$P < 0.001$ ***; $P < 0.01$ ** $P < 0.05$ *.

RESULTS

Preliminary analyses/effective experimental treatments—

Water-limited (drought) blocks were maintained at consistently lower soil water potentials (i.e., less available soil water) than watered blocks (significant treatment effect, $F = 201.47$, $df = 1$, $P < 0.0001$, in a repeated-measures model that contained the effects of drought treatment and block nested within drought treatment). In early June, mean soil water potential ± 1 SE was -0.029 ± 0.006 MPa in the drought blocks, and -0.015 ± 0.004 MPa in the watered blocks, whereas, in July, mean soil water potential was -0.038 ± 0.004 MPa in the drought blocks and -0.021 ± 0.003 MPa in watered blocks. Soil water potential less than or equal to -0.030 MPa is stressful to *I. capensis* and results in wilting (M. S. Heschel, unpublished data). Thus, plants in drought blocks experienced relatively dry conditions early in the season. On average, June is wetter than July in this region, so plants were drought stressed at an earlier developmental stage than usual (see Heschel and Hausmann, 2001, for details). Moreover, rainfall totals in June 1999 were 8.08 cm below normal (Newport, Rhode Island U.S. Weather Station), making this the driest June in a century (Newport, Rhode Island U.S. Weather Station). Because land-use history suggests that the populations in this study are probably no older than 100 yr, the early onset of drought observed here might have represented a novel stress.

Significant selection for phenological and physiological traits in drought—

In the field, significant selection was detected in both treatments for early flowering (Table 1). A significant selection differential was also detected for stomatal conductance in water-limited conditions (Table 1), such that plants with higher stomatal conductance values had higher fitness. While the selection gradient for stomatal conductance was not significant in either treatment, a significant flowering time by stomatal conductance interaction (correlative selection) was observed in water-limited conditions (Table 1). This interaction indicated that early-flowering lines with higher stomatal conductances were more fit in dry conditions (Fig. 1). Nonparametric Spearman correlations indicated that WUE and relative fitness were negatively associated in water-limited conditions ($r = -0.12$, $P = 0.05$), but uncorrelated in watered conditions ($r = -0.081$, $P = 0.19$). The selection gradients and differentials did not differ across treatments for both stomatal conductance and flowering time (Table 1). However, the flowering time by stomatal conductance gradient (correlative selection) differed marginally across treatments (Table 1). No selection was observed for leaf size in either water treatment (Table 1). No stabilizing or disruptive selection was detected for any trait.

Population differentiation in responses to water limitation—

Lines from the dry-site population flowered significant-

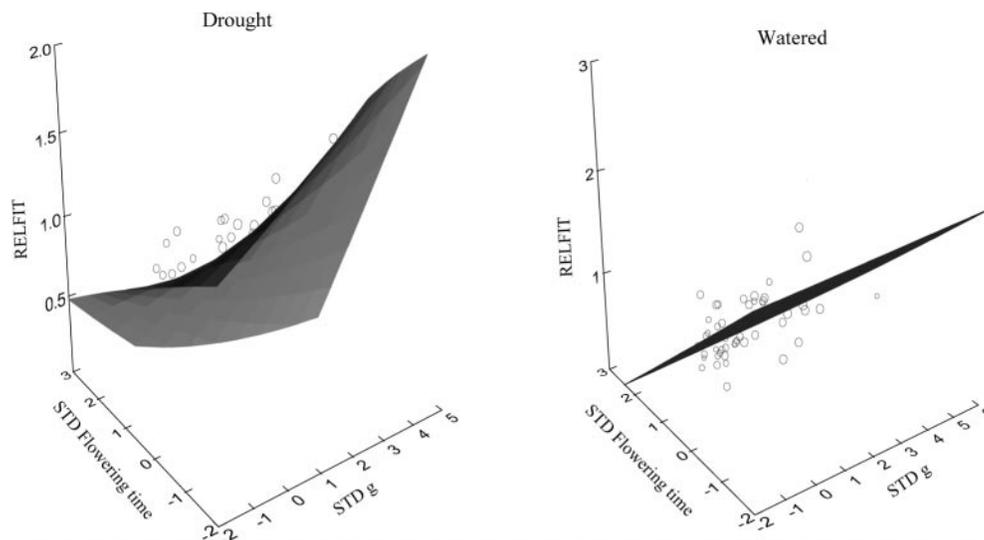


Fig. 1. Relative fitness (RELFIT) plotted against standardized (STD) flowering time and stomatal conductance (g) values across both populations in water-limited (drought) and well-watered conditions.

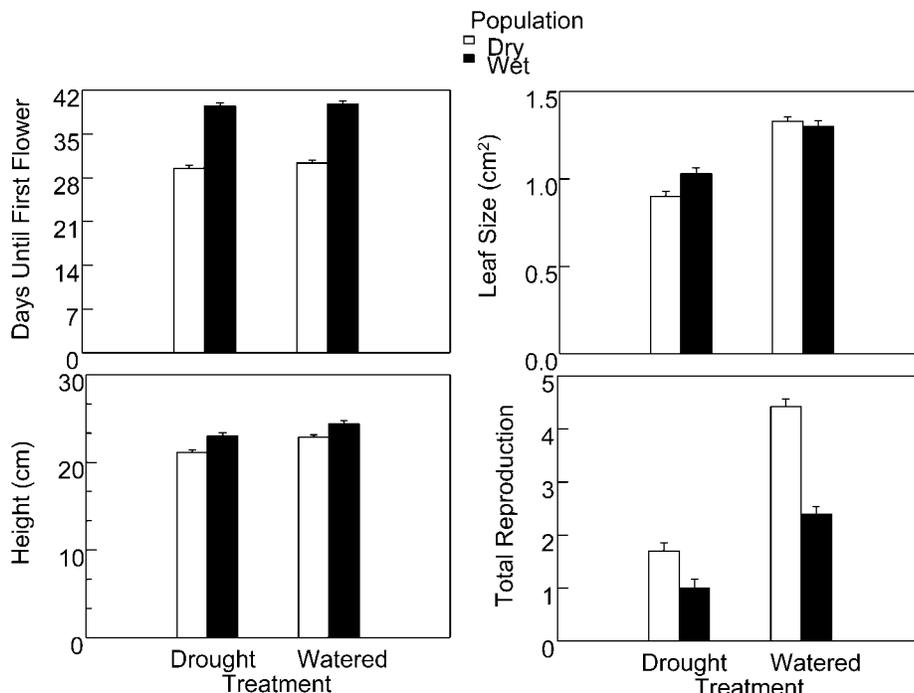


Fig. 2. The number of days until first flower, the area of the most-recently-fully-expanded leaf (leaf size), plant height after 1.5 mo of growth, and natural log of reproductive fitness (total number of flowers, fruits, and pedicels) for the wet and dry populations in water-limited (drought) and well-watered conditions. Least-square means \pm 1 SE are shown.

ly earlier than lines from the wet-site population in both the unwatered (drought) and watered treatments (significant population effect; Fig. 2; Table 2). The populations also differed significantly in WUE (Fig. 3). In the drought treatment, plants from the dry-site population had higher water-use efficiencies than wet-site population plants (Fig. 3; $\chi^2 = 12.20$, $df = 1$, $P = 0.0005$), whereas, in the watered treatment, the populations did not differ in WUE (Fig. 3; $\chi^2 = 1.96$, $df = 1$, $P = 0.16$). These population differences in WUE were predominantly due to different responses in stomatal conductance between the two environments (Fig. 3), and mirror the genetic differentiation observed between these populations in two other studies (Heschel and Hausmann, 2001; Heschel et al., 2002). The carbon assimilation rate was lower in drought than in watered plots for both populations (significant treatment effect; Table 2; Fig. 3), but populations differed in their stomatal sensitivities to watering treatment (marginally significant population by treatment effect, but significant contrasts within each watering treatment; Table 2; Fig. 3). Dry-site population lines had significantly lower stomatal conductances when unwatered than wet-site population lines (planned contrast: $F = 4.87$, $df = 1$,

$P = 0.03$), but in watered plots the populations had equivalent stomatal conductances (planned contrast: $F = 0.14$, $df = 1$, $P = 0.71$). Significant genetic variation for A and marginally significant genetic variation for g was detected, but no genotype by environment interactions were found for gas exchange traits (Table 2). Carbon assimilation rates were generally lower than values observed in a greenhouse experiment (Heschel and Hausmann, 2001); however, the plants in this field experiment were shorter and had fewer nodes than did greenhouse plants from the same lines. Plants in the second pilot experiment were of the same size and developmental stage as plants in the field experiment and as a result, gas exchange data were of the same magnitude in both studies. Moreover, plants used for previous gas exchange measurements were grown in light-saturated, high-resource greenhouse conditions, while the plants in the field experiment were acclimated to the canopy shade.

Leaf size plasticity also differed between populations (significant population by treatment effect; Table 2; Fig. 2). In water-limited conditions, dry-site population plants had significantly smaller leaves than wet-site population plants ($F =$

TABLE 2. Analysis of variance for population and drought treatment on physiological and morphological traits. F statistics and P values are reported. A = carbon assimilation rate; g = stomatal conductance; — = no covariate used in the analysis.

	Block (Drought trt)	Drought trt	Line (Pop)	Population	TrtXLine (Pop)	PopXTrt	Initial height
A	9.86***	43.75**	1.95*	3.05+	0.97	0.52	—
g	6.17***	35.54**	1.26+	0.019	1.15	2.86+	—
Leaf size	18.33***	8.78*	3.58***	0.74	0.89	10.18**	44.02***
Height	7.69***	7.19*	1.98**	14.17***	1.99***	0.34	505.52***
Flowering time	5.18***	0.15	9.69***	37.83***	0.99	0.47	7.83**
Total reproduction	7.89***	47.18**	2.66***	37.91***	1.42*	8.75**	—

*** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$; + $P < 0.10$.

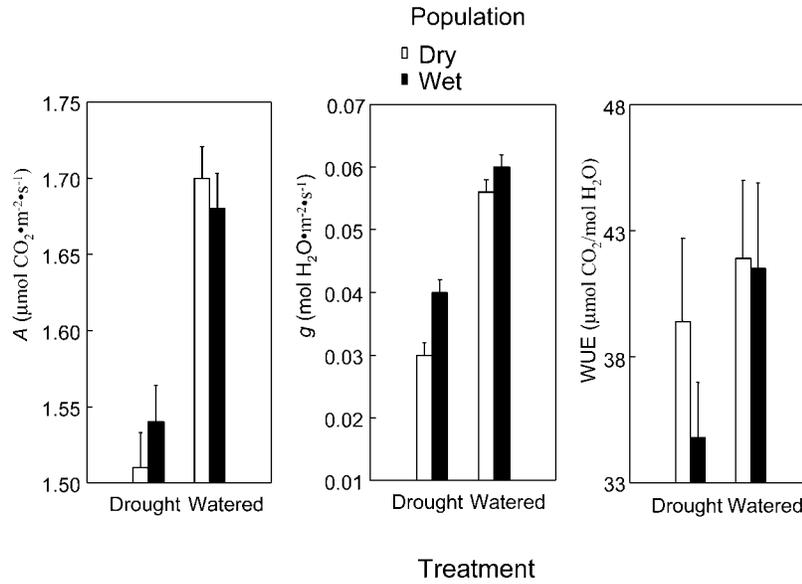


Fig. 3. Carbon assimilation rate (A), stomatal conductance (g), and water-use efficiency ($WUE = A/g$) values for the wet and dry populations in water-limited (drought) and well-watered conditions. Least-square means \pm 1 SE are shown.

7.56, $df = 1$, $P = 0.008$; Fig. 2), but in watered plots, wet- and dry-site population plants had equivalent leaf sizes ($F = 0.55$, $df = 1$, $P = 0.46$). Wet-site population plants were taller than dry-site population plants regardless of treatment (Fig. 2; Table 2). A significant line by drought treatment effect was also detected for height, indicating genetic variation for plasticity of height in response to water-limited conditions.

Across both treatments, dry-site population plants had higher fitness than wet-site population plants (drought conditions: $F = 21.62$, $df = 1$, $P < 0.001$; watered conditions: $F = 83.44$, $df = 1$, $P < 0.001$). However, the fitness difference between populations was greater in watered plots (Fig. 2; significant population and population by treatment effects; Table 2). Significant genetic variation was detected within both populations for the fitness responses of lines to water limitation (significant line by treatment effect; Table 2). That is, norms of reaction

crossed within both populations (Fig. 4). Although a limited number of lines exhibited high fitness only in watered conditions, suggesting high-water specialist genotypes, a greater number of lines showed similar fitness in both environments, suggesting generalist genotypes. It is unlikely that high-water specialists are a result of adaptation to greenhouse conditions because lines were single-seed derived and few lines were lost during successive generations of inbreeding.

DISCUSSION

The direction of selection on physiological and phenological traits may depend on the timing of the onset of drought. Significant selection was detected for early flowering time, increased stomatal conductance, and decreased WUE when water became limiting early in the growth season. That is, water-

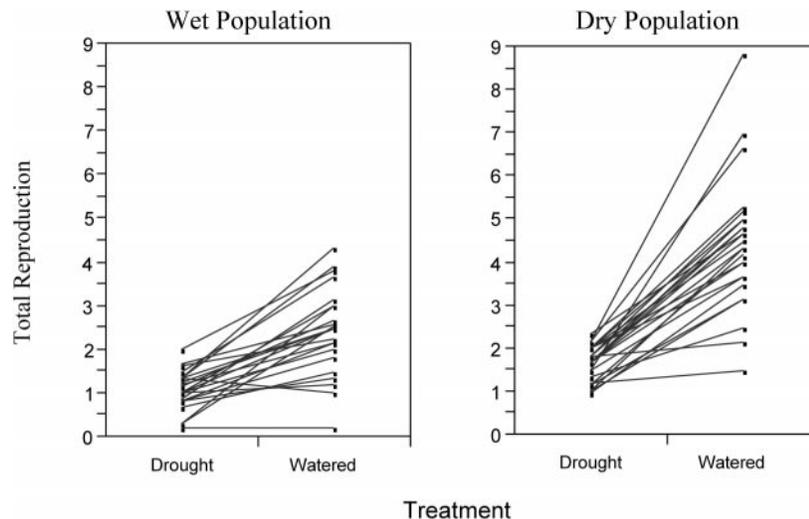


Fig. 4. Total reproductive fitness (total number of flowers, fruits, and pedicels) of each dry and wet population inbred line in water-limited (drought) and well-watered conditions. Least-square means are shown.

limited plants that conserved less water, assimilated more carbon, and flowered early had the highest fitness. Therefore, during early-season stress, selection was detected for drought avoidance rather than drought tolerance.

Selection for drought tolerance or avoidance—Contrary to functional predictions for longer lived annuals, selection was detected for increased stomatal conductance and early flowering (correlative selection, Fig. 1) and low WUE in water-limiting conditions. Also, direct selection was not observed on leaf size when holding the effects of stomatal conductance and flowering time constant. Therefore, increasing stomatal conductance, decreasing WUE, and flowering early maximized fitness when soil water was limiting in 1999, while decreasing leaf size was of no adaptive value. This pattern of selection for relatively high water use and rapid development may have been due to the early onset of drought in 1999. Soil water was extremely limiting in early June in this experiment; whereas in the past, similar soil water conditions have not been reached until late July (Heschel and Hausmann, 2001; Heschel et al., 2002). When soil water became limiting later in the season at the same site in 1997, increased WUE and lower stomatal conductance resulted in increased reproductive fitness (Heschel et al., 2002). However, here, because plants were water limited early in the life history of *I. capensis*, increased WUE and lower stomatal conductance resulted in decreased reproductive fitness. This pattern is similar to that observed by Donovan and Ehleringer (1992), in that plants in early life-history stages showed lower WUE and higher gas exchange rates than those at later life-history stages. Alternatively, lower soil nitrogen availability could have resulted in selection for decreased WUE and increased stomatal conductance in this experiment (Livingston et al., 1999; Arntz et al., 2000). However, because our data were collected in exactly the same microsite as in Heschel et al. (2002), we hypothesize that the observed differences in selective patterns between 1997 and 1999 were due to the principal factor that varied between these experiments, namely the timing of drought stress.

Population differences in stress response—Plants from the dry-site population responded to water limitation by decreasing stomatal conductance to a greater degree than plants from the wet-site population. As a result, the dry-site population was more water-use efficient than the wet-site population in drought conditions. Interestingly, the water-conserving responses of the dry-site lines (higher WUE and lower stomatal conductance) may have been maladaptive for the drought experienced here; selection was detected for decreased WUE and increased g in water-limited conditions. These seemingly maladaptive physiological responses of dry-site lines may have depressed the fitness difference between the wet and dry populations in the drought treatment since the fitness difference was greater in watered conditions (Fig. 2). Nonetheless, the dry-site population had a higher mean fitness than the wet-site population regardless of treatment, potentially due to generalist lines (Fig. 4) that flowered earlier in drought and well-watered conditions. Flowering early may have increased fitness to a greater extent than other traits (Table 1). As such, the early flowering of the dry-site population may have allowed it to have higher average fitness than the wet population in drought despite the maladaptive increases in WUE for dry-site lines. In fact, early flowering is a drought avoidance strategy (Farris and Lechowicz, 1990; Geber and Dawson, 1990, 1997; Ben-

nington and McGraw, 1995; Stanton et al., 2000; McKay et al., 2003). Therefore, we surmise that drought-avoiding generalist lines would be selected for in unpredictable soil moisture environments (Schneider, 1989; Fry, 1996).

Conclusions—Early-season drought avoidance involved selection for different responses than late-season drought tolerance. Here, when water became limiting early in the season, lines with low WUE, increased stomatal conductance, and early flowering were the most fit, whereas, in studies when water became limiting later in the growth season at the same microsite and in a greenhouse (Heschel, 2000; Heschel et al., 2002) lines with high WUE and decreased stomatal conductance had the highest fitness. When confronted with early-season drought, it may be adaptive for *I. capensis* to maximize carbon assimilation by means of low WUE and high rates of gas exchange and flower early in order to reproduce quickly (Cohen, 1970; Mooney et al., 1976; Geber and Dawson, 1990). Correlative selection was detected for early flowering combined with high stomatal conductance in water limiting conditions (Fig. 1). Thus, early flowering and stress avoidance may be key mechanisms behind adaptation to early-season drought at this site. In contrast, when water becomes limiting later in its life cycle, *I. capensis* should adopt a drought tolerance strategy regardless of light environment (Heschel et al., 2002). In this case, increased WUE and decreased stomatal conductance maximize fitness as predicted by Zangerl and Bazzaz (1984) and Dudley (1996a). Overall, the physiology of water relations may be on a complex evolutionary trajectory that will vary by year. Such annual variation in selection for WUE may promote the persistence of stress avoiding and tolerating water-use physiology among natural populations of annual plants (cf. Geber and Dawson, 1997).

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