

POPULATION DIFFERENTIATION AND NATURAL SELECTION FOR WATER-USE EFFICIENCY IN *IMPATIENS CAPENSIS* (BALSAMINACEAE)

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In xeric environments, water-use efficiency (WUE) is likely to be a target of natural selection. Higher WUE is expected to confer a fitness advantage in drought conditions, but this prediction has rarely been tested. To examine the relationships between WUE and fitness, two common garden experiments were conducted. Inbred lines of *Impatiens capensis* derived from wet and relatively dry sites were planted into dry field conditions in an open and a woodland site. The lines from the dry population increased their instantaneous WUE in response to the dry conditions of the field to a greater degree than lines from the wet population. High instantaneous WUE also conferred a fitness advantage in the stressful conditions of the field. Thus, WUE may be an important trait for *I. capensis* populations in adapting to drier conditions.

Keywords: Balsaminaceae, drought stress, *Impatiens capensis*, population differentiation, instantaneous water-use efficiency.

Introduction

Relatively little is known about microevolutionary adaptation to drought in plants (Gurevitch et al. 1986; Farris 1987). Although several studies have shown that water availability can be a significant selective agent on traits within plant populations (Farris 1988; Dudley 1996a), few studies have demonstrated adaptive genetic differentiation in response to variation in drought conditions (Schemske 1984; Bennington and McGraw 1995; Dudley 1996a, 1996b; M. S. Heschel and C. Riginos, unpublished manuscript).

Limited water availability can exert selection on drought tolerance through selection on instantaneous water-use efficiency (WUE), that is, carbon uptake per unit of water lost to stomatal transpiration. As measured with a gas exchange system, WUE is an instantaneous indicator of drought tolerance because changes in carbon assimilation and stomatal conductance can occur within minutes of water deficit (Larcher 1995). Therefore, population differentiation in response to environments that vary spatially and temporally in water availability is likely to be manifested as differentiation in WUE (Silander and Antonovics 1979; Zangerl and Bazzaz 1984; Quick et al. 1992; Basnayake et al. 1995; Dudley 1996a).

Maximizing carbon gain in an environment with heterogeneous water availability is important to herbaceous annuals with no seed bank because population persistence is highly

dependent on the reproductive output of a given season. Herbaceous annuals are therefore predicted to make maximal use of water when it is available by fully opening their stomata and assimilating as much carbon as possible, thereby exhibiting low WUE (Cohen 1970; Cowan 1986). However, when water supply is limited, drought-tolerant taxa should minimize water loss by greatly increasing WUE (Zangerl and Bazzaz 1984; Cowan 1986). In drought conditions, plants with high WUE would be predicted to accumulate more biomass per unit water lost than plants with low WUE (Givnish 1986; Dudley 1996a). Thus, WUE is expected to have different optimal values depending on water availability.

Here we assess the degree to which two natural populations of a native North American annual, *Impatiens capensis* Meerb. (Balsaminaceae), have differentiated in instantaneous WUE in response to drought stress, and we measure the strength of natural selection on WUE in drought conditions. This species is ideal for studying the evolution of WUE because it lacks cambium, significant prop roots, and a thick, waxy cuticle. Thus, changes in plant osmotic potential are expected to affect instantaneous WUE. In addition, changes in gas exchange traits are easily quantifiable since *Impatiens* does not close its stomata at midday (Schulz et al. 1993). Finally, several studies of *Impatiens* have demonstrated population differentiation and genetic substructure, indicating that adaptive differentiation can occur (Schemske 1984; Schoen et al. 1986; Schoen and Latta 1989; Schmitt and Gamble 1990; Argyres and Schmitt 1991; Schmitt 1993; Bennington and McGraw 1995; Dudley and Schmitt 1995; Donohue et al. 2000a, 2000b, 2001; Heschel and Hausmann 2001).

To investigate the extent to which WUE and its plasticity have differentiated in two natural populations of *I. capensis* from different soil moisture environments and to measure the strength of selection on instantaneous WUE, we conducted experiments in two field sites using inbred lines from these populations. Specifically, we addressed the following ques-

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tions: (1) Has instantaneous WUE differentiated between populations experiencing different levels of soil moisture? (2) What is the magnitude of natural selection on instantaneous WUE in field conditions? We predicted that the population that evolved under conditions of low and variable water availability would exhibit higher instantaneous WUE under drought conditions. We further predicted that instantaneous WUE would be high in a drought-stressed environment and that the WUE expressed in these different environments would increase fitness.

Material and Methods

Organism and Study Sites

Jewelweed, *Impatiens capensis* Meerb. (Balsaminaceae), is an annual, self-compatible herb of North American deciduous forests and wetlands. The species has a mixed mating system, producing both cleistogamous (selfing) and chasmogamous (potentially outcrossing) flowers from June to October (Waller 1979). Seeds disperse ballistically, usually less than 1.5 m from parent plants (Schmitt et al. 1985; Kelly 1997). However, long-distance dispersal can occasionally occur by flotation, so new populations are typically established on the banks of waterways and in wetlands (Gleason and Cronquist 1963; Leck 1979, 1996; George 1997).

While most *Impatiens* populations inhabit mesic areas, some experience variable soil moisture conditions (Gleason and Cronquist 1963; Leck 1979, 1996; George 1997). Two populations at Brown University's Haffenreffer Reserve in Bristol, Rhode Island, provide an excellent opportunity to study differentiation for drought stress tolerance. One of the populations occupies a sunny, uniformly wet site (hereafter referred to as the "wet population"), while the other experiences a shaded, variably dry environment (hereafter referred to as the "dry population") (Heschel and Hausmann 2001; fig. 1). These populations are separated by ca. 1 km.

Common Garden Experiments

A reciprocal transplant experiment was conducted to assess the WUE of the populations under study in field conditions. Inbred lines from both populations were maintained for six generations by single-seed descent in a greenhouse to minimize parental environmental effects specific to site of origin. Lines were started from seedlings that were transplanted to the Brown University greenhouse in 1996. These seedlings were randomly chosen from evenly spaced microsites that covered the entire area of each population in both the dry and wet sites.

The inbred lines were planted in the field in Bristol as part of a larger experiment (Donohue et al. 2000a, 2000b, 2001). Cleistogamous seeds from 15 wet and 13 dry population lines were stratified at 4°C for 4 mo in distilled water. In April 1997 these seeds were planted into flats with 4-cm-diameter cells filled with Scott's Metro-Mix 350 (Scotts-Sierra Horticultural Products, Marysville, Ohio). Seeds and seedlings were top-watered in the greenhouse and kept continuously moist to ensure establishment. In early May 1997, ca. 2 wk after emergence of most seedlings, replicates from each line were reciprocally transplanted into the site of the wet population and

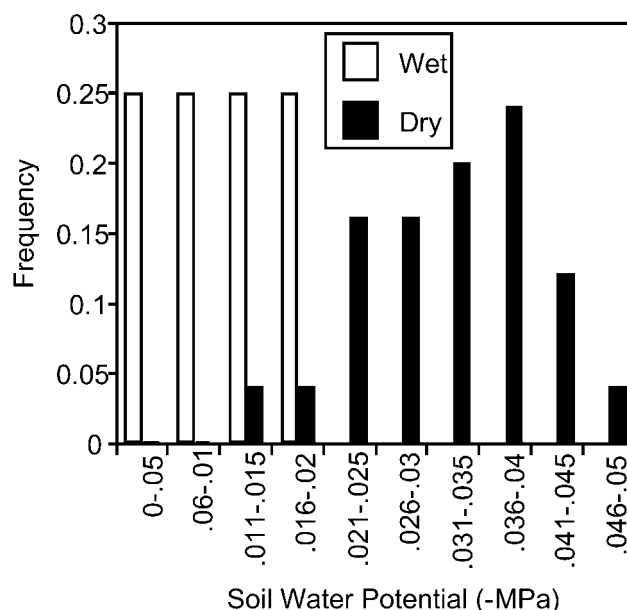


Fig. 1 Soil water potential (MPa) for the wet and dry populations for late July/early August 1996, determined with a tensiometer 6 cm below the soil surface. Means across all transects \pm 1 SE are shown for each population. More negative values are indicative of drier soil conditions. Tensiometer measurements were taken every 5 m along 25-m transects through the two populations.

the site of the dry population. Up to four replicates per inbred line were randomly distributed among three blocks at 15 cm spacing (53 plants m^{-2}) at each site. Before planting, the blocks were cleared of all existing vegetation. Seedlings were watered for 2 d after being transplanted into the field to ensure establishment, and plant height and physiological (gas exchange) measurements were taken simultaneously during July when plants were ca. 2.5 mo old and drought stressed. At the dry site, gas exchange measurements were made on a subset of the planted lines because many plants had died as a result of heat and extreme drought stress. Reproductive fitness was estimated by censusing flower and fruit production every 2 wk from July until all plants had died.

The site of the dry population experienced 10–200 $\mu mol m^{-2} s^{-1}$ of light intensity at midday and -0.05 to -0.07 MPa soil water availability; the site of the wet population experienced ca. 1000–1200 $\mu mol m^{-2} s^{-1}$ of light intensity at midday and -0.05 to -0.06 MPa soil water availability during the experiment because of a late-season drought. The -0.05 to -0.06 MPa value did not represent typical summer water availability for the wet site (usually 0 to -0.02 MPa; Heschel and Hausmann 2001) and was the result of removing all aboveground vegetation for the experimental plots. Therefore, both the field environments represented conditions that impose drought stress.

Physiological Measurements

Carbon assimilation rate (A ; $\mu mol CO_2 m^{-2} s^{-1}$) and stomatal conductance (g ; $mol H_2O m^{-2} s^{-1}$) were measured with an ADC Infrared Gas Analyzer (IRGA), model LCA 4, for

calculations of instantaneous WUE. IRGA measurements were taken between the hours of 10:00 A.M. and 3:00 P.M. Water-use efficiency was estimated as the ratio of A to g (units of measure are micromoles of CO_2 per mole of H_2O) (Dudley 1996a). The LCA 4 was “environmentally controlled” and had a PAR adjustable light source and a Peltier cooling unit built into the Parkinson Leaf Chamber. Light levels were kept consistently between 760 and $800 \mu\text{mol m}^{-2} \text{s}^{-1}$, and the chamber temperature was kept between 27° and 29°C . In *I. capensis*, maximum carbon assimilation rates are attained at $750 \mu\text{mol m}^{-2} \text{s}^{-1}$ of light, so the light used was at saturating levels (M. S. Heschel, unpublished data). Relative humidity ranged from ca. 60% to 70% across both experimental sites. Effects of time of measurement and date were factored out by adjusting IRGA values with residuals from linear regression models (Type I sums of squares) that included time and date (Farris and Lechowicz 1990; Dudley 1996a). Temperature and light levels were not included in the final regression models because they did not significantly explain variation among measurements. Separate models for the field measurements determined that the effect of inbred line did not significantly predict leaf temperature. Boundary-layer conductances were estimated with moist Whatman filter paper leaf mimics (Parkinson 1985). To correct for different leaf areas in the Parkinson leaf chamber during measurements, individual leaf areas were calculated and used by the IRGA in each calculation.

Data Analysis

All statistical analyses were performed with JMP (version 3.1, SAS Institute, 1994). For each field site, nested ANOVA models were used to determine whether populations differed significantly in WUE, A , and g . Separate models were used for each site because of imbalance in line representation between the two experimental sites. For both models, population was

a fixed effect; block and inbred line (nested within population) were random effects. The main effect of population was tested over the line effect. Interactions with block were pooled with the error.

Phenotypic selection analysis was used to determine the strength of natural selection on WUE, A , and g (Lande and Arnold 1983). Relative fitness (individual fitness divided by mean fitness: total number of flowers and fruits \div mean in each site) was the response variable and WUE, A , or g were the predictors. For each site, data from both populations were pooled to increase statistical power. The slope of the regression line estimates the strength of selection, or selection differential, S . In other words, the degree to which WUE, A , or g predict relative fitness is conveyed by the parameter estimates or slope terms. Selection differentials are usually expressed in units of phenotypic standard deviations (Lande and Arnold 1983) called standardized selection differentials, S' , or selection intensity. Therefore, WUE, A , and g values were standardized with respect to one standard deviation from the mean. Population was initially included as a class variable to control for differences in fitness between populations due to unmeasured characters that may have covaried with WUE, A , or g . Since the population term was never significant, it was dropped from all linear regression models. Multivariate selection gradients (Lande and Arnold 1983) were not estimated because A , g , and WUE were highly collinear. Regression models with quadratic terms were used to test for stabilizing or disruptive selection. Because significant quadratic terms were not detected, these nonlinear selection models are not reported.

Results

Population Differentiation

In both field experiments, the dry population had a significantly higher mean water-use efficiency than the wet popu-

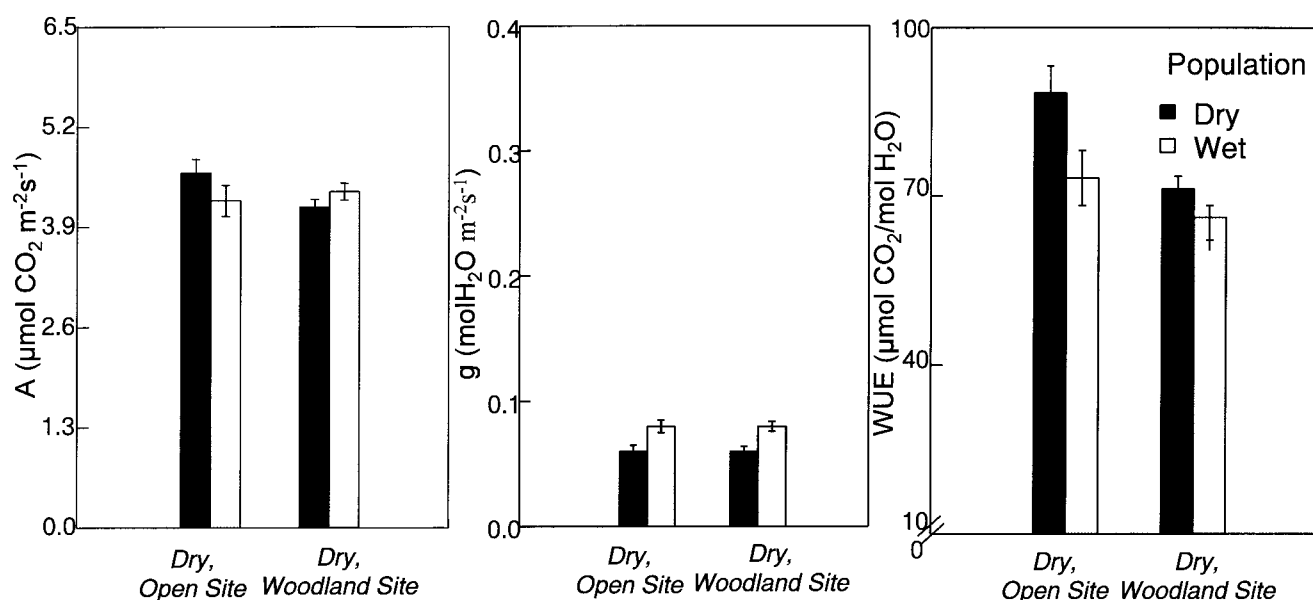


Fig. 2 Carbon assimilation rate (A), stomatal conductance (g), and instantaneous water-use efficiency (WUE) values for the wet and dry populations in the field experiments. Means ± 1 SE are shown.

Table 1
ANOVA for Population on Water-Use Efficiency (WUE), Carbon Assimilation Rate (A), and Stomatal Conductance (g) in the High-Light, Open Site

Source	WUE				A				g			
	SS	df	F	P	SS	df	F	P	SS	df	F	P
Block	1050.62	2	0.22	0.80	0.055	2	0.010	0.99	0.00005	2	0.034	0.97
Population	11,182.2	1	9.33	0.004	9.88	1	2.64	0.12	0.00013	1	0.14	0.71
Line[Pop]	27,899	26	0.46	0.99	100.48	26	1.44	0.09	0.024	26	1.35	0.14
Residual	348,235.65	148			396.69	148			0.10	148		

Note. For the low-light, woodland site, the block and line terms were excluded due to a lack of replication, so *t*-tests were conducted (WUE: $N = 28$, $t = -2.36$, $df = 26$, $P = 0.03$; A: $N = 28$, $t = -1.32$, $df = 26$, $P = 0.2$; g: $N = 28$, $t = 1.04$, $df = 26$, $P = 0.31$).

lation (fig. 2; table 1). The observed population differences in instantaneous WUE resulted primarily from changes in stomatal conductance between the two populations (fig. 2). The observed population differences in A and g were not significant, however (table 1). No variation among lines was detected for WUE in either experimental site; however, lines varied significantly in A and g across both field sites (table 1).

Phenotypic Selection Analysis

Plants grown in open, dry field conditions experienced selection for increased WUE and A (table 2). In woodland, dry field conditions, selection differentials were of the same sign and stronger in slope but only marginally significant because of extremely low sample sizes due to mortality ($N = 28$). Marginally significant selection was also detected for lower g in low-light conditions (table 2). In open conditions, lower stomatal conductance tended to translate into increased fitness, but this relationship was not statistically significant. Thus, directional (linear) selection favored higher WUE, higher A, and lower g in the field under drought conditions. No stabilizing or disruptive selection was found in any environment.

Discussion

The two populations differed genetically in instantaneous WUE expression. The observed genetic differentiation in WUE was primarily due to differences in stomatal conductance. As predicted, the population that evolved in the more heterogeneous water environment (the dry site population) had greater instantaneous WUE in drought conditions than the population from the more homogeneously wet site. This result is in agreement with previous studies in greenhouse conditions (Heschel and Hausmann 2001; M. S. Heschel, unpublished data). In these two studies, lines from the dry population had greater

WUE than the wet population lines in dry compared with watered conditions.

The observed differentiation may be adaptive (Cohen 1970; Cowan 1986): lines derived from a population where water is an ephemeral resource should have high WUE when water is limited. When water is plentiful, herbaceous annuals are expected to open their stomata fully, i.e., to exhibit low WUE in order to assimilate as much carbon as possible. Because fitness of annuals without a seed bank depends entirely on reproductive output within a single season, the resulting augmentation of assimilated carbon should lead to increased fitness (Givnish 1986). However, under dry conditions, an optimal strategy would be to limit water loss at the expense of decreased carbon uptake but with the potential benefit of increased viability (Dudley 1996a, 1996b). The observed differentiation in WUE would be adaptive if high WUE was selectively favored under drought conditions. The selection analysis supports an interpretation of adaptive divergence in WUE expression in dry conditions. In this experiment, high WUE was associated with higher relative fitness under late-season drought conditions, as expected. Therefore, the observed increase in WUE under drought conditions may be adaptive.

In drought conditions, high instantaneous WUE also predicted increased fitness irrespective of light environment; in both a sunny and a shaded site, plants with increased WUE had higher fitness. Increases in WUE are at the expense of higher potential carbon gains since stomata are closed to conserve water. Thus, conserving water at the expense of lower carbon acquisition increased fitness under drought in high- and low-light conditions. The importance of maintaining positive turgor pressure during drought was highlighted by the marginally significant selection for lower stomatal conductance in low-light conditions. Nonetheless, in both high- and low-light drought conditions, positive selection was detected for increased carbon assimilation. This result underscored the es-

Table 2
Phenotypic Selection Analysis

Environment	A			g			WUE		
	S'	t	P	S'	t	P	S'	t	P
Open site	0.12 \pm 0.04	2.94	0.004	-0.013 \pm 0.06	-0.23	0.82	0.076 \pm 0.04	2.09	0.03
Woodland site	0.28 \pm 0.15	1.89	0.07	-0.38 \pm 0.22	-1.75	0.09	0.27 \pm 0.15	1.82	0.07

Note. S' = standardized selection differential values ± 1 SE, in standard deviation units. A = carbon assimilation rate; g = stomatal conductance; WUE = water-use efficiency.

sence of the trade-off between water conservation and carbon assimilation under drought conditions: although higher water-use efficiency is selectively advantageous, higher carbon assimilation rate is also adaptive. Therefore, a physiological response to drought should result in water conservation, but this benefit will be partially outweighed by decreases in net carbon acquisition (Geber and Dawson 1997). In fact, water conservation may be selectively advantageous only during late-season drought. In another field experiment, under early-season drought conditions, high carbon assimilation still translated into fitness gains, but because of the life history emphasis on reproducing as quickly as possible, selection paradoxically favored low instantaneous WUE (M. S. Heschel and C. Riginos, unpublished manuscript). During early-season drought, wasting water to increase short-term carbon gains was selected for. Thus, adaptation to drought is a dynamic process.

Overall, *Impatiens capensis* populations have differentiated for drought tolerance. This study documented significant se-

lection on instantaneous WUE under natural, late-season drought conditions irrespective of light environment. It also suggests that past selection on WUE may have led to the observed population divergence in instantaneous WUE. The direction of population divergence was in the direction hypothesized for adaptive divergence in response to drought stress.

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