Testing for stress-dependent inbreeding depression in *Impatiens capensis* (Balsaminaceae)[1]

M. Shane Heschel,[2] Neil Hausmann, and Johanna Schmitt

Department of Ecology and Evolutionary Biology, Brown University, Box G-W, Providence, Rhode Island 02912 USA

The relevance of inbreeding depression to the persistence of plant populations can depend upon whether stress magnifies inbreeding depression for fitness-related traits. To examine whether drought stress exacerbates inbreeding depression in gas exchange traits and biomass, we grew selfed and outcrossed progeny of inbred lines from two populations of *Impatiens capensis* in a greenhouse experiment under water-limited and moist soil conditions. Drought stress did not magnify the degree of inbreeding depression for any of the traits measured. In fact, in one population there was a trend for stronger inbreeding depression under well-watered, benign conditions. Furthermore, significant inbreeding depression for carbon assimilation rate and stomatal conductance was only detected in the lines from one population. In contrast, inbreeding depression for biomass was detected within both populations and differed among lines. Drought stress exerted significant selection on physiological traits, favoring increased carbon assimilation rates and decreased stomatal conductance in drought-stressed plants. Patterns of selection did not differ between inbred and outcrossed plants but did differ marginally between populations. Thus, estimates of selection were not biased by the mixed mating system per se, but may be biased by combining individuals from populations with different histories of selection and inbreeding.

Key words: carbon assimilation rate; drought stress; inbreeding depression; natural selection; stomatal conductance.

Inbreeding has been shown to have negative fitness consequences for natural plant populations (e.g., Schemske, 1978; Waller, 1984; Dudash, 1990; Schmitt and Ehrhardt, 1990; Schmitt and Gamble, 1990; Willis, 1993; McCall et al., 1994; Heschel and Paige, 1995; Paige and Heschel, 1996; Donohue, 1998; Frankham and Ralls, 1998; Byers and Waller, 1999; Cheptou et al., 2000; Fishman, 2001). Most of this fitness disadvantage (i.e., inbreeding depression) is probably attributable to the expression of recessive deleterious alleles in the homozygous condition (Charlesworth and Charlesworth, 1999). Moreover, the severity of this fitness disadvantage may depend on environmental conditions (Dudash, 1990; Miller, 1994; Pray et al., 1994; Byers and Waller, 1999). For example, drought stress in plants may exacerbate inbreeding depression in fitness traits (Hauser and Loeschcke, 1996), change the degree of heterozygote advantage (Pederson, 1968), and lead to local extinction (Smith et al., 1993). However, drought-dependent inbreeding depression could potentially facilitate purging of deleterious alleles in populations with a long-term history of drought stress, leading to reduced inbreeding depression (e.g., Charlesworth and Charlesworth, 1987). Thus, the impact of inbreeding on natural plant populations may vary according to the frequency and severity of stresses like drought.

Plant drought response involves coordinated changes in morphological traits such as leaf area, carbon allocation to roots, or cell elasticity (Chapin et al., 1993). Additionally, physiological traits like stomatal conductance to water vapor and carbon assimilation rate are expected to be important de-terminants of fitness in drought conditions because of their relevance to water-use efficiency and photosynthesis (Cohen, 1970; Dudley, 1996; Geber and Dawson, 1997; Heschel and Hausmann, 2001; Heschel et al., 2002; Heschel and Riginos, 2005). Inbreeding depression in these traits could therefore reduce fitness under water-limitation. For example, Norman et al. (1995) demonstrated that inbreeding might reduce the expression of water-use efficiency, thereby causing a fitness reduction for self-fertilized plants in stressful conditions. Thus, inbreeding may affect the nature of selection on physiological traits.

Inbreeding depression may also influence measures of natural selection on physiological traits associated with stress response. If physiological traits are subject to inbreeding depression, measures of selection on those traits in species with a mixed mating system may be biased. This bias arises because inbreeding can create a spurious correlation between a trait and fitness by reducing both (Willis, 1996). That is, selfed plants may have lower stomatal conductance and fitness than outcrossed plants, even if there is no causal connection between the two traits. A similar argument also applies to selection experiments with individuals from several populations with different histories of inbreeding or bottlenecks. Plants from a population with a recent history of inbreeding or a high genetic load (e.g., Heschel and Paige, 1995) may have lower stomatal conductance and fitness than plants from a large, out-crossing population. Such spurious correlations can confound measurements of selection. Therefore, the degree of inbreeding depression in particular traits needs to be measured to accurately assess relationships between traits and fitness.

If inbreeding depression can alter measurements of selection on physiological traits, then environmental differences in expression of inbreeding depression may cause the appearance of environmental differences in selection on those traits. In particular, if inbreeding depression is exacerbated by stress, the relationship between physiological traits and fitness may differ between stressful and benign environments, independent of ecological differences in the causes of selection between those environments (Wade and Kalisz, 1990). Therefore, the

[2] Author for correspondence (e-mail: sheschel@oeb.harvard.edu), present address: Harvard University Herbaria, 22 Divinity Avenue, Cambridge, Massachusetts 02138 USA.

strength of selection on a particular trait may be biased by inbreeding and environmental covariance (Willis, 1996). To date, however, only a few studies have tested for drought stress-dependent inbreeding depression (Pederson, 1968; Nason and Ellstrand, 1995; Hauser and Loeschcke, 1996; Cheptou et al., 2000) and even fewer have measured inbreeding depression for physiological traits (Norman et al., 1995). To our knowledge, the data presented here represent the first examination of the effects of drought stress on inbreeding depression, as well as their joint impact on the relationship between physiological traits and fitness.

The annual plant *Impatiens capensis* Meerb. (Balsaminaceae) provides a good model with which to investigate the relationship between inbreeding and drought stress. *Impatiens* typically inhabits water-saturated environments (Leck, 1996) and thus is very sensitive to slight decreases in water potential (Schulz et al., 1993). Levels of drought stress vary within and among years and can differ consistently among natural *Impatiens* populations (Heschel and Hausmann, 2001). There is also evidence for drought-dependent selection on physiological traits in this species (Heschel et al., 2002; Heschel and Riginos, 2005). *Imaptiens capensis* has a mixed mating system with frequent cleistogamous flower production. Thus, natural populations of *Impatiens* are highly inbred as revealed by high inbreeding coefficients for allozyme alleles within populations (Knight and Waller, 1987). Although the possibility of the purging of deleterious alleles makes inbreeding depression less likely in a highly selfing annual (Charlesworth and Charlesworth, 1987), inbreeding depression has been observed in populations of *I. capensis* (Waller, 1984; Schmitt and Ehrhardt, 1990; Schmitt and Gamble, 1990; McCall et al., 1994). In this species, intraspecific competition exacerbates inbreeding depression (Schmitt and Ehrhardt, 1990), and stress may exaggerate differences between inbred and outcrossed progeny (Waller, 1984). It is therefore of interest to test for drought-dependent inbreeding depression in this system and to examine the possible consequences for selection on physiological traits in dry and wet sites.

Here we address the following questions: (1) Is inbreeding depression in fitness and physiological traits exacerbated by drought stress in *Impatiens capensis*? (2) How does inbreeding affect the relationship between physiological traits and fitness in drought-stressed and benign environments?

MATERIALS AND METHODS

Study species and source populations—*Impatiens capensis* and its congeners have a mixed mating system of cleistogamous (selfing) and chasmogamous (outcrossing) flower production in which selfing is ubiquitous (Waller, 1979; Waller, 1980). Seeds disperse ballistically, usually less than 1.5 m from parent plants (Schmitt et al., 1985; Kelly, 1997). As a result, microgeographic population genetic structure has been documented within and between natural populations of *Impatiens* (e.g., Schemske, 1984; Schoen et al., 1986; Knight and Waller, 1987; Schoen and Latta, 1989; Schmitt and Gamble, 1990; Argyres and Schmitt, 1991; Schmitt, 1993; Donohue et al., 2000a).

Inbred lines of *I. capensis* were originally collected at Brown University's Haffenreffer Reserve in Bristol, Rhode Island, USA. Twenty-five seedlings were randomly collected in April 1996 from each of two previously studied sites (Schmitt, 1993; Dudley and Schmitt, 1996; Donohue and Schmitt, 1999; Donohue et al., 2000a, b) and then each of these seedlings was used to establish an inbred line by six generations of single seed descent under greenhouse conditions. The two sites are separated by less than one kilometer: a sunny clearing site with an estimated population size of approximately 3000 plants in 1996 and a shaded understory site growing under a hickory–oak

canopy with approximately 2000 plants in 1996 (M. S. Heschel, unpublished data). Substantial genetic variation in life-history traits exists both within and between *I. capensis* populations in these sites (Argyres and Schmitt, 1991; Schmitt, 1993; Donohue and Schmitt, 1999; Donohue et al., 2000b; Heschel, 2000). During the growing season, the site located in the clearing (henceforth the "wet" site) is sunny and wet, experiencing soil water potential values from -0.005 to -0.025 MPa; whereas, the wooded site (henceforth the "dry" site) is shaded and heterogeneously dry, experiencing soil water potentials from -0.015 to -0.065 MPa (Heschel and Hausmann, 2001). Severe plant wilting does not occur until soil water potentials drop below -0.030 MPa (M. S. Heschel, unpublished data).

These populations also differ in outcrossing rates in the field. In 1996, plants in the wet population site produced significantly more chasmogamous (CH) flowers per plant than in the dry population site (wet population (mean $\pm$ 1 S.E.): 0.95 ± 0.38, dry population: 0.14 ± 0.17 CH flowers per plant; $F = 4.31$, df $= 1$, $P = 0.05$; Heschel, 2000). This is consistent with Waller's (1980) observation that *I. capensis* populations in wetter sites outcross more than populations in dry sites. Thus, the history of inbreeding may differ among these populations.

Experimental design—To test for an impact of drought stress on inbreeding depression, we compared physiological and fitness traits of inbred and outcrossed plants from both populations under water-limited and irrigated treatments in a greenhouse experiment. Because the lines had been self-fertilized for six generations by single-seed descent, the degree of homozygosity for the lines would have been high, with less than 0.5% of heterozygous loci in the initial parents being heterozygous within individuals of these lines. Therefore, crosses between the lines should result in individuals heterozygous at loci polymorphic between the parental lines (Falconer and Mackay, 1996). To establish inbred and outcrossed plants, nine "focal" individuals were randomly chosen from the existing lines for each population. Seeds were collected from two cross-types: fruits generated from a cross with a line descended from a "near neighbor" in the natural population (5–10 m distance) and fruits produced through self-fertilization. Pollen-donor lines for the "near neighbor" crosses were randomly chosen from among the nine focal plants, with no two identical crosses among the focal plants. These crosses represent a realistic realm of possible matings in natural populations of *I. capensis* as most crosses involve either self-pollination via cleistogamy or pollination within the 5–10 m range via chasmogamy (Waller, 1984).

In November 1998, four replicate seeds for each watering treatment were collected from each of the 18 crosses (2 cross-types × 9 lines). All 288 seeds (4 replicates × 2 treatments × 2 cross-types × 9 lines × 2 populations) were individually weighed and then stratified in distilled water in microtiter trays at 4°C for 4 months. In March 1999, seeds were planted individually into 10 cm² pots filled with MetroMix 360 (Scotts-Sierra Horticultural Products, Marysville, Ohio, USA) in the Brown University greenhouse. The eight inbred and eight outcrossed plants per focal line were then divided into four drought (water-limited) and four irrigated (moist) blocks, with two replicate individuals randomly assigned to each block. All plants were bottom-watered throughout the experiment, except for a 1-week treatment period during which the drought blocks were not watered (see next paragraph).

After 2 months of growth, withholding water for 1 week imposed the drought treatment. Immediately following the end of the drought treatment, maximum photosynthetic rate (A_{max}, hereafter referred to as A) and stomatal conductance (g_{st}, hereafter referred to as g) were measured. One month later, plants were harvested for aboveground biomass (dry weight) measurements. To estimate total aboveground biomass, stems were cut at the soil surface, and the aboveground tissue was placed in a drying oven at 65°C for 2 days. Shoot biomass is a good estimate of reproductive fitness in *I. capensis* (Waller, 1979).

Carbon assimilation rate (A) (μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$) and stomatal conductance to water vapor (g) (mol $H_2O \cdot m^{-2} \cdot s^{-1}$) were measured with an LCA4 (ADC Bioscientific Ltd., Hoddesdon, UK) infrared gas analyzer (IRGA). All gas exchange was measured between 1000 and 1500 hours over a 5-day period during mid-May. The LCA 4 was "environmentally controlled," with an adjustable PAR (photosynthetically active radiance) light source and a Peltier

cooling unit built into the Parkinson leaf chamber. Photon flux density (PFD) was kept consistently at about 800 μmol · m⁻² · s⁻¹ and the chamber temperature was kept between 23 and 25°C. Maximum carbon assimilation rates are attained at PFD levels of approximately 800 μmol · m⁻² · s⁻¹ in *I. capensis*, so light was at saturating levels (Heschel et al., 2004). Ambient relative humidity varied from 45 to 60%. Effects of the time and date of the measurements 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, 1996). Light and temperature levels were not included in the regression models because they did not significantly explain variation among measurements. Boundary-layer conductances were estimated with moist filter paper leaf mimics (Parkinson, 1985). Leaf temperatures did not vary among lines.

Data analysis—All statistical analyses were performed with JMP (version 3.2; SAS Institute, 1998). Nested ANCOVAs were used to test for the effects of drought treatment, cross-type, and population on *A, g,* and biomass. Drought treatment, cross-type, and population source were considered fixed factors and inbred line (nested within population) and experimental block (nested within drought treatment) were considered random factors (mixed model, Type III sums of squares). The effect of treatment was tested over a synthetic denominator composed of block and drought treatment × line effects, the effect of population was tested over the line term, and the effect of cross-type was tested over the cross-type × line term. Seed weight was used as a covariate to control for effects of initial size differences. Linear contrasts were calculated on model least square means to determine the source of differences within interaction terms. All models were examined for homoscedasticity and normality of residuals. Seed weight (covariate) by treatment interactions were initially included in the models and then removed if nonsignificant.

Differences in inbreeding depression between drought and irrigated conditions might be a consequence of differences in phenotypic variance between drought treatments (D. Waller, University of Wisconsin, personal communication). To test for this possibility, the degree of inbreeding depression (δ) was calculated as 1 − (trait value of selfed plants divided by trait value of outcrossed plants). A reliable index for variance expression differences across environments is the opportunity for selection (I) or the square of the coefficient of variation for a particular trait (CV²) (Crow, 1989). For each line and within each drought treatment, inbreeding depression and CV² for biomass, *A,* and *g* were calculated across selfed and outcrossed replicates. For each line, the change in δ across drought treatments was regressed against the change in CV² across drought treatments. A strong positive relationship would indicate that the detection of changes in inbreeding depression across environments depended on the amount of phenotypic variance expressed for biomass, *A,* and *g*. In other words, the opportunity to detect inbreeding depression would have been constrained by the amount of variance observed for the traits. These analyses were conducted pooling data across populations as well as splitting data by population. Pooling and splitting by population generated very similar results, so the pooled analyses are presented to increase statistical power.

Selection differentials—Phenotypic selection analysis was used to estimate the strength of natural selection in each treatment on the following traits: stomatal conductance, carbon assimilation rate, and seed weight (Lande and Arnold, 1983). The trait variables were standardized within each treatment to a mean of zero and a variance of one (Lande and Arnold, 1983), so the slope of the regression line in a univariate analysis estimates the selection differential, S (Heschel et al., 2002; Heschel and Riginos, 2005). Because the regression coefficient is a covariance divided by a variance, setting the trait variance to one yields simply the covariance between the trait and fitness, i.e., the selection differential. For each drought treatment, separate regression analyses were performed for each trait. For these analyses, relative fitness (individual biomass divided by mean biomass) served as the response variable. The predictor variables were *A, g,* or seed weight, in combination with block, population, and cross-type. Block and population were included to control for differences in fitness between blocks and populations that were due to un-
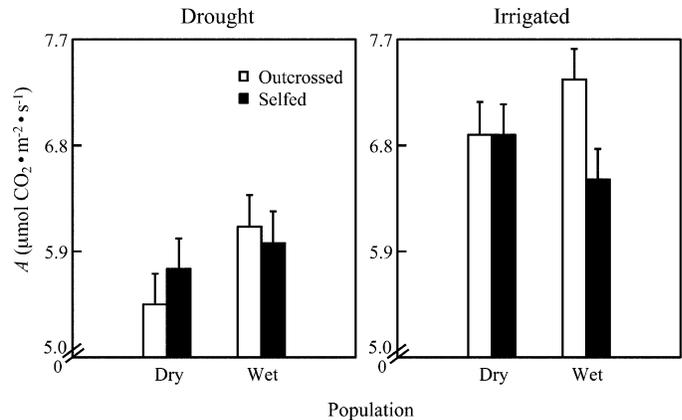

Fig. 1.   Population-dependent inbreeding depression for carbon assimilation rate (*A*) across drought and irrigated conditions. Least-square means ± 1 SE are shown.

measured characters (Heschel et al., 2002). Cross-type was included to control for differences in fitness due to inbreeding depression (Willis, 1996). A cross-type × trait interaction was also included in all models. A significant interaction between cross and trait would indicate that the regression of fitness on a trait depends on whether plants are selfed or outcrossed. No interaction between cross and a trait, but a significant effect of covariate (trait) would indicate there is some relationship between the trait and fitness. Finally, no interaction between cross and a trait, but a significant effect of cross would indicate that the slopes of the relationship between the trait and fitness are the same for selfed and outcrossed plants, but their *y*-intercepts differ. Variation in inbreeding leads to biased (inflated) estimates of selection whenever the first or last cases are operating (Willis, 1996). We also examined the potential for biases in estimates of selection arising from among-population variation in inbreeding history by including population × trait interactions in the analyses. Trait variables were examined for normality and natural-log transformed when necessary. Regression models with quadratic terms were used to estimate stabilizing or disruptive selection.

Selection gradients—Multivariate phenotypic selection analysis (Lande and Arnold, 1983) was used to estimate direct selection on each trait while holding the value of other traits constant and while controlling for population and cross-type effects on fitness. For drought and irrigated conditions, separate multiple regression models were used that included all of the following predictors: block (random), population (fixed), cross-type (fixed), *A, g,* and seed weight. Cross-type × trait and population × trait interactions were included in both the drought and irrigated treatment models to control for the effects of inbreeding and population of origin on a particular trait. Relative fitness, which was calculated from individual biomass within each treatment, was the response variable. Standardized *A, g,* and seed weight values were used in both models.

RESULTS

Effects of drought and inbreeding—Overall, plants in the drought treatment had significantly lower carbon assimilation rates (Fig. 1) and lower biomass (irrigated treatment LSM = 11.35 ± 0.36 g; drought treatment LSM = 8.95 ± 0.37 g) than irrigated plants (Table 1), indicating that the drought treatment imposed a substantial stress. Carbon assimilation and biomass did not have significant main effects of cross-type (Table 1); however, significant inbreeding depression was observed for stomatal conductance (Fig. 2). Drought stress did not exacerbate inbreeding depression because cross-type × treatment interactions were not significant for any trait. The ability to detect inbreeding depression in biomass and gas ex-

TABLE 1.   ANCOVA for population, drought treatment, and cross-type on physiological and morphological traits. A = maximal carbon assimilation rate; g = stomatal conductance. F values are reported.

Treatment	A	g	Biomass
Block [Drought]	2.59*	9.79***	2.86**
Drought	11.05**	2.13	21.64**
Line [Population]	1.54	0.60	0.83
Population	1.78	1.28	4.51*
Cross	0.14	4.74*	0.0040
Drought × Cross	2.14	1.07	0.75
Drought × Population	0.85	1.62	0.92
Cross × Population	3.17+	4.34*	0.24
Drought × Population × Cross	0.22	3.77+	2.34
Drought × Line	1.51	1.32	2.29*
Cross × Line	1.20	1.62	7.05***
Drought × Cross × Line	0.89	0.76	0.73
Seed Weight	10.60***	0.96	1.59

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

Fig. 2.   Population-dependent inbreeding depression for stomatal conductance (g) across drought and irrigated conditions. Least-square means ± 1 SE are shown.

change traits between the drought treatments did not depend on differences in phenotypic variance expression for biomass, A, or g between treatments (biomass: $r^2 = 0.0068$, $N = 18$, $P = 0.75$; A: $r^2 = 0.081$, $N = 18$, $P = 0.25$; g: $r^2 = 0.12$, $N = 18$, $P = 0.16$). Initial seed mass was positively associated with carbon assimilation rate but had no effect on any other variable (Table 1).

Inbreeding effects for physiological traits tended to differ between populations, as indicated by significant or marginally significant cross-type × population interaction effects for stomatal conductance and carbon assimilation, respectively (Table 1). Outcrossed plants from the wet, more outcrossing population had significantly higher overall maximal carbon assimilation rates (LSM contrast, $F = 4.21$, df = 1, $P = 0.04$) and stomatal conductances (LSM contrast, $F = 12.73$, df = 1, $P = 0.0004$) than inbred plants. Also, the differences in g between outcrossed and selfed plants from this population were greater in the irrigated treatment, contrary to the prediction of stress-dependent inbreeding depression (Fig. 2; drought: $F = 0.043$, df = 1, $P = 0.84$; irrigated: $F = 4.97$, df = 1, $P = 0.04$). However, outcrossed and inbred plants from the dry population did not differ significantly in either physiological trait in the irrigated treatment (LSM contrasts: g: $F = 0.10$, df = 1, $P = 0.75$; A: $F = 0.22$, df = 1, $P = 0.64$; Figs. 1,2).

Effects of inbreeding also differed among lines within populations. Lines differed significantly in the direction and magnitude of inbreeding effects on biomass, as indicated by a significant cross-type × line interaction (Table 1, Fig. 3). Some lines exhibited inbreeding depression, whereas others responded little to inbreeding or actually showed evidence of outbreeding depression (Fig. 3). However, no significant line effects or interactions were detected for physiological traits.

Phenotypic selection analysis—Directional (linear) selection favored higher A and lower g under drought conditions (selection differential, S, Table 2), but under irrigated conditions no significant selection on gas exchange traits was detected. No stabilizing or disruptive selection was found when quadratic terms were included in the models (not shown). Selection differentials did not differ significantly between inbred and outcrossed plants (nonsignificant cross by trait interactions, Table 3), suggesting that inbreeding per se did not alter the relationship between physiological traits and fitness in either drought or irrigated conditions. However, ANCOVA de-
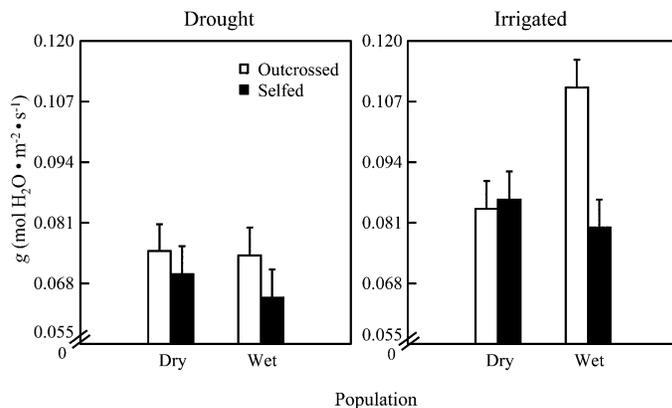
tected marginally significant effects of population and of population by trait interactions in the irrigated treatment, suggesting that population differences could potentially contribute to biased estimates of selection on physiological traits in well-watered conditions (Table 3).

In drought conditions, the selection differential for A was smaller and not significant when population and cross-type were included in the model (Table 2), suggesting that the apparent selection on carbon assimilation under water limitation was actually due to selection on other traits that covaried with population source. Selection on g was identical in magnitude and significance in drought conditions regardless of the inclusion of population and cross-type, indicating that this result was not an artifact of inbreeding or population differences. In irrigated conditions marginally significant selection was detected for decreased stomatal conductance but only after adjustment for effects of population and cross-type (Table 2).
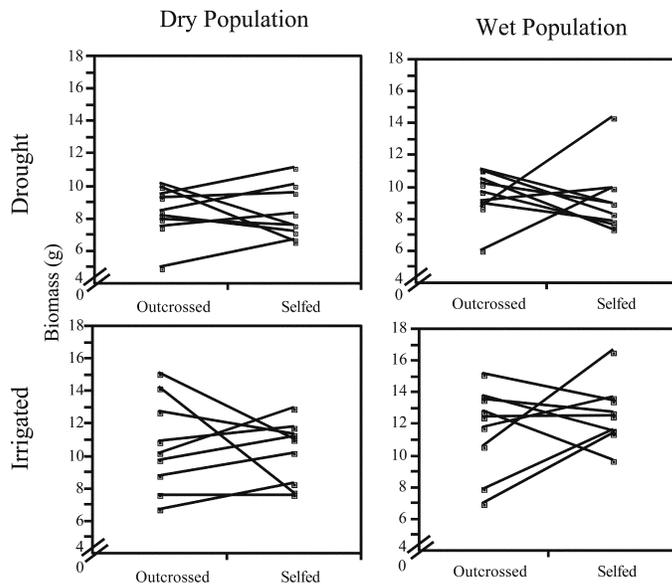

Fig. 3.   Genetic variation for changes in biomass across drought and irrigated conditions for lines from each population (crossing reaction norms). Least-square means of selfed and outcrossed plants from dry and wet population lines are shown.

TABLE 2. Results of selection analyses. Standardized selection differentials (S), standardized differentials adjusted for the effects of population and cross (S_{adj}), and standardized selection gradients (β) are shown for each drought treatment. A = carbon assimilation rate; g = stomatal conductance.

Trait	Drought			Irrigated		
	S	S_{adj}	β	S	S_{adj}	β
A	0.048*	0.027	0.087*	0.022	0.0032	0.020
g	−0.066**	−0.066**	−0.10***	−0.023	−0.037[+]	−0.042[+]
Seed Weight	−0.021	−0.011	−0.019	−0.015	0.033	0.027

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

The marginally significant population interaction for g (Table 3) suggests that failure to account for population of origin in this selection analysis could have biased estimates of selection downward for this trait in benign, irrigated conditions.

A selection gradient analysis including effects of A, g, or seed weight detected direct selection for increased carbon assimilation and decreased stomatal conductance in drought conditions (Table 2). These selection estimates remained significant when effects of population, cross-type, and their interactions were included in the model (Table 4). The effects of population and cross-type were not significant, indicating that differences in fitness were completely explained by differences in physiology under water limitation (Table 4). In irrigated conditions, the selection gradient analysis did not detect selection either on carbon assimilation or on stomatal conductance. When population and cross effects were included in the gradient analysis for irrigated plants, a significant population effect was detected, indicating that unmeasured traits that covaried with population source may have explained differences in fitness, but not A, g, or seed weight. However, the effects of measured traits on fitness depended neither on cross-type nor on population source (nonsignificant cross and population interaction terms, Table 4). Seed weight had no effect on fitness in either treatment.

DISCUSSION

Stress-dependent inbreeding depression may play a large role in determining population persistence by reducing the viability of small populations in stressful habitats (e.g., Heschel and Paige, 1995; Keller and Waller, 2002). It also may help determine the outcome of selection on traits contributing to stress tolerance (e.g., Willis, 1993, 1996). Thus, stress-dependent inbreeding depression may represent an important but relatively understudied factor affecting the evolution of plant stress response. Here, the expression of inbreeding depression depended on environmental context, but contrary to theoretical predictions, inbreeding depression for physiological traits was actually greater in irrigated plants than in drought-stressed plants. The expression of inbreeding depression also differed between populations from different sites of origin. The relationship between gas exchange and fitness depended on drought conditions, but did not differ between inbred and outcrossed plants. Thus, drought stress exerts significant selection on physiological traits in *I. capensis*, and this selection does not appear to be an artifact of the mixed mating system within populations. However, population differences in fitness under irrigated conditions suggest the potential for biased measurements of selection on physiological traits in common garden experiments such as ours.

Stress-dependent inbreeding depression—Exacerbation of inbreeding depression by drought stress has been observed in several plant species (Heschel and Paige, 1995; Hauser and Loeschcke, 1996; Cheptou et al., 2000; but see Nason and Ellstrand, 1995), suggesting that drought may be an important factor in plant conservation and mating system evolution. However, in our study, drought stress did not exacerbate inbreeding depression in *I. capensis*. In fact, inbreeding depression for physiological traits was greater in irrigated plants, suggesting that inbreeding depression for such traits may only be expressed under benign conditions. Similarly, Norman et al. (1995) detected stronger inbreeding depression in *Scheidia lydgatei* under high nutrient conditions than under nutrient stress. Both Waller (1984) and Norman et al. (1995) hypothesized that stress hinders growth to such a degree that differences between inbred and outcrossed progeny are never realized in stressful conditions. That is, trait variance expression in stressful conditions is lower than in benign conditions such that differences between inbred and outcrossed individuals become undetectable in stressful environments. This scenario seems plausible for the data presented here because drought-stressed plants had significantly lower biomass, A, and g than irrigated plants across both populations. However, differences in biomass, A, and g between selfed and outcrossed progeny did not depend upon phenotypic variance expression between environments. Therefore, the opportunity to detect inbreeding depression was probably not entirely due to the variance structure of the data.

TABLE 3. Results of standardized selection differential analyses (S_{adj}), adjusting for effects of inbreeding. For each water treatment ANCOVA is presented for population, cross-type, and physiological/morphological traits on relative fitness. A = carbon assimilation rate; g = stomatal conductance. F values are reported.

Trait	Drought				Irrigated			
	Population	Cross	Population × trait	Cross × trait	Population	Cross	Population × trait	Cross × trait
A	1.53	0.43	0.73	1.33	2.64	0.23	2.93[+]	0.78
g	1.70	1.59	0.24	0.46	4.27[+]	0.058	3.60[+]	1.64
Seed Weight	1.98	0.36	0.72	0.013	3.76[+]	0.25	0.16	1.26

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

TABLE 4. Results of standardized selection gradient analyses (β), adjusting for effects of inbreeding. For each water treatment, the ANCOVA is presented for population, cross-type, and physiological/morphological traits on relative fitness. A = carbon assimilation rate; g = stomatal conductance. F values are reported.

Treatment	Population	Cross	A	g	Seed weight	Population $\times A$	Population $\times g$	Population $\times$ seed weight	Cross $\times A$	Cross $\times g$	Cross $\times$ seed weight
Drought	0.57	1.82	8.76**	14.97***	0.36	2.11	0.91	1.18	0.13	0.62	0.0005
Irrigated	4.86*	0.42	0.71	3.36+	0.96	0.59	2.17	0.12	1.92	1.61	0.74

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

Differences between inbred and outcrossed progeny might have been amplified in the water-limited treatment if there had been asymmetric water competition between inbred and outcrossed plants in combination with drought. For example, in *Drosophila melanogaster*, Miller (1994) found that temperature and lead stress only amplified inbreeding depression when these stresses were combined with inter- or intraspecific competition. Similarly, Cheptou et al. (2000) found that inbreeding depression might only be realized when plants are competing. These authors hypothesized that outcrossed plants might have higher relative growth rates and when in competition, the fitness of inbred plants might be reduced because outcrossed plants ''steal'' resources faster than inbred plants. Because inbreeding depression is magnified by competition in *I. capensis* (Schmitt and Ehrhardt, 1990), the degree of inbreeding depression observed here might have been greater in drought conditions if inbred and outcrossed progeny had been competing for the same resources. Thus, although our experiment did not detect stress-dependent inbreeding depression, the possibility exists that drought could exacerbate inbreeding depression under asymmetric competitive conditions.

Population differences—Expression of inbreeding depression also differed among lines and between populations. For aboveground biomass, significant genetic variation for the direction and degree of inbreeding depression was present within both populations, suggesting the potential for selection to act upon mating system variation. Plants from the wet population site displayed inbreeding depression for A and g under irrigated conditions, but no inbreeding depression was detected for these traits in plants from the dry population site. These population differences in inbreeding depression may be related to the different histories of inbreeding as well as different histories of selection in the two sites. Generally, populations in stressful areas are expected to experience different selection pressures and therefore have different levels of inbreeding depression than populations growing under optimal conditions (Levin, 1984; Hauser and Loeschcke, 1996). Moreover, plants in the dry site produce fewer outcrossing, chasmogamous flowers than plants in the wet site. Thus, the more inbreeding *Impatiens* population from the dry site may have experienced stronger purging of deleterious recessive alleles than the population from the wet site. Alternatively, modifier loci, maintained in the more outcrossing wet population, may have been selected for at the wet site. These loci would have enhanced the expression of adaptive traits, such as gas exchange, in the heterozygous condition and resulted in stress-dependent inbreeding depression for the wet population plants (Otto and Bourguet, 1999). Nonetheless, in another experiment with these lines (C. Riginos, M. S. Heschel, and J. Schmitt, unpublished data), effects of maternal inbreeding were detected for seedling physiological traits in lines from both populations. Thus, the expression of population differences in inbreeding

depression depends upon the developmental stage and trait considered.

Selection analyses—Willis (1996) cautions that inbreeding depression in trait expression may result in biased measures of selection in species with a mixed mating system, such as *I. capensis*. That is, if studies of selection do not account for variation in cross-type as well as inbreeding history, they will observe upwardly biased estimates of selection (e.g., Table 2) because of a decline in both traits and fitness for inbred individuals and inbred populations. We detected little evidence for such a bias in this study. Inbreeding depression was observed for physiological traits, but cross-type per se did not affect the relationship between those traits and fitness. For instance, decreased stomatal conductance to water vapor was favored by selection under drought conditions across selfed and outcrossed plants irrespective of population source. However, in irrigated conditions, the relationship of physiological performance to fitness seemed to depend upon the population of origin, possibly due to differences in inbreeding history between the populations. This suggests that different histories of inbreeding might lead to nonrandom associations between quantitative traits like gas exchange parameters and fitness (Ritland, 1996) and that these associations should depend upon environmental conditions. The effect of such associations should resemble that of linkage disequilibrium between a trait and fitness in altering trait expression patterns (Willis, 1993) and make the efficacy of directional selection dependent on the degree of inbreeding in a mixed-mating population. Here, population differences affected estimates of selection on gas exchange only in benign, irrigated conditions. Estimates of selection were downwardly biased when analyses did not account for population structure; in contrast with the upward bias, we would expect from pooling populations with different levels of genetic load. Thus, our results suggest that the evolutionary trajectories of gas exchange traits within populations will depend primarily upon the selective environment, rather than upon the mating system.

Conclusions—Although drought stress did not exacerbate inbreeding depression, it resulted in strong selection on physiological traits in this experiment. Selection favored increased maximal assimilation rates and decreased stomatal conductance in drought-stressed plants, whereas there was little evidence for selection on gas exchange traits for irrigated plants. A similar pattern of selection was also observed in a field experiment in which plants experienced a late season drought (Heschel et al., 2002). On the other hand, selection actually favored higher stomatal conductance in drought stressed plants, but not in irrigated plants in another field experiment in which plants experienced a severe early season drought (Heschel and Riginos, 2005). Thus, drought is an important selective agent for *I. capensis*, but the nature of selection de-

pends upon seasonal timing. The results of this study suggest that inbreeding depression will not influence responses to that selection.

LITERATURE CITED

ARGYRES, A. Z., AND J. SCHMITT. 1991. Microgeographic genetic structure of morphological and life history traits in a natural population of *Impatiens capensis*. *Evolution* 45: 178–189.

BYERS, D. L., AND D. M. WALLER. 1999. Do plant populations purge their genetic load? Effects of population size and mating history on inbreeding depression. *Annual Review of Ecology and Systematics* 30: 479–513.

CHAPIN III, F. S., K. AUTUMN, AND F. PUGNAIRE. 1993. Evolution of suites of traits in response to environmental stress. *American Naturalist* 142: 78–92.

CHARLESWORTH, D., AND B. CHARLESWORTH. 1987. Inbreeding depression and its evolutionary consequences. *Annual Review of Ecology and Systematics* 18: 237–268.

CHARLESWORTH, D., AND B. CHARLESWORTH. 1999. The genetic basis of inbreeding depression. *Genetical Research* 74: 329–340.

CHEPTOU, P. O., A. BERGER, A. BLANCHARD, C. COLLIN, AND J. ESCARRE. 2000. The effect of drought stress on inbreeding depression in four populations of the Mediterranean outcrossing plant *Crepis sancta* (Asteraceae). *Heredity* 85: 294–302.

COHEN, D. 1970. The expected efficiency of water utilization in plants under different competition and selection regimes. *Israel Journal of Botany* 19: 50–54.

CROW, J. F. 1989. Fitness variation in natural populations. *In* W. G. Hill and T. F. C. Mackay [eds.], Evolution and animal breeding: reviews on molecular and quantitative approaches in honor of Alan Robertson, 91–97. CABI, New York, New York, USA.

DONOHUE, K. 1998. Effects of inbreeding on traits that influence dispersal and progeny density in *Cakile edentula* var. *lacustris* (Brassicaceae). *American Journal of Botany* 85: 661–668.

DONOHUE, K., AND J. SCHMITT. 1999. The genetic architecture of plasticity to density in *Impatiens capensis*. *Evolution* 53: 1377–1386.

DONOHUE, K., D. MESSIQUA, E. HAMMOND PYLE, M. S. HESCHEL, AND J. SCHMITT. 2000a. Evidence of adaptive divergence in plasticity: density- and site-dependent selection on shade-avoidance responses in *Impatiens capensis*. *Evolution* 54: 1956–1968.

DONOHUE, K., D. MESSIQUA, E. HAMMOND PYLE, M. S. HESCHEL, AND J. SCHMITT. 2000b. Density-dependence and population differentiation of genetic architecture in *Impatiens capensis* in natural environments. *Evolution* 54: 1969–1981.

DUDASH, M. R. 1990. Relative fitness of selfed and outcrossed progeny in a self-compatible, protandrous species, *Sabatia angularis* L. (Gentianaceae)—a comparison in three environments. *Evolution* 44: 1129–1139.

DUDLEY, S. A. 1996. Differing selection on plant physiological traits in response to environmental water availability: a test of adaptive hypotheses. *Evolution* 50: 92–102.

DUDLEY, S. A., AND J. SCHMITT. 1996. Testing the adaptive plasticity hypothesis: density-dependent selection on manipulated stem length in *Impatiens capensis*. *American Naturalist* 147: 445–465.

FALCONER, D. S., AND T. F. C. MACKAY. 1996. Introduction to quantitative genetics. Longman, Essex, UK.

FARRIS, M. A., AND M. J. LECHOWICZ. 1990. Functional interactions among traits that determine reproductive success in a native annual plant. *Ecology* 71: 548–557.

FISHMAN, L. 2001. Inbreeding depression in two populations of *Arenaria uniflora* (Carophyllaceae) with contrasting mating systems. *Heredity* 86: 184–194.

FRANKHAM, R., AND K. RALLS. 1998. Inbreeding leads to extinction. *Nature* 392: 441–442.

GEBER, M. A., AND T. E. DAWSON. 1997. Genetic variation in stomatal and biochemical limitations to photosynthesis in the annual plant, *Polygonum arenastrum*. *Oecologia* 109: 535–546.

HAUSER, T. P., AND V. LOESCHCKE. 1996. Drought stress and inbreeding depression in *Lychnis flos-cuculi* (Caryophyllaceae). *Evolution* 50: 1119–1126.

HESCHEL, M. S. 2000. The evolution of drought tolerance in *Impatiens capensis*. Ph.D. dissertation, Brown University, Providence, Rhode Island, USA.

HESCHEL, M. S., AND N. J. HAUSMANN. 2001. Population differentiation for abscisic acid sensitivity in response to drought in *Impatiens capensis* (Balsaminaceae). *International Journal of Plant Sciences* 162: 1253–1260.

HESCHEL, M. S., AND K. N. PAIGE. 1995. Inbreeding depression, environmental stress, and population size variation in scarlet gilia (*Ipomopsis aggregata*). *Conservation Biology* 9: 126–133.

HESCHEL, M. S., AND C. RIGINOS. 2005. Mechanisms of selection for drought stress tolerance and avoidance in *Impatiens capensis* (Balsaminaceae). *American Journal of Botany* 92: 37–44.

HESCHEL, M. S., K. DONOHUE, N. J. HAUSMANN, AND J. SCHMITT. 2002. Population differentiation and natural selection for water-use efficiency in *Impatiens capensis* (Balsaminaceae). *International Journal of Plant Sciences* 163: 907–912.

HESCHEL, M. S., J. STINCHCOMBE, K. E. HOLSINGER, AND J. SCHMITT. 2004. Natural selection on light response curve parameters in the herbaceous annual, *Impatiens capensis*. *Oecologia* 139: 487–494.

KELLER, L. F., AND D. M. WALLER. 2002. Inbreeding effects in wild populations. *Trends in Ecology and Evolution* 17: 230–241.

KELLY, J. K. 1997. Fitness variation across a subdivided population of the annual plant *Impatiens capensis*. *Evolution* 51: 1100–1111.

KNIGHT, S. E., AND D. M. WALLER. 1987. Genetic consequences of outcrossing in the cleistogamous annual, *Impatiens capensis*. I. Population-genetic structure. *Evolution* 41: 969–978.

LANDE, R., AND S. J. ARNOLD. 1983. The measurement of selection on correlated characters. *Evolution* 37: 1210–1226.

LECK, M. A. 1996. Germination of macrophytes from a Delaware River tidal freshwater wetland. *Bulletin of the Torrey Botanical Club* 123: 48–67.

LEVIN, D. A. 1984. Inbreeding depression and proximity-dependent crossing success in *Phlox drummondii*. *Evolution* 38: 116–127.

MCCALL, C., D. M. WALLER, AND T. MITCHELL-OLDS. 1994. Effects of serial inbreeding on fitness components in *Impatiens capensis*. *Evolution* 48: 818–827.

MILLER, P. S. 1994. Is inbreeding depression more severe in a stressful environment? *Zoo Biology* 13: 195–208.

NASON, J. D., AND N. C. ELLSTRAND. 1995. Lifetime estimates of biparental inbreeding depression in the self-compatible annual plant *Raphanus sativus*. *Evolution* 49: 307–316.

NORMAN, J. K., A. K. SAKAI, S. G. WELLER, AND T. E. DAWSON. 1995. Inbreeding depression in morphological and physiological traits of *Schieda lydgatei* (Caryophyllaceae) in two environments. *Evolution* 49: 297–306.

OTTO, S. P., AND D. BOURGUET. 1999. Balanced polymorphisms and the evolution of dominance. *American Naturalist* 153: 561–574.

PAIGE, K. N., AND M. S. HESCHEL. 1996. Inbreeding depression in scarlet gilia: a reply to Ouborg and Van Groenendael. *Conservation Biology* 10: 1292–1294.

PARKINSON, K. J. 1985. A simple method for determining the boundary layer resistance in leaf cuvettes. *Plant, Cell & Environment* 8: 223–226.

PEDERSON, D. G. 1968. Environmental stress, heterozygote advantage and genotype-environment interaction in *Arabidopsis*. *Heredity* 23: 127–138.

PRAY, L. A., J. M. SCHWARTZ, C. J. GOODNIGHT, AND L. STEVENS. 1994. Environmental dependency of inbreeding depression: implications for conservation biology. *Conservation Biology* 8: 562–568.

RITLAND, K. 1996. Inferring the genetic basis of inbreeding depression in plants. *Genome* 39: 1–8.

SCHEMSKE, D. W. 1978. Evolution of reproductive characteristics in *Impatiens* (Balsaminaceae): the significance of cleistogamy and chasmogamy. *Ecology* 59: 596–613.

SCHEMSKE, D. W. 1984. Population structure and local selection in *Impatiens pallida* (Balsaminaceae), a selfing annual. *Evolution* 38: 817–832.

SCHMITT, J. 1993. Reaction norms of morphological and life-history traits to light availability in *Impatiens capensis*. *Evolution* 47: 1654–1668.

SCHMITT, J., AND D. W. EHRHARDT. 1990. Enhancement of inbreeding depression by dominance and suppression in *Impatiens capensis*. *Evolution* 44: 269–278.

SCHMITT, J., AND S. E. GAMBLE. 1990. The effect of distance from the parental site on offspring performance and inbreeding depression in *Impatiens capensis*: a test of the local adaptation hypothesis. *Evolution* 44: 2022–2030.

SCHMITT, J., D. W. EHRHARDT, AND D. SWARTZ. 1985. Differential dispersal of self-fertilized and outcrossed progeny in jewelweed (*Impatiens capensis*). *American Naturalist* 126: 570–575.

SCHOEN, D. J., AND R. G. LATTA. 1989. Spatial autocorrelation of genotypes

in populations of *Impatiens pallida* and *Impatiens capensis*. *Heredity* 63: 181–189.

SCHOEN, D. J., S. C. STEWART, M. J. LECHOWICZ, AND G. BELL. 1986. Partitioning the transplant site effect in reciprocal transplant experiments with *Impatiens capensis* and *Impatiens pallida*. *Oecologia* 70: 149–154.

SCHULZ, K. E., M. SMITH, AND Y. WU. 1993. Gas exchange of *Impatiens pallida* Nutt. (Balsaminaceae) in relation to wilting under high light. *American Journal of Botany* 80: 361–368.

SMITH, M., Y. WU, AND O. GREEN. 1993. Effect of light and water-stress on photosynthesis and biomass production in *Boltonia decurrens* (Asteraceae), a threatened species. *American Journal of Botany* 80: 859–864.

WADE, M. J., AND S. KALISZ. 1990. The causes of natural selection. *Evolution* 44: 1947–1955.

WALLER, D. M. 1979. The relative cost of self- and cross-fertilized seeds in *Impatiens capensis* (Balsaminaceae). *American Journal of Botany* 66: 313–320.

WALLER, D. M. 1980. Environmental determinants of outcrossing in *Impatiens capensis* (Balsaminaceae). *Evolution* 34: 747–761.

WALLER, D. M. 1984. Differences in fitness between seedlings derived from cleistogamous and chasmogamous flowers in *Impatiens capensis*. *Evolution* 38: 427–440.

WILLIS, J. H. 1993. Effects of different levels of inbreeding on fitness components in *Mimulus guttatus*. *Evolution* 47: 864–876.

WILLIS, J. H. 1996. Measures of phenotypic selection are biased by partial inbreeding. *Evolution* 50: 1501–1511.