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Natural selection on light response curve parameters in the herbaceous annual, *Impatiens capensis*

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Abstract We tested for genetic variation in light response curves and their acclimation to sun versus shade in recombinant inbred lines (RILs) of the annual species *Impatiens capensis* derived from a cross between sun and shade populations. We exposed replicates of 49 RILs to experimentally manipulated light levels (open versus shade) in a greenhouse and measured photosynthetic light response curves, height, biomass, and reproduction. Plants were taller in the shade treatment, but we were unable to detect differences between light treatments (i.e., acclimation) in the maximal rate of photosynthesis, the light compensation point, or the quantum efficiency of photosynthesis. Genotypic selection analyses indicated that higher maximal rates of carbon assimilation and higher light compensation points (typical of sun-acclimated light curves) were favored by natural selection in both light treatments. Thus, it appears that the pattern of selection on photosynthetic parameters may not depend on light environment in this species.

Keywords Photosynthetic acclimation responses · Sun–shade ecotypes · *Impatiens capensis* · Natural selection

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Introduction

The distribution and abundance of plant species is strongly influenced by physiological tolerances (Field 1988; Mooney 1991; Hacker and Bertness 1995). As a result, the range of light environments that a particular species can inhabit is likely to depend on the extent to which it can acclimate to new light levels by changing its photosynthetic response. The ability of plants to acclimate to shade environments by changing their daily photosynthetic response curves—their light response curves—is often taken as a central tenet of plant physiological ecology. In particular, the light response curves of sun and shade-acclimated phenotypes typically differ in the following ways (Lambers et al. 1998):

1. Sun leaves fix greater amounts of carbon at high light levels than shade leaves, i.e., the maximal rate of photosynthesis ($A_{\max}$) for sun leaves is greater than for shade leaves (Pearcy and Sims 1994).
2. High maximal rates of photosynthesis are associated with high rates of dark respiration (R), i.e., $A_{\max}$ is approximately proportional to R (Hirose and Werger 1987; Field 1988; Givnish 1988; Larcher 1995; Davies 1998).
3. Carbon assimilation often increases more slowly with increasing light levels in sun leaves than in shade leaves, i.e., the quantum efficiency of photosynthesis (A_{qe}) may be lower in sun leaves than in shade leaves (Larcher 1995).
4. Sun leaves need more light to reach a positive carbon balance than do shade leaves, i.e., the light compensation point (L_{CP}) is greater for sun leaves than for shade leaves (Clough et al. 1981; Davies 1998).

While many studies have documented differences in light curve physiology in sun and shade environments similar to those listed above (e.g., Chazdon and Pearcy 1986), other studies have failed to detect such differences in sun–shade physiology (e.g., Baskauf and Eickmeier 1994). Part of the reason is likely to be that whole-plant factors other than leaf-level physiology, like plant architecture and biomass allocation, play a large role in adaptation to different light environments (Givnish 1988).

It may also be that life-history characteristics have an important influence on mechanisms of adaptation to different light environments (Arntz and Delph 2001). Studies showing marked sun–shade responses have typically involved longer lived perennial species (e.g., Ehleringer 1978; Chazdon and Pearcy 1986; Pearcy and Sims 1994; Buchmann et al. 1996; Davies 1998). There are, however, relatively few light curve studies with herbaceous annual (e.g. Mooney et al. 1981) or short-lived perennial (e.g. Mulkey et al. 1991) species. Generally, natural selection may favor different responses in annuals and perennials. On the one hand, in longer lived perennials, natural selection may favor genotypes that are able to acclimate leaf-level photosynthetic responses to the light environment prevalent during their development. On the other hand, in annuals and short-lived perennials, which have to complete their growth and reproduction quickly in a given season, natural selection may favor relatively high maximal rates of photosynthesis in all light environments (Mulkey et al. 1991).

While studies have confirmed the relationships between $A_{\max}$, A_{qe}, L_{CP}, and carbon fixation, relatively few studies have examined the relationship between the physiological parameters involved in sun–shade acclimation and reproductive fitness (Farris and Lechowicz 1990; Dudley 1996; Arntz and Delph 2001; Heschel et al. 2002). Moreover, few studies have tested for within-population genetic variation in sun–shade physiology (but see Teramura and Strain 1979; Scheiner et al. 1984; Garbutt 1986; Geber and Dawson 1990; Sultan and Bazzaz 1993; Sims and Kelly 1998). Here we test the adaptive significance of light curve parameters in shaded and open environments by measuring selection on photosynthetic parameters in recombinant inbred lines of *Impatiens capensis*, created from crosses between inbred lines derived from open and shaded populations.

Materials and methods

Study species and quantitative genetic design

Impatiens capensis Meerb. (Balsaminaceae), is an annual, self-compatible herb of North American deciduous forests and wetlands and is an excellent model for studying the adaptive nature of light responses. Populations occur naturally under a wide range of light conditions, and earlier studies have documented substantial within and among population variation in many morphological, life-history, and physiological straits (Schemske 1984; Waller 1984; Schoen et al. 1986; Schoen and Latta 1989; Argyres and Schmitt 1991; Schmitt 1993; Bennington and McGraw 1995; Dudley and Schmitt 1996; Paoletti and Holsinger 1999; Donohue et al. 2000, 2001; Heschel and Hausmann 2001; Heschel et al. 2002). In natural populations, seedlings typically emerge early in spring under high light conditions, and grow rapidly prior to canopy closure. Because *I. capensis* does not close its stomata at mid-day (Schulz et al. 1993), gas exchange measurements can be made from 10 a.m. until 4 p.m. Most importantly, the populations from which the inbred lines used here are derived have already been shown to be locally adapted to different light conditions (Schmitt 1993; Dudley and Schmitt 1996; Donohue et al. 2000).

In 1996, 50 inbred lines were established from seedlings collected from open- and woodland populations in Brown University's Haffenreffer Reserve (Bristol, R.I., USA). These lines were maintained for at least eight generations of single-seed descent through self-fertilized seed (derived from cleistogamous flowers). As a result, less than 0.5% of heterozygous loci in the initial parents are heterozygous within individuals of these lines and all homozygotes within a line carry the same allele. In 1998, a cross between a single inbred line from the woodland population and a single inbred line from the open population produced about 100 F1 progeny that have been propagated by single-seed descent through self-fertilized seed for at least six generations to create recombinant inbred lines. The genetic structure of such lines is analogous to that of many natural *Impatiens* populations in which rare outcrossing events may be followed by several generations of self-fertilization (Paoletti and Holsinger 1999).

Experimental design

As part of a larger experiment on the evolution of quantitative genetic traits that are continuous functions of environmental variables (e.g., Kirkpatrick et al. 1990; Kingsolver et al. 2001; J. R. Stinchcombe, unpublished data), two replicates of 49 of these recombinant inbred lines were grown under neutral shade conditions (mean of 173 μmol m^{-2} s^{-1} during mid-day) and high-light or "sun" conditions (mean of 778 μmol m^{-2} s^{-1} during mid-day) in the Brown University greenhouse. The shade treatment represents a 78% reduction in available photosynthetically active radiance (PAR); these light conditions are within the range of natural levels at the shaded and open sites (Heschel and Hausmann 2001), and thus represent ecologically relevant alternative light environments. Seeds from these 49 recombinant inbred lines had been collected and stratified in distilled water in microtiter trays at 4°C for 4 months prior to planting. In the greenhouse, seeds were initially planted into pine cells or "cone-tainers" containing MetroMix 360 (Scotts-Sierra Horticultural, Marysville, Ohio, USA) during the last week of April 2002. The pine cells were randomly distributed on a greenhouse bench, top-watered until saturated, and kept consistently moist. After seedling establishment under high-light conditions (approximately 1 week of growth), replicates of the 49 lines were moved onto two greenhouse benches or blocks. On each bench plants were randomly positioned in both high- and low-light treatments (i.e., a split-plot design); one replicate for each line was positioned within each treatment. Planting density was approximately 144 plants per 1 m^2 in a 10×5 array with adjacent plants separated by approximately 11.5 cm. Blocks were surrounded by aluminum foil to prevent incident light from affecting treatments.

After seedling establishment, all plants were watered daily using Ebb-Flo flood irrigation to ensure that soil moisture levels were the same in both light treatments and that plants did not suffer from water stress (Heschel and Hausmann 2001). Plants were fertilized bi-weekly with Peter's N/P/K (Scotts-Sierra Horticultural, Marysville, Ohio, USA). Photosynthetic light curves were determined for all the lines during the second and third weeks of June (see below). During the last week of June plants were harvested: final height, reproductive fitness, and aboveground biomass were measured for all individuals. For each plant, reproductive fitness was estimated as the total number of flowers, fruits, and pedicels (Donohue et al. 2000). The duration of the experiment approximated a short growing season for *I. capensis*; short seasons can occur in natural populations because of unpredictable summer droughts (M.S. Heschel, unpublished data). Biomass was determined after drying aboveground parts of plants at 65°C for approximately 48 h. Plant height and dry aboveground biomass were measured to evaluate the effectiveness of the sun–shade treatments (Schmitt 1993).

Light curves were determined for 1-month-old replicates of all lines across both light treatments with an ADC LCA3 infrared gas analyzer (IRGA). Two replicates were measured per line per treatment, with each replicate taken from a different greenhouse bench. IRGA measurements were taken over the course of 10 days, with all the bench 1 replicates being measured during the first 5 days, so that day covaried with bench; on a given day, measurements were taken on high- and low-light treatment plants in a staggered fashion such that light treatment did not covary with time of day.

Gas exchange was measured on the most recent fully expanded leaf of each replicate at six light levels (0.32, 19.5, 70, 245, 625, and 1,068 μmol m^{-2} s^{-1}). All IRGA measurements were taken within a shade enclosure to ensure that the six light levels were consistent between line replicates; a halogen lamp provided PAR light that was heat-filtered through water. Differing light levels were obtained by shading the IRGA cuvette with varying numbers of nylon screens; gas exchange measurements were always started at 0.32 μmol m^{-2} s^{-1} (the most nylon screens) and progressed to 1,068 μmol m^{-2} s^{-1} (the fewest nylon screens). Screens were removed in succession to assess carbon assimilation rate at each of the six light levels, and were changed after gas exchange readings had stabilized (typically about 1 min) and been recorded. Over the 10 days in which IRGA measurements were made ambient CO_2 levels ranged from 360 to 380 ppm and ambient relative humidity ranged from 60 to 75% in the greenhouse. On a given measurement day, ambient CO_2 varied by 5–7 ppm and RH varied by 10%. For a given set of six light curve measurements, ambient CO_2 varied by only 3–4 ppm, RH varied by about 5%, and time of individual measurement did not significantly predict assimilation rate (M.S. Heschel and J.R. Stinchcombe, unpublished data). Leaf temperatures did not vary among lines (M.S. Heschel and J.R. Stinchcombe, unpublished data). 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 (PLC), individual leaf areas were calculated and gas exchange values were adjusted for each measurement.

Statistical analysis

Sun–shade treatment effectiveness

Prior to analyzing the carbon assimilation data, we determined whether the high- and low-light treatments were effective in producing plants with differing morphological phenotypes. We examined treatment differences in height and biomass with a split-plot analysis in Proc Mixed of SAS, using final plant height and dry biomass as response variables in successive analyses. Independent variables included bench and treatment (sun vs shade) as fixed effects, and line, line × treatment, and bench × treatment as random effects. Denominator degrees of freedom for hypothesis testing were determined by Satterthwaite approximation. For these and all subsequent analyses, bench was used as a predictor to control for microenvironmental effects in the greenhouse. Bench × treatment was included as a random effect to ensure the proper denominator for the treatment *F*-test in a split-plot design (Littell et al. 1996).

Curve-fitting analysis of carbon assimilation

To fit carbon assimilation rates with light curves, three model types are currently in use by physiologists: a non-rectangular hyperbola (Thornley 1976; Farquhar and Sharkey 1982), a modified Michaelis–Menten function (Marshall and Biscoe 1980; Farquhar and Sharkey 1982), and the Mitscherlich function (Potvin et al. 1990). The Mitscherlich function,($A = A_{\max}[1 - e^{-A_{qe}(PPF - L_{CP})}]$ where PPF = photosynthetic photon flux), is easiest to fit to observed responses and often fits the data better than the quadratic models, and as such is becoming the accepted means of light curve fitting in physiological ecology (Peek et al. 2002). The results presented here are based on fitting the Mitscherlich function with the NLIN procedure in the SAS statistical package (Potvin et al. 1990; Peek et al. 2002). Initial seed estimates for parameter estimation were calculated by the methods described by Potvin et al. (1990).

After fitting a Mitscherlich function for each plant, the three light curve parameters ($A_{\max}$, A_{qe}, L_{cp}) were recorded and analyzed as response variables in mixed-model, split-plot ANOVAs using Proc Mixed in SAS. In these analyses, bench and treatment were included as fixed effects, and line, line × treatment, and bench × treatment terms were included as random effects; denominator degrees of freedom for hypothesis testing were determined by Satterthwaite approximation. To test the significance of the line and line × treatment random effects, we compared the −2 log likelihoods of full models containing all terms and reduced models with only the random effect of interest removed. The difference between −2 log likelihoods is approximately chi-square distributed, with degrees of freedom equal to the difference in model dimension. In our analyses all models compared differed by only one parameter, so the chi-square had one degree of freedom. Because the hypothesis being tested was directional (i.e., whether a genetic variance was significantly *greater* than zero), the *P*-values from these chi-square significance tests were divided by two (Littell et al. 1996, p 44).

Repeated measures analysis of carbon assimilation

As a complement to the curve fitting approach, we also analyzed the raw carbon assimilation data with repeated measures ANOVA (RM-ANOVA). Each IRGA light level was treated as a time point by using the “REPEATED” option of Proc Mixed. The results of RM-ANOVA can be sensitive to the assumed variance–covariance structure of the data (Potvin et al. 1990)—i.e., whether the correlation between two measures taken close in time (or at similar light levels) is equal to the correlation between two measures taken further apart. One approach to addressing this sensitivity is to test whether the data satisfy the assumptions of compound symmetry or sphericity (Potvin et al. 1990). An alternative approach, which we used here, is to model the variance–covariance structure of the data, and then perform RM-ANOVA in the context of the variance–covariance structure that provides the best fit to the data. We compared the fit of four types of covariance structures (compound symmetric, first-order autoregressive, spatial power, and unstructured), which make different assumptions about how variances and covariances change across measurement levels (for more details see Littell et al. 1996, pp 94–102; Littell et al. 1998). We selected the covariance structure that best fit the data by comparing Akaike information criteria (AIC) scores. The AIC is a likelihood-based criterion that imposes a penalty for the number of parameters examined—thus the model with the best AIC score provides the best fit to the data, after applying a complexity penalty related to the number of parameters. Preliminary analyses suggested an unstructured variance–covariance matrix provided the best fit to the data, and results of RM-ANOVAs based upon this assumption are reported. Although the RM-ANOVA approach does not provide a direct test of light treatment differences in specific physiological parameters (i.e., A_{qe}, $A_{\max}$, and L_{CP}), it has the advantage of utilizing fewer statistical tests to examine the same data set. Therefore, the results of both the curve-fitting and RM-ANOVA analyses are reported (e.g., Peek et al. 2002).

Selection analyses

The relationships between total reproductive fitness and the light curve parameters were examined across the light treatments with selection analyses (Lande and Arnold 1983). Multiple regression (PROC GLM) of relative fitness on all light curve parameters was used to estimate selection gradients; all traits were standardized to a mean of zero and a standard deviation of one, and relative fitness was calculated with respect to the mean number of flowers, fruits, and pedicels across both light treatments. To reduce the influence of microenvironmental/bench-related variation in determining the relationship between traits and fitness, genotypic (line) trait means were used for selection analyses (Rausher 1992; Stinchcombe et al. 2002). Selection gradients were estimated separately by treatment. Quadratic terms were initially included in all models to test for nonlinear selection, but no significant stabilizing/disruptive selection was detected, so these terms were dropped from the regression models.

To determine if the pattern of natural selection on light curve parameters differed between treatments, we used ANCOVA. The ANCOVA included relative fitness as the response variable, and the following independent variables: $A_{\max}$, A_{qe}, L_{CP}, Treatment, $A_{\max}$ × Treatment, A_{qe} × Treatment, and L_{CP} × Treatment. We interpreted any significant interaction terms as evidence that the pattern of natural selection on that trait differed between treatments (e.g., Stinchcombe and Rausher 2001).

Results

Sun–shade treatment effectiveness

Plants in the shade treatment were significantly taller than plants in full sun (Table 1; high light mean ± 1 SE = 15.4 ±0.87 cm; low light mean ± 1 SE = 24.93±0.88 cm). There were no significant effects of sun–shade treatments on dry biomass (Table 1; high light mean ± 1 SE = 0.21±0.01 g; low light mean ± 1 SE = 0.20±0.01 g). These data indicate that our sun and shade treatments were effective at producing different morphological phenotypes: the shade plants were taller and spindlier than the sun plants (i.e., less mass per unit height), suggesting a shade avoidance strategy on the part of plants in the shade treatment.

Curve-fitting analysis of carbon assimilation

The analysis of the Mitscherlich function parameters indicated significant differences in the estimated light compensation points for plants grown on adjacent greenhouse benches (Table 1), suggesting microenvironmental differences in greenhouse conditions. Although the sun–shade treatments produced differing phenotypes in terms of height and biomass, we failed to document any significant effects of the sun–shade treatment on estimated light curve parameters (Table 1; Fig. 1). Moreover, at 1,068 μmol m^{-2} s^{-1}, we detected only minimal differences in stomatal conductance values between the sun–shade treatments (means ± 1 SE, sun treatment: 0.47±0.06 mol H_2O m^{-2} s^{-1}; shade treatment: 0.40±0.06 mol H_2O m^{-2} s^{-1}). These relatively high stomatal conductances suggest that plants were not water-stressed in either light treatment (Heschel et al. 2002). The 49 RILs used in the experiment exhibited significant genetic variation in final plant height and dry biomass, and marginally significant genetic variation in maximum photosynthetic rate ($A_{\max}$). Since $A_{\max}$ is a fitted parameter that is itself estimated with some error, the fact that we were able to detect marginal evidence of significant genetic variation suggests that our RIL population was in fact genetically variable for $A_{\max}$ but that we would have needed to include more lines or more replicates within each line-treatment combination to detect significant genetic variation at the α = 0.05 level. In addition, previous studies of inbred lines derived from open and shade populations (the source of the RILs used here) have detected significant genetic variation within the populations for maximal carbon assimilation rate (M.S. Heschel, unpublished data), suggesting that this trait should be genetically variable for the lines used here.

Interestingly, light response curves for some lines showed an acclimation response in which carbon assimilation rates were uniformly higher for individuals grown in one light environment rather than another (Fig. 2). For example, in line 42 net carbon assimilation rates were higher in the low-light treatment than the high-light treatment at higher light levels (Fig. 2). In contrast, for line 37, high- versus low-light treatment differences were in the direction predicted by theory (Fig. 2). Despite these

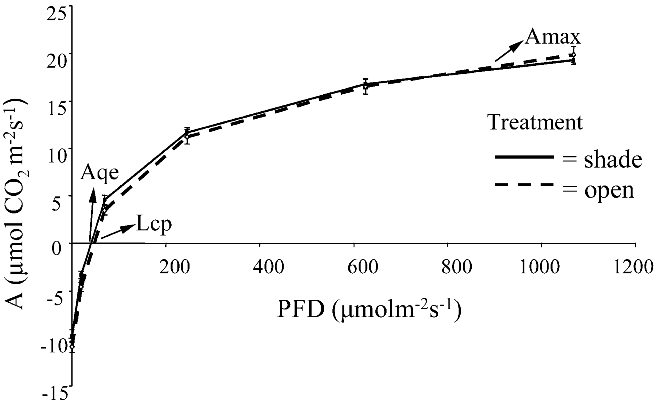

Fig. 1 Mean carbon assimilation rate (*A*) values at each measured light level. Least-square means ± 1 SE are shown. *PFD* photon flux density

Table 1 Analysis of variance for line and light treatment on physiological light curve parameters and morphological traits. *F*-statistics and *P*-values for fixed effects are reported from mixed-model, split-plot ANOVAs calculated by maximum likelihood with Proc Mixed. Likelihood ratio test statistics and *P*-values are reported for random effects. (A_{qe} quantum efficiency of photosynthesis, L_{CP} light compensation point, $A_{\max}$ maximal rate of photosynthesis)

	Bench	Sun–shade treatment	Line	Line × treatment
Final height	$F_{1,0.99}$=4.10, P=0.29	$F_{1,1.2}$ = 73.99, P=0.05	LLR = 30.2, P<0.0001	LLR = 1.2, P=0.14
Dry biomass	$F_{1,110}$=24.80, P<0.001	$F_{1,111}$=2.26, P=0.14	LLR = 61.2, P<0.0001	LLR = 0, P>0.99
A_{qe}	$F_{1,0.99}$=8.73, P=0.21	$F_{1,1.04}$=0.6, P=0.58	LLR = 1.4, P=0.12	LLR = 0, P>0.99
$A_{\max}$	$F_{1,\ 121}$=2.39, P=0.13	$F_{1,124}$=0.62, P=0.43	LLR = 1.8, P=0.09	LLR = 0, P >0.99
L_{CP}	$F_{1,\ 95.5}$=4.08, P=0.05	$F_{1,\ 51.6}$=2.54, P=0.12	LLR = 0.7, P=0.20	LLR = 0.3, P=0.29

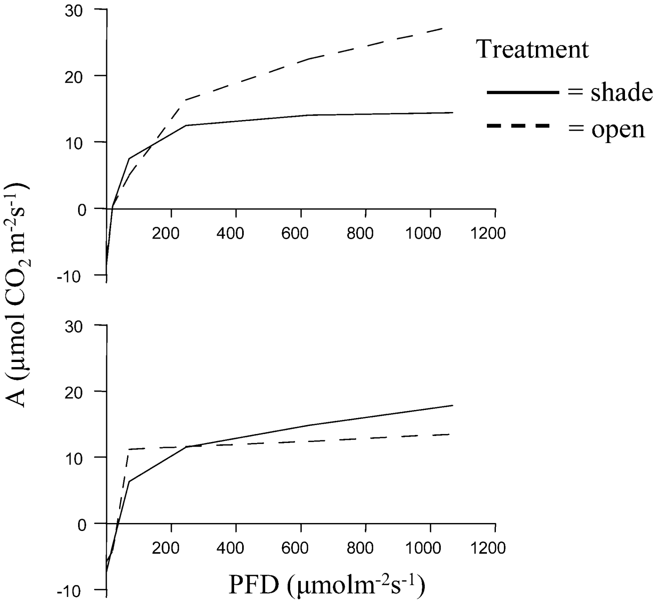

Fig. 2 Light response curves for recombinant inbred lines 37 (*top panel*) and 42 (*bottom panel*) grown in low- and high-light environments. *PFD* photon flux density

intriguing examples, we failed to detect a significant line × treatment interaction for any light curve parameter.

Repeated measures analysis of carbon assimilation

Results from the RM-ANOVA were generally in concordance with the curve-fitting approach (Table 2). For example, with RM-ANOVA we failed to detect significant sun–shade treatment effects or treatment × light level interactions. The absence of significant treatment or treatment × light level interactions suggests that there was no difference in either the mean carbon assimilation values across the sun–shade treatments or in the shape of the light curves between the sun–shade treatments. These results are supported by our failure to detect significant treatment effects on fitted light curve parameters (see above): light curve parameters that do not differ between treatments will describe light curves of the same elevation and general shape. The significant bench effect detected with RM-ANOVA (Table 2) illustrates the benefit of curve fitting as a complement to RM-ANOVA: while the significant bench term in the RM-ANOVA indicated significant differences in carbon assimilation across greenhouse benches, it was possible neither to determine which specific aspect of the light curve differed between benches nor to assess whether there was any biological significance to this effect. The analysis of the Mitscherlich function parameters suggests that this significant bench effect was most likely due to significant differences in the light compensation point (and possibly $A_{\max}$) between different greenhouse benches. In general, the agreement between the RM-ANOVA and curve-fitting analyses indicates that conclusions based upon fitting the raw assimilation data to the Mitscherlich function were not simply due to the arbitrary choice of light-curve function.

Selection analyses

In the separate analyses by treatment, A_{max} was significantly and positively related to reproductive fitness only in the low light treatment (Table 3). In the ANCOVA for relative fitness (Table 4), which is a more powerful analysis (note the difference in error df between Tables 3 and 4), we detected significant effects of $A_{\max}$ on relative fitness regardless of light treatment. These data suggest that in the experiment as a whole, $A_{\max}$ was significantly related to relative fitness; the separate analyses by treatment suggest that the direction of this effect was positive. In the ANCOVA we also detected marginally significant effects of L_{CP} on relative fitness ($P = 0.06$; Table 4); the coefficients from the separate analyses by treatment also suggest that this relationship was positive, with higher L_{CP} values associated with increased relative fitness. Variation in A_{qe} was not associated with relative fitness. The significant treatment effect in Table 4 indicates that once physiological parameters are accounted for, mean relative fitness was higher in the shaded treatment (low light mean ± 1 SE = 1.70±0.32; high light mean ± 1 SE = 0.42±0.33). Nonetheless, despite significant effects of $A_{\max}$ and treatment on relative fitness, we failed to detect significant differences in selection gradients between the high- and low-light treatments (non-significant light curve parameter × treatment interactions; Table 4). These results suggest that, contrary to expectation, selection may not favor differing light response curves in sun versus shade habitats in *I. capensis*.

Although plants in the low light treatment did not experience light levels similar to those at which $A_{\max}$ was estimated, carbon assimilation was positively correlated across adjacent light levels. In other words, lines with higher estimated values for $A_{\max}$ also had higher carbon assimilation rates at the 70, 245, 625, and 1,068 µmol m^{-2} s^{-1} light levels (M.S. Heschel and J.R. Stinchcombe, unpublished data). Thus, the observed selection to increase $A_{\max}$ may reflect correlated selection to increase carbon assimilation under lower light levels.

Table 2 Repeated measures analysis of variance for raw carbon assimilation values, based upon an unstructured variance–covariance matrix

Source	Numerator *df*	Denominator *df*	*F*	*P>F*
Bench	1	118	16.78	<0.0001
Sun/shade treatment	1	123	1.07	0.30
IRGA light levels	5	158	418.36	<0.0001
IRGA light levels × treatment	5	158	1.07	0.38

Table 3 Selection Gradients for light curve parameters in sun and shade treatments. (A_{qe} quantum efficiency of photosynthesis, L_{CP} light compensation point, A_{max} maximal rate of photosynthesis. Error degrees of freedom was 40 for the sun treatment and 43 for the shade treatment)

Trait	β (SE)	*P*
High light		
A_{qe}	0.54(0.41)	0.20
L_{CP}	0.39 (0.36)	0.28
A_{max}	0.34 (0.30)	0.26
Low light		
A_{qe}	0.17 (0.50)	0.73
L_{cp}	0.62 (0.38)	0.12
A_{max}	1.45 (0.70)	0.05

Table 4 ANCOVA for relative fitness to test whether the pattern of selection on light curve parameters differed between treatments. Abbreviations are as in Table 3

Source	*df*	Type III SS	*F*-value	*P*
A_{qe}	1	5.52	1.21	0.27
L_{CP}	1	16.64	3.64	0.06
A_{max}	1	26.64	5.83	0.02
Light treatment	1	34.10	7.46	0.008
A_{qe} × treatment	1	1.44	0.32	0.58
L_{CP} × treatment	1	0.85	0.19	0.67
A_{max} × treatment	1	10.11	2.21	0.14
Error	83	379.41		

Discussion

Ecophysiology studies have documented genetic differentiation among populations in light curve parameters (e.g., Sims and Kelley 1998) as well as the acclimation of light curves to differing light environments (e.g., Ehleringer 1978; Chazdon and Pearcy 1986; Pearcy and Sims 1994; Buchmann et al. 1996; Davies 1998). However, the generality of these results for shorter lived species, like herbaceous annuals, remains unclear. For *I. capensis*, such acclimation does not appear to occur, at least across a range of light environments commonly encountered by this species. In addition, the results of our selection analyses indicate that the pattern of natural selection on ecophysiological traits in *I. capensis* is similar in sun and shade environments, suggesting that genetic differentiation of light curve parameters in *I. capensis* is unlikely.

Light treatment effects

We failed to observe significant effects of the sun–shade treatments on any of the measured light curve parameters (Tables 1, 2; Fig. 1). There are several potential explanations for these data. First, it is possible that our experiment lacked sufficient statistical power to detect acclimation in light curves. Several observations suggest that this is unlikely. For example, we detected significant differences between treatments in final height (Table 1), internode lengths (J.R. Stinchcombe, unpublished data), and relative fitness (Table 4), as well as significant bench effects for dry biomass and L_{CP} (Tables 1, 2). In addition, the mean carbon assimilation values for the sun and shade treatments were nearly identical, often differing by only 0.02 μmol CO_2 m^{-2} s^{-1}, showing that there was almost no difference in carbon assimilation rates in the two environments. A second potential explanation of these data is that the light levels were too severe in the sun treatment, or not severe enough in the shade treatment, or both. If the high light treatment was too severe it may have resulted in photoinhibition or high temperature stress, or a combination of these two factors (Valladares et al. 2000). High light and heat stress would have depressed carbon assimilation values for the sun treatment plants, resulting in sun and shade plants converging on similar light curve responses. Alternatively, the shade treatment may not have been severe enough to produce the typical acclimation response of sun-shade light curves. Although the shade treatment used in the greenhouse produced a 78% reduction in available PAR, and the average PPF under the shade treatment (173 μmol m^{-2} s^{-1}) was well below the light levels at which carbon fixation became asymptotic (e.g., Fig. 2), it is possible that a more severe shade treatment might have produced sun–shade acclimation in light response curves. For example, Wulff (1989) suggested that acclimation of light response curves is possible in *I. capensis* grown under extreme shade (6% of ambient light). Nonetheless, the 78% reduction in PAR in the shade treatment of our study failed to produce a significant reduction in biomass (Table 1), suggesting that *I. capensis* was able to tolerate or compensate for reduced PAR in the shade treatment, rather than acclimate to it. Moreover, it is worth noting that the two treatments here were representative of naturally occurring light levels in common microsites within the open and shaded source populations (Heschel, unpublished data) of these lines. Accordingly, even if an acclimation response could be detected with a more severe shade treatment, our data suggest that acclimation responses are lacking across an ecologically relevant (cf. Arnold and Peterson 2002) range of light environments for *I. capensis*. This inference is supported by our selection analyses, which suggest that natural selection favors high rates of photosynthesis in both low and high light, rather than the plastic ability to acclimate light response curves.

Selection on light curve parameters

Differences in shade-avoidance morphology have been shown to be adaptive in the source populations from which the RILs used in this experiment were derived (e.g., Donohue et al. 2000). However the fitness differences described by Donohue et al. (2000) between open and woodland genotypes planted in the woodland site could not be entirely explained by selection on shade avoidance traits or flowering time, suggesting that local adaptation might also have favored shade-tolerant physiological traits in the woodland site. As a result, we expected that natural selection would also favor different combinations of light curve traits in the sun–shade treatments, which were designed to mimic light environments of the populations from which they were derived. We expected light curve parameters that produced a "sun" light curve to be favored in the sun treatment, and light curve parameters that produced a "shade" light curve to be favored in the shade treatment. In particular, greater A_{max}, L_{CP} and lower A_{qe} values were expected to be selectively advantageous for high-light treated lines and lower L_{CP} and A_{qe} values for low-light treated lines. However, only higher A_{max}, and possibly L_{CP}, were significantly related to reproductive fitness. Moreover, higher maximal carbon assimilation rates and L_{CP} values were selected for in both high- and low-light environments: different light response curves were not favored in different light environments (Table 4). Rather, the predominant pattern of natural selection was toward a more "sun-like" light response curve.

The absence of diversifying selection that would produce genetic differentiation of sun and shade light response curves could be due to the life-history of *I. capensis*. Because *I. capensis* is an annual with a low tolerance for severe stress, an adaptive strategy seems to entail reproducing as quickly as possible early in a given season to avoid such stress, especially when ecological cues indicate that season length or longevity may be limited (M.S. Heschel and Riginos, unpublished data; Bennington and McGraw 1995; Donohue et al. 2000). Thus, when ecological cues suggest shorter season lengths, it may be adaptive to assimilate carbon at the highest rate possible regardless of the light environment experienced (cf. Geber and Dawson 1997). Here, light conditions were homogeneously high or low; however, increased values of A_{max} and L_{CP} may also be favored in more realistic and variable light conditions. For example, in heterogeneous light environments "sun-like" photosynthetic responses might promote a stress avoidance strategy by allowing plants to capitalize on periodic sun flecks (Chazdon 1992). Interestingly, the highest light level used in gas exchange measurements (1,068 μmol m^{-2} s^{-1}) may have simulated a sun fleck for the shade-acclimated lines, making this hypothesis more plausible. Of course, the results presented here are based on recombinant inbred lines in a greenhouse experiment. Differences between naturally occurring genotypes in the field may lead to different patterns of selection in natural populations. Thus, the observed pattern of selection on A_{max}, L_{CP} and A_{qe} must be further tested with field experiments to assess its importance under natural conditions. In particular, it would be of interest to examine multiple, replicated sun- and shade-adapted populations to determine if population differentiation of light curve parameters is absent, as would be predicted based on our selection analysis.

Conclusions

Sun–shade acclimation in photosynthetic light curves was observed for a small subset of the lines studied here (Fig. 2), although we failed to detect an overall pattern of sun–shade acclimation. Moreover, in both light treatments only higher A_{max} was significantly associated with reproductive fitness. Taken together, these results suggest that photosynthetic light responses of *I. capensis* do not acclimate to the variation in light availability represented by our experimental treatments, and that selection in moderately shady and sunny light environments typical of natural populations will not produce genetically differentiated sun and shade light-curve ecotypes. High- and low-light treated lines were equally able to attain rapid, positive carbon balance. These data suggest that maintaining the photosynthetic machinery necessary for attaining high rates of carbon assimilation is not costly in this species (cf. Mulkey et al. 1991). Rather, lacking the ability to acquire maximal carbon entails a fitness cost. However, this association between $A_{max}$and fitness is also likely to depend on season length. In two studies with *I. capensis*, shortening the season length (similar to what was done here) led to selection for higher carbon assimilation and greater water loss irrespective of the soil moisture environment experienced (Heschel and Riginos, unpublished data). Therefore, in this annual species which initiates early growth and development prior to canopy closure, shorter season lengths may favor high maximal rates of carbon assimilation and early reproduction. These observations suggest that for annual species, photosynthetic acclimation responses to light may be more important in delineating ecological range limits in regions with longer growing seasons.

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