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Interspecific competition alters natural selection on shade avoidance phenotypes in *Impatiens capensis*

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Summary

- Shade avoidance syndrome is a known adaptive response for *Impatiens capensis* growing in dense intraspecific competition. However, *I. capensis* also grow with dominant interspecific competitors in marshes. Here, we compare the *I. capensis* shade-avoidance phenotypes produced in the absence and presence of heterospecific competitors, as well as selection on those traits.
- Two treatments were established in a marsh; in one treatment all heterospecifics were removed, while in the other, all competitors remained. We compared morphological traits, light parameters, seed output and, using phenotypic selection analysis, examined directional and nonlinear selection operating in the different competitive treatments.
- Average phenotypes, light parameters and seed production all varied depending on competitive treatment. Phenotypic selection analyses revealed different directional, disruptive, stabilizing and correlational selection. The disparities seen in both phenotypes and selection between the treatments related to the important differences in elongation timing depending on the presence of heterospecifics, although environmental covariances between traits and fitness could also contribute.
- Phenotypes produced by *I. capensis* depend on their competitive environment, and differing selection on shade-avoidance traits between competitive environments could indirectly select for increased plasticity given gene flow between populations in different competitive contexts.

Introduction

Competition plays a fundamental role in structuring ecological communities and is especially important for plants because of their sessile nature (Goldberg & Fleetwood, 1987; Tilman, 1994). Competitive interactions between plants often have significant effects on individual fitness (Cipollini, 2004; Grace & Tilman, 1990), population growth (Haag *et al.*, 2004), community structure and dynamics (Goldberg & Barton, 1992; Gurevitch *et al.*, 2006), and natural selection (Weinig, 2000). The outcome of competition for light between plants is typically determined by their relative sizes, with larger plants having the advantage (Zobel, 1992). Phenotypically plastic changes in size and phenology in response to competitors have been hypothesized as mechanisms that diminish the negative effects of competition, and potentially facilitate the coexistence of competing species (Callaway *et al.*, 2003).

Phenotypic plasticity, the ability of an individual to adjust its phenotype based on environmental variables, has been studied for decades and is a ubiquitous phenomenon among species (Bradshaw, 1965; Sultan, 2000; Miner *et al.*, 2005). For plants, plastic responses to light cues are particularly important (Neff *et al.*, 2000; Donohue, 2003; Gurevitch *et al.*, 2006). Plants sense competitors through decreases in the red to far-red ratio (R : FR) of light (for reviews see Schmitt *et al.*, 2003, and Franklin, 2008). This reliable, early warning of competition induces a suite of plastic responses in many plant species known as the 'shade-avoidance syndrome' (Dudley & Schmitt, 1996; Franklin, 2008). This includes stem elongation (e.g. elongated hypocotyls and internodes), and if shading by other vegetation continues, accelerated reproduction (Donohue *et al.*, 2001; Franklin, 2008).

Several past studies examining *Impatiens capensis* suggest that the shade-avoidance syndrome is adaptive (Dudley & Schmitt, 1996; Donohue *et al.*, 2000; Huber *et al.*, 2004). For example, Dudley & Schmitt (1996) found that plants that elongated in response to R : FR cues had higher fitness in high-density environments but not low-density environments. In a later study, Donohue *et al.* (2000) examined two populations of *I. capensis* – one from a wooded area and one from a clearing site. They found that plasticity was adaptive in the clearing site, where the R : FR ratio was a reliable cue of conspecific competitors (Donohue *et al.*, 2000). Lines from the woodland site were less plastic, and there was adaptive differentiation between the populations (Donohue *et al.*, 2000).

Past studies of the shade avoidance syndrome in *I. capensis* have only examined the effects of varying intraspecific competition. However, in addition to growing in dense monocultures in which it dominates the understory and herbaceous layer (Winsor, 1983), *I. capensis* also frequently grows with much larger competitor species in marshes and wetlands. These interspecific competitors have diverse resource requirements, growth rates, and flowering times (Firbank & Watkinson, 1990), are frequently perennial, and often surpass the maximum height of *I. capensis*, suggesting that the typical *I. capensis* shade-avoidance phenotype seen in monocultures is unlikely to be effective. Thus, the presence of heterospecifics may alter natural selection on shade-avoidance phenotypes. One approach to testing this hypothesis is to compare the strength and mode of natural selection (Lande & Arnold, 1983) between environments with and without heterospecifics.

The habitats where *I. capensis* grow are extremely variable: interspecific competition, intraspecific density and canopy cover all differ among patches. Such spatial heterogeneity is expected to favour plasticity (van Tienderen, 1991). If marsh populations are plastic, the shade-avoidance syndrome could allow them to attain a more adaptive phenotype for their competitive surroundings. As suggested by Callaway *et al.* (2003), plastic responses could mitigate the negative effects of competition and allow *I. capensis* survive in both dense monoculture and marsh habitats. However, it remains unknown whether marsh and wetland environments are net population sinks, and thus contribute little to the evolution of plasticity in *I. capensis*; theoretical models note that the scale of population regulation and whether populations have above or below average fitness can dramatically affect the evolution of plasticity (Via & Lande, 1985; Gomulkiewicz & Kirkpatrick, 1992). Marsh habitats may be a sink because *I. capensis* are found in them at much lower densities than in monoculture patches (B. V. McGoey & J. R. Stinchcombe, pers. obs., see later). If marshes with interspecific competitors are inherently poor or stressful habitats that necessarily lead to low fitness, one would predict that plants growing in the marsh habitat would have lower per capita reproductive output than plants in monoculture populations.

To examine the effects of interspecific competition on the evolution of shade avoidance phenotypes, we established experimental blocks in a marsh where plants faced intense interspecific competition and removed heterospecifics from half of each block. From this experiment, we sought to answer the following questions. How does the removal of heterospecific competitors affect shade-avoidance phenotypes such as height, total number and lengths of internodes, reproductive timing, and the number of cleistogamous and chasmogamous flowers? Does the per capita reproductive output of plants in a marsh suggest that this habitat is a net population sink? Does natural selection on these phenotypes differ depending on the presence or absence of heterospecific competitors?

Materials and Methods

Study species

Impatiens capensis Meerb. is a native North American annual commonly found in forested areas and wetlands (Schemske, 1978; Kelly, 1997; Huber *et al.*, 2004; Steets *et al.*, 2006, 2007). This species has a mixed mating system (Waller, 1980): individuals produce self-fertilizing cleistogamous flowers and, if large enough, orange, showy, outcrossing, chasmogamous flowers. *Impatiens capensis* plants grow in heterogeneous light environments (Schmitt, 1993), in both monocultures and in diverse marsh communities. Seeds typically disperse ballistically 1.5 m away from parental plants (Schmitt *et al.*, 1985; Kelly, 1997), although genetic marker data show evidence for longer distance gene flow, presumably from secondary water dispersal (von Wettberg *et al.*, 2008). Plants can reach heights of *c.* 2 m (Schmitt *et al.*, 1985; J. R. Stinchcombe & B. V. McGoey, pers. obs.) and competitive hierarchies are established early in the growing season. We studied natural populations of *I. capensis* at the Koffler Scientific Reserve (44°03′ N, 79°29′ W, north of Toronto, Ontario, Canada; http://www.ksr.utoronto.ca).

Experimental design

We used a manipulative field experiment to study how interspecific competition affected shade-avoidance traits in the marsh environment where *I. capensis* naturally occurs with a diverse group of heterospecifics; no dense monocultures of *I. capensis* are found in this habitat.

We randomly selected patches for 21 blocks throughout a large marsh where *I. capensis* grow with several heterospecifics. The competitors included cattails (*Typha latifolia*), marsh marigold (*Caltha palustris*), stinging nettle (*Urtica dioica*), horsetails (*Equisetum fluviatile*) and a diverse group of unidentified grasses. Each 1 × 0.5 m block was split into two plots, with each plot randomly assigned to either the removal or interspecific competition treatment. Above-ground interspecific competition was eliminated from removal treatment on a weekly basis with clipping shears. Because the interspecific competition and removal plots are immediately adjacent to each other, and surrounded by the rest of the marsh, our experimental design is likely to be conservative in its ability to detect effects of interspecific competition. Interspecific competitors, plant density, and moisture levels all naturally varied across blocks. Intraspecific density ranged from 20 to 160 plants m^{-2}, and heterospecific density ranged from 70 to 200 plants m^{-2}.

We tagged *I. capensis* plants in May before they grew true leaves and tracked individuals over the season to assess height, growth rate, flowering time and type, mortality and reproductive success. Plant height was measured once a month throughout the 5-month growing season, and in late August (approx. 120 d after the start of the experiment), we measured the total number of internodes and internode lengths. To estimate light parameters that affect growth and elongation, at each census we used a Field Scout Red/Far Red Meter and a Field Scout Quantum Meter (both Spectrum Technologies Inc., Plainfield, IL, USA). These measurements gave an indication of light quality (the R : FR ratio) and quantity (total photosynthetic active radiation) for each of the plots. We measured both PAR (photosynthetically active radiation) and R : FR ratio four times in each plot just above and below the top of the *I. capensis* canopy.

Starting in early July, we censused plants twice a week for flowers to assess the date of first flowering. Mortality was tracked over the summer as the percentage of plants that survived between height measurements. We estimated fitness as the total number of seeds produced over a plant's lifetime. To do this, we marked flowers as either cleistogamous or chasmogamous when they emerged, and counted both types of fruits bi-weekly throughout August and September to estimate the total number of cleistogamous and chasmogomous fruits per plant. Fruit number was converted to seed number by multiplying these values by the average number of seeds per fruit type, as determined from a sample of 40 cleistogamous and chasmogamous fruits. Plants that died before reproduction were assigned a fitness estimate of zero. Importantly, our estimates do not include any possible effects of seed quality such as size, or maternal effects, which could affect the realized fitness of *I. capensis* (McCall *et al.*, 1991).

To qualitatively evaluate whether the marsh is poor habitat for *I. capensis* that leads to poor reproductive performance, we compared per capita reproductive output of plants in the marsh with those of plants in four monoculture, nonmarsh populations. For these four populations, we tracked survivorship, flowering and fruit production as described earlier for 200 individuals in each population. These four monoculture populations had initial densities ranging from 154 to 350 plants m^{-2} (i.e. at or on the higher end of the marsh densities) and are in sites where *I. capensis* is a habitat dominant (*sensu* Winsor, 1983) in the understory from year to year (J. R. Stinchcombe, unpublished). Although drought stress can impose selection on *I. capensis* (Huber *et al.*, 2004; Heschel & Riginos, 2005), we did not observe leaf wilting or signs of drought stress in monoculture blocks during the summer.

Data analysis

Light measurements We used repeated measures ANOVAs to evaluate changes in light quality (R : FR) and quantity (PAR) over the season. Analyses were conducted for both R : FR and PAR measurements taken above and below the canopy of *I. capensis*, for a total of four repeated measures ANOVAs. Each included treatment and time as factors repeated for each block at each census.

Phenotypic comparisons between marsh treatments To assess the effects of treatment and block on height over time, we used a repeated measures ANOVA. In these models, we interpreted an effect of treatment as indicating that vertical position of height profiles differed between the removal and control treatments, while a treatment × time interaction was interpreted as indicating that treatment effects were variable over time.

For traits for which we had single measurements, ANOVA was used to compare removal and interspecific competition plants. We tested for differences between treatments in the total numbers of chasmogamous and cleistogamous fruits, days to first flower, internode lengths, hypocotyl lengths and total seed number (our fitness estimate). Because the removal treatment was applied to half a block at a time, rather than individual plants, we tested the treatment effect in our ANOVAs over the block × treatment interaction to obtain *F*-statistics for hypothesis testing.

We do not present results for block or block × treatment interactions because testing for and generalizing about spatial variation in traits and fitness was not one of our primary goals, and preliminary inspection of block × treatment interactions revealed that they were universally driven by changes in the magnitude of the treatment effect, rather than reversals in its direction.

Fitness effects and natural selection Abiotic and biotic factors could have direct effects on the fitness of plants in each plot. To evaluate this, we tested the correlation between light parameters (R : FR and PAR), and total plant density with fitness for each experimental treatment. For these analyses, we used mean fitness of each plot, as the light measures were plot-level rather than individual characteristics.

Phenotypic selection analysis was used to determine which traits were important for fitness in the two treatments. To test the impact of *I. capensis* traits on fitness, as well as any differences in their fitness effects between treatments, we conducted both an ANCOVA and phenotypic selection analyses. In these analyses, the relative fitness was estimated as the total number seeds produced per plant, divided by the mean seed number for all the plants in the marsh. Mean-standardized values were used for traits (Hereford *et al.*, 2004; Hansen & Houle, 2008) to facilitate comparisons between traits with different units.

An ANCOVA was performed for relative fitness with traits that we identified a priori as likely to be under selection. The traits were: maximum height, early growth rate, late growth rate, hypocotyl length, first internode length, and days until flowering. We estimated early growth and late growth rates by calculating the difference in vertical height between the second and first census, the fifth and fourth census, respectively, divided by the number of elapsed days. Our approach to estimating growth rate only captures how quickly vertical height changed throughout the season (i.e. cm d^{-1}), and not the biomass of these plants (which would have required destructive harvests and altered the competitive environments) or the developmental rate of plants (which we examine later, using the total number of internodes). Treatment, block and their interaction, as well as each trait's interaction with treatment were included in the model. We interpret a significant effect of a trait on relative fitness as evidence for natural selection, and trait × treatment interactions as evidence that selection is affected by competitive context.

To complement the ANCOVA, which used *a priori* selected traits, we also used a model-selection approach to characterize the traits that were most important for fitness in the two treatments in an unbiased, systematic manner. Models describing the important traits for relative fitness were developed separately for the interspecific competition and removal treatments. We used mean-standardized traits for all models, and statistical models were selected using Akaike's information criterion (AIC). The coefficients for each trait were taken as directional selection gradients on that trait. To examine quadratic selection, models that included the traits (used in the ANCOVA), squares of the traits, and their cross-products were used. Quadratic regression coefficients were converted to selection gradients (γ) by doubling them and their respective standard errors (Stinchcombe *et al.*, 2008). To check if significant nonlinear selection represented true stabilizing selection, or if it was curvilinear selection, we examined partial regression plots and looked for an intermediate minima (cf. Mitchell-Olds & Shaw, 1987). Preliminary analysis of variance inflation factors suggested that multicollinearity did not affect partial regression coefficients. Because of highly nonnormal residuals, we calculated confidence intervals for the coefficients by bootstrapping the residuals.

Canonical analysis of the γ matrix To facilitate interpretation of the patterns of stabilizing, disruptive, and correlational selection between treatments, we performed a canonical analysis of the γ matrix. Briefly, this approach rotates the γ matrix to eliminate correlational selection, leaving only stabilizing/disruptive selection on synthetic traits that are linear combinations of the original traits (Phillips & Arnold, 1989; Simms, 1990; Blows & Brooks, 2003; Stinchcombe *et al.*, 2008). To do this, we applied principal component analysis (PCA) to the γ matrix. The eigenvalues of this PCA are measures of nonlinear (stabilizing/disruptive) selection on the synthetic traits; the eigenvectors of each PC describe the contributions of the original traits to the synthetic traits. Similar to traditional PCA, one can estimate the proportion of variation that is associated with a given canonical axis (m_i).

To compare the similarity of the two γ matrices, we used the subspace similarity method described by Blows *et al.* (2004). To do this, we saved the first three PCs of each γ matrix as columns in two matrices, **A** and **B**, respectively. We then calculated the matrix **S**, according to $\mathbf{S} = \mathbf{A}^{T}\mathbf{B}\mathbf{B}^{T}\mathbf{A}$, where superscript T indicates matrix transposition. The eigenvalues of **S**, in turn, can be used to find the similarity between the two matrices **A** and **B**: the sum of the eigenvalues of **S** will range from 0 to k (where k = the number of PCs entered into the columns of **A** and **B**). Sums close to zero indicate near orthogonality (i.e. complete dissimilarity) of the two matrices, while sums close to k indicate that the two subspaces share similar orientations (Blows *et al.*, 2004). We elected to use three PCs of the γ matrices because these PCs explain > 99% of the variation in the γ matrix, and because the subspace similarity method can only utilize less than half of the PCs of a matrix (Blows *et al.*, 2004).

Table 1 Light parameters in May and August for the removal and interspecific competition blocks

		(a) Removal	(b) Interspecific competition
R : FR	May	1.079 ± 0.021	0.952 ± 0.052
above	August	0.978 ± 0.054	0.907 ± 0.070
R : FR	May	0.630 ± 0.051	0.444 ± 0.052
below	August	0.579 ± 0.06	0.247 ± 0.033
PAR	May	1554 ± 84	1204 ± 114
above	August	989 ± 108	400 ± 112
PAR	May	158 ± 20	97 ± 35
below	August	426 ± 78	120 ± 35

PAR, photosynthetically active radiation; R : FR, red to far-red ratio.

Data analysis was performed with R (R Development Core Team, 2007), SAS (v. 9.1.3; SAS Institute, Cary, NC, USA), and the PopTools add-in for Excel (Hood, 2006), depending on ease of use for particular analyses.

Results

Light characteristics

There were differences in light parameters in the two treatments, as well as changes through time (see Table 1 for May and August measurements, representing the beginning and end of the season). Both above and below the *I. capensis* canopy R : FR ratios were significantly higher in removal versus interspecific competition plots (for both $P < 0.0001$). In a rm-ANOVA, there were significant effects of time for the R : FR measurements both above and below the *I. capensis* canopy ($P < 0.05$ for both), with R : FR ratio decreasing over the season in both treatments. For both above and below the *I. capensis* canopy R : FR measurements, there were significant interactions between treatment and time, with the R : FR ratio declining more rapidly over time in the interspecific competition treatment (Table 1).

Total photosynthetically active radiation was also significantly lower in the interspecific competition treatment than in the removal treatment ($P < 0.0001$) (Table 1). There was a significant effect of time for both the above ($P < 0.0001$) and below ($P < 0.0001$) *I. capensis* canopy PAR measurements, with PAR

Table 2 Mean phenotypic traits (± 1 SE) for *Impatiens capensis* plants from removal and interspecific competition treatments, along with *F*-statistics for the treatment effect

Trait (units)	Removal	Interspecific competition	$F_{1,20}$	P
May height (cm)***	**6.1 ± 0.091**	**8.3 ± 0.015**	**35.16**	**< 0.0001**
June height (cm)***	**20.5 ± 0.34**	**26.8 ± 0.52**	**34.45**	**< 0.0001**
July height (cm)	62.5 ± 1.13	56.7 ± 1.23	0.93	0.35
August height (cm)**	**82.8 ± 1.59**	**67.8 ± 1.60**	**9.82**	**0.0052**
September height (cm)	94.8 ± 1.52	90.7 ± 1.70	0.01	0.9157
Maximum height (cm)**	**69.9 ± 1.94**	**56.4 ± 1.73**	**8.99**	**0.0071**
Flowering time (d)*	**61.0 ± 0.2**	**61.9 ± 0.27**	**5.36**	**0.0313**
Hypocotyl length (cm)	8.5 ± 0.17	8.8 ± 0.19	2.52	0.1279
First internode length (cm)**	**8.0 ± 0.14**	**9.4 ± 0.18**	**8.34**	**0.0091**
Total number of internodes**	**11.9 ± 0.175**	**9.8 ± 0.177**	**15.33**	**0.0009**
Early growth rate ($\mathrm{cm\ d^{-1}}$)***	**0.47 ± 0.011**	**0.62 ± 0.012**	**23.78**	**< 0.0001**
Late growth rate ($\mathrm{cm\ d^{-1}}$)**	**0.65 ± 0.037**	**0.35 ± 0.035**	**19.09**	**0.0003**
Absolute fitness (seeds)**	**43.5 ± 3.2**	**21.6 ± 2.0**	**12.14**	**0.0023**

*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. Values in bold are significantly different between treatments.

declining significantly over the season, in both marsh treatments. Only the below canopy measurements showed a significant interaction between treatment and census ($P = 0.014$); PAR increased over the season below the *I. capensis* canopy in the removal treatment.

Phenotypic comparisons between marsh treatments

Removal of marsh vegetation had significant effects on a host of plant phenotypes, including fitness. Plants in the removal treatment, compared with the interspecific competition treatment, differed significantly in several phenotypes: they were shorter early in the season (May and June), taller later in the season (August), reached taller maximum heights, had shorter first internodes and more total internodes (Table 2a). Several of these differences support the hypothesis that plants growing in interspecific competition elongate earlier, and the greater number of internodes suggests accelerated developmental rates. In contrast to expectations based on the shade avoidance syndrome, plants in the interspecific competition treatment flowered later than those in the removal treatment (Table 2a).

Our finding that plants in the removal treatment were shorter early in the season and taller late in the season suggests temporal shifts in growth, and two analyses support this view. First, when heights were examined over the entire season with a repeated measures ANOVA, there was a significant interaction between treatment and time ($P = 0.024$). Second, when we tested for treatment effects on early and late growth rate ($\mathrm{cm\ d^{-1}}$ growth between the first and last two censuses) we found that plants in the removal treatments grew significantly slower early in the season (Table 2a). Plants in the removal treatment had higher late season growth rates, but the difference was not significant (Table 2a).

Plants in the removal treatment also set significantly more seeds, having an absolute fitness of approximately twice that of plants in the interspecific competition treatment (Fig. 1b). The

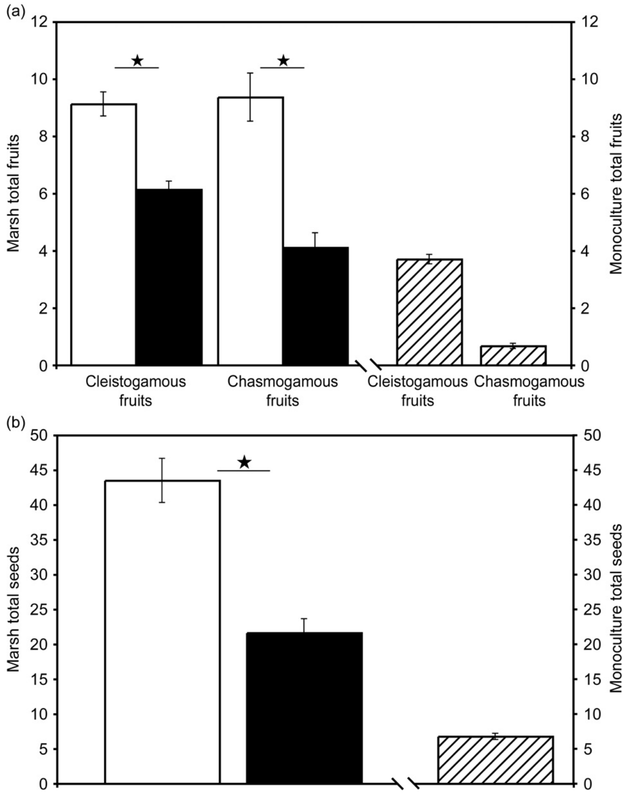

Fig. 1 Mean fruit and seed production of *Impatiens capensis* in the marsh experiment and natural, monoculture populations. (a) Mean cleistogamous and chasmogamous fruit production (± 1 SE) in the two marsh treatments (open bars, removal; dark-tinted bars, interspecific competition). The difference between means is highly significant according to ANOVA (Table 2). For purposes of illustration, the mean fruit production (± 1 SE) of plants growing in the four monoculture populations (hatched bars) is shown. Note the separate *y*-axis for monoculture populations on the right, using the same scale. No formal comparison was made between monoculture and marsh treatments. (b) Total seed production in the two marsh treatments, and four monoculture populations, as in (a).

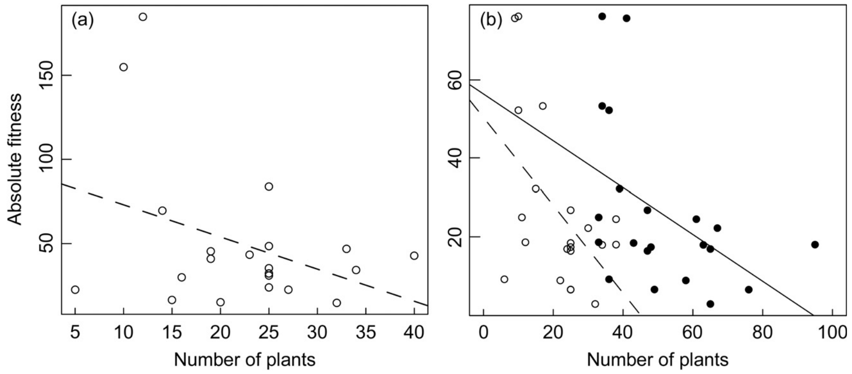

Fig. 2 The effects of intraspecific and total plant density on *Impatiens capensis* fitness. (a) The effect of intraspecific density on *I. capensis* fitness in the removal treatment. Plotted points are mean *I. capensis* density per plot and mean seed production per plot. (b) The effects of intraspecific density (open circles, dashed line) and total density (closed circles, solid line) on *I. capensis* fitness in the interspecific competition treatment. Plotted points are mean densities per plot and mean seed production per plot.

differences in average seed number per plant between the two treatments appears to be driven by plants in the removal treatment producing significantly more of both types of fruits, rather than their relative production (chasmogamous and cleistogamous; Fig. 1a). Per capita fruit and seed production in the marsh also exceeded that of the monoculture populations (Fig. 1). While these data do not preclude the possibility of dispersal from monocultures to the marsh, they do indicate that the marsh is not so poor or stressful a habitat that it necessarily leads to low reproductive success.

Fitness effects and directional natural selection

In the interspecific competition treatment, there was a significant negative relationship between total plant density (both intraspecific and interspecific competitors) and *I. capensis* fitness ($r = -0.472$, $P = 0.031$, Fig. 2b). There were also negative correlations between fitness and total conspecifics ($r = -0.516$, $P = 0.017$), as well as fitness and total heterospecifics ($r = -0.271$, $P = 0.23$), although only the former correlation was significant. In removal plots (which contain only *I. capensis*), there was a negative correlation between number of *I. capensis* and fitness (see Fig. 2a), but it was only marginally significant ($r = -0.375$, $P = 0.094$). Light quantity (PAR) and quality (R : FR) also had significant effects on fitness. There were positive correlations between fitness and R : FR ratio ($r = 0.462$, $P = 0.035$), as well as fitness and PAR ($r = 0.545$, $P = 0.011$) for the interspecific competition plots. For removal plots, only PAR ($r = 0.326$, $P = 0.015$) was positively correlated with fitness.

Of the traits we expected a priori to be under selection, maximum height and early growth were significant terms in the ANCOVA (see Table 3). The interaction between treatment and hypocotyl length, treatment and maximum height, and treatment and late growth rate were the only interaction terms that were significant. Together, these data suggest early growth was under selection in the marsh experiment but that the strength of selection did not differ across treatments. By contrast, hypocotyl length, maximum height and late growth showed evidence of being under variable selection between the two treatments. Comparison of the ANCOVA with the model selection analyses (i.e. Table 3 vs Table 4), suggests that the significant trait × treatment interactions were driven by traits being under significant selection in one treatment but not the other (hypocotyl and maximum height, which were not retained in the removal treatment model), or variation in the strength of selection (late growth).

Table 3 Results of ANCOVA for relative fitness of *Impatiens capensis* plants

Source	SS	*F*	*P*
Treatment	0.52	0.219	0.64
Block	**8.46**	**3.58**	**0.059**
Block × treatment	0.98	0.41	0.52
Maximum height***	**274.46**	**116.33**	**< 0.0001**
Days until flowering	0.004	0.0017	0.97
Hypocotyl length	8.1	3.43	0.064
First internode length	4.79	2.03	0.15
Early growth*	**13.76**	**5.83**	**0.016**
Late growth	4.58	1.94	0.16
Treatment × maximum height**	**23.27**	**9.86**	**0.0017**
Treatment × days	0.17	0.078	0.79
Treatment × hypocotyl length*	**12.82**	**5.43**	**0.020**
Treatment × first internode length	0.16	0.066	0.80
Treatment × early growth	6.22	2.64	0.11
Treatment × late growth**	**17.34**	**7.35**	**0.0069**

Traits were standardized to a mean of 1 before analysis. The overall model had 596 error degrees of freedom.
*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. Values in bold are significant.

Directional selection varied between treatments (Table 4). For example, one notable difference between the two marsh treatments was directional selection for higher late growth rates in the interspecific competition treatment but not in the removal treatment. There were also similarities: maximum height was under significant positive directional selection in both competition treatments in the marsh. Total internode number was not a significant predictor of relative fitness in either treatment.

Nonlinear selection and canonical analysis of γ

In both the interspecific competition and removal treatments there was significant nonlinear selection on maximum height (Table 5a,b). Inspection of partial regression plots suggested

Table 4 Directional phenotypic selection gradients (β, 95% CL) for interspecific competition and removal treatments, as determined by model selection using Akaike's information criterion (AIC) for each treatment

	β (95% CL)	
Traits	Removal	Interspecific competition
Early growth	NA	0.04398 (–0.288, 0.438)
First internode length	**–0.8605**** **(–1.578, –0.28)**	**–0.476*** **(–0.869, –0.127)**
Height in May	–0.789* (–1.622, 0.038)	**–0.443*** **(–0.980, –0.071)**
Hypocotyl	NA	0.310 (0.0095, 0.797)
Late growth	NA	**0.2023*** **(0.0439, 0.396)**
Maximum height	**2.697***** **(2.15, 3.42)**	**1.879***** **(1.39, 2.55)**
Total internodes	NA	NA

Traits were standardized to a mean of 1 before analysis. *P*-values are from standard parametric hypothesis tests, while confidence intervals are from bootstrapping the residuals of the multiple regression. Traits which are denoted as 'NA' were not included in the model selected by AIC.
*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. For values in bold, the confidence intervals do not cross zero.

that maximum height was under curvilinear selection, rather than true disruptive selection with an intermediate fitness minima. The nonlinearity in this relationship suggests that there were accelerating fitness benefits of larger size (i.e. plants at the top of the height distribution received more fitness benefit per unit of height than those in the middle or bottom of the height distribution). In the removal treatment, there was significant stabilizing selection on late growth, indicating that intermediate late growth rates had higher fitness than faster or slower late growth rates. For the interspecific competition treatment, the point estimate of selection on late growth was also negative, although nonsignificant.

We detected several significant correlational selection gradients in each treatment (Table 5a,b). Inspection of individual coefficients suggests that in the interspecific competition treatment, positive correlations among phenology, size and growth traits were favoured (hypocotyl length, days to flowering, maximum height and late growth), while in the removal treatment, negative correlations between growth rates (early and late) and size components (hypocotyl length and maximum height) were favoured. However, visualizing correlational selection for multiple traits is difficult (Phillips & Arnold, 1989; Blows, 2007a) and inspection of a handful of coefficients of a matrix can be misleading (cf. Walsh, 2007).

Canonical analysis of the γ matrices for the interspecific competition and removal treatments revealed that the overall pattern of nonlinear selection was disruptive. In the removal treatment, the first axis (m_1) explained 98% of the variation (Table 6a) and indicated significant disruptive selection. Inspection of the eigenvectors of this axis revealed strong contributions from days to flower and first internode length (eigenvectors of Table 6a). The second and third most important axes (m_2 and m_7) each explained < 1% of the variation in the curvature of the fitness surface. The m_2 axis appeared to be driven by opposing contributions from maximum height on the one hand, and hypocotyl length and height in May on the other. The m_7 axis, which is indicative of stabilizing selection on the synthetic trait (negative coefficient), is statistically significant but explains a low percentage of the curvature in the fitness surface.

In the interspecific competition treatment, the first axis (m_1) explained only 89% of the variation in the nonlinear fitness surface and appeared to be driven strongly by days to flower, with additional contributions from hypocotyl length (Table 6b). In contrast to the removal treatment, the second and third most important axes (m_2, m_7) explained appreciable variation (approx. 5%). The m_2 axis was driven by contributions from maximum height, hypocotyl length, and early growth (Table 6b). Similar to the removal treatment, the m_7 axis is subject to stabilizing selection; however, in this case it appears to reflect a larger percentage of the variation in the curvature of the fitness surface (approx. 4.5%). The m_7 axis appears to be largely a function of positive contributions from late growth, hypocotyl length, and first internode length, and opposing contributions from height in May and days to flower.

Comparison of the traditional analysis of the γ matrix with the canonical analysis reveals several results. First, in the traditional analysis, only four or five of the original selection gradients were significant (out of 28 estimated for each treatment). By contrast, in the canonical analysis, six of the seven canonical gradients are significant in each treatment. The differences in significance are likely caused by increased power as a result of fewer parameters in the model. Comparison of the quadratic selection gradients (diagonal of Table 5) with the eigenvalues of Table 6 suggests differences in the strength of stabilizing/disruptive selection. Using the traditional method, the median γ_{ii} was 1.08 in the removal treatment and 0.526 in the interspecific competition treatment; the median λ_i values from the canonical analysis were 3.039 and 0.9, respectively. These figures suggest that there is more curvature in relative fitness along the axes described by the synthetic traits than along the axes described by the original traits (Blows & Brooks, 2003). As Blows & Brooks (2003) emphasize, canonical analysis does not discover new evidence of selection that had not previously been present, but instead reallocates it to different coefficients (i.e. among 7 λ_i values instead of among 21 γ_{ij} values and 7 γ_{ii} values).

The subspace similarity method allows an estimation of the similarity between the two estimated γ matrices. We find that the sum of the eigenvalues of **S** equalled 2.03 (out of a maximum of 3). These data suggest that the two nonlinear fitness surfaces, as described by their first three PCs, share similar, but not identical geometric orientations.

Table 5 Stabilizing, disruptive and correlational phenotypic selection gradients (γ, CI) for interspecific competition and removal treatments

	Early growth	Late growth	Height in May	Hypocotyl length	First internode length	Days to flower	Maximum height
(a) Removal treatment							
Early growth	–0.237						
	(–3.44, 5.1)						
Late growth	**–1.44** ***	**–0.277** *					
	(–2.41, –0.313)	**(–0.5108, 0.1012)**					
Height in May	0.197	0.621	0.0718				
	(–3.58, 3.17)	(–0.522, 1.70)	(–3.74, 4.88)				
Hypocotyl length	2.198	0.149	–0.348	–1.08			
	(–0.782, 4.83)	(–0.669, 1.08)	(–2.72, 1.99)	(–2.76, 1.04)			
First internode length	–0.529	0.621	–1.23	1.02	–1.23		
	(–3.94, 2.20)	(–0.361, 1.39)	(–4.63, 2.48)	(–1.42, 3.56)	(–5.08, 3.38)		
Days to flower	0.169	0.400	–3.62	–2.36	8.10	57.0	
	(–10.2, 137)	(–3.88, 5.11)	(–18.2, 9.14)	(–13.3, 8.73)	(–6.49, 24.4)	(–7.12, 78)	
Maximum height	0.837	**0.762** *	–1.66	**–2.95** *	–0.68	–2.82	**3.16** *
	(–3.15, 3.77)	**(0.306, 1.65)**	(–5.04, 1.67)	**(–5.80, –0.417)**	(–3.22, 2.09)	(–14.8, 8.1)	**(0.184, 7.18)**
(b) Interspecific competition							
Early growth	0.817						
	(–0.643, 2.14)						
Late growth	0.00976	–0.145					
	(–0.441, 0.531)	(–0.440, 0.168)					
Height in May	–0.286	0.337	0.526				
	(–1.428, 0.899)	(–0.256, 0.937)	(–0.913, 2.17)				
Hypocotyl length	–0.119	–0.0925	0.0848	–0.0520			
	(–1.15, 0.887)	(–0.656, 0.391)	(–0.989, 1.06)	(–1.48, 1.43)			
First internode length	–0.677	–0.246	0.311	–0.124	–0.395		
	(–1.86, 0.344)	(–0.896, 0.345)	(–1.17, 1.74)	(–1.29, 1.01)	(–2.46, 1.89)		
Days to flower	–1.20	–0.167	–0.0697	**3.71** *	1.07	9.29	
	(–5.82, 3.30)	(–3.36, 3.45)	(–4.94, 4.78)	**(–0.957, 8.23)**	(–4.24, 6.79)	(–19.0, 36.8)	
Maximum height	0.232	**0.480** *	–1.065	**1.36** **	0.140	–0.921	**1.28** *
	(–0.858, 1.32)	**(0.111, 1.00)**	(–2.50, 0.430)	**(0.147, 2.60)**	(–1.19, 1.55)	(–8.20, 6.05)	**(0.422, 3.02)**

Traits were standardized to a mean of 1 before analysis. *P*-values are from standard parametric hypothesis tests, while confidence intervals are from bootstrapping the residuals of the multiple regression.

*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. For values in bold, the confidence intervals do not cross zero.

Discussion

Changes in light resources due to competition can have direct effects on plant fitness and plasticity. Our experiment had three major findings. First, we found that heterospecific competitors affected light resources and cues (i.e. PAR and R : FR ratio), and shade-avoidance phenotypes. Second, censuses of per capita reproductive output in marsh and monoculture populations suggest that marsh habitats are not inherently poor habitats that lead to low reproductive success. Third, the presence or absence of heterospecifics significantly altered natural selection on traits expected to be involved in shade avoidance responses. We discuss these findings below, along with the potential caveats that apply to our results, in the context of their implications for the evolution of phenotypic plasticity.

Phenotypic differences and light resources

Our results demonstrate that numerous plant traits, including total reproductive output, are affected by the presence of heterospecifics. The direction and timing of these effects can be explained by changes in light quantity and quality. By inducing plastic changes in shade-avoidance phenotypes that are subject to natural selection (see below), heterospecifics have the potential to alter the evolution of the shade-avoidance syndrome. For example, early in the season, R : FR ratios were lower in the removal treatment (Table 1), which would signal the presence of competitors to the focal *I. capensis*; early in the season, the average height in interspecific competition treatment was greater than the removal treatment. Later in the season, both PAR and R : FR decreased in the removal treatment (due to shading from conspecifics and heterospecifics in the surrounding marsh), and at this time the height trends had reversed. In essence, our removal treatment delayed the onset of the R : FR signal until later in the season when plants had been growing in higher PAR for 2 months (Table 1). These higher PAR levels are indicative of greater light resources, which may have allowed the removal plot plants to elongate late in the season, whereas the plants growing with heterospecifics were likely too energy-limited to continue responding to low R : FR.

Table 6 Canonical analysis of the γ matrices, including the eigenvalues (λ_i ± 1 SE) and the eigenvectors of the canonical axes (m_i): (a) removal treatment; (b) interspecific competition treatment

m_i	λ_i	% Variance	Early growth	Late growth	Height in May	Hypocotyl length	First internode length	Days to flower	Maximum height
(a) Removal treatment									
1	58.5582 ± 20.56*	98.13	–0.001	0.007	–0.062	–0.034	0.135	0.987	–0.048
2	5.1301 ± 0.52*	0.75	–0.071	0.093	–0.239	–0.436	–0.073	0.021	0.856
3	1.9130 ± 0.48*	0.10	0.715	–0.473	–0.330	0.344	–0.003	0.005	0.194
4	0.3326 ± 0.4	< 0.001	–0.225	0.380	–0.649	0.375	0.483	–0.097	–0.007
5	–0.3985 ± 0.16*	< 0.001	0.447	0.672	0.448	0.289	0.067	0.036	0.241
6	–3.0394 ± 0.72*	0.26	–0.069	–0.350	0.405	–0.049	0.814	–0.076	0.193
7	–5.0882 ± 0.88*	0.74	0.478	0.221	–0.207	–0.681	0.275	–0.093	–0.363
(b) Interspecific competition treatment									
1	10.8468 ± 3.4*	89.26	–0.123	–0.021	0.007	0.313	0.093	0.936	–0.049
2	2.5740 ± 0.22*	5.03	0.208	0.087	–0.428	0.346	–0.088	–0.032	0.799
3	0.9001 ± 0.48†	0.61	0.832	–0.126	–0.166	–0.009	–0.376	0.131	–0.324
4	0.4493 ± 0.14*	0.15	0.239	0.559	0.731	0.241	–0.123	–0.022	0.150
5	–0.3176 ± 0.10*	0.08	–0.259	0.701	–0.415	–0.207	–0.430	0.087	–0.185
6	–0.6939 ± 0.22*	0.37	0.353	0.380	–0.162	–0.362	0.750	0.104	0.023
7	–2.4377 ± 0.48*	4.51	–0.013	0.167	–0.238	0.742	0.282	–0.296	–0.444

Significance of the eigenvalues was estimated with a quadratic regression containing synthetic traits estimated from the eigenvectors and the original traits. *, $P < 0.01$; †, $P = 0.0584$.

The resulting differences in early and late growth rates (Table 2) were themselves subject to variable natural selection (Tables 3, 4).

Our results are consistent with other studies that indicate that the presence of heterospecific competitors can affect the evolution of shade-avoidance traits by modifying their effectiveness, expression or timing. For example, Weinig (2000) found that when *Abutilon theophrasti* plants were grown under weeds, they lacked the carbon resources necessary to elongate, despite low R : FR cues. Other studies have found that the presence of heterospecific leaf litter can alter selection on early elongation traits in *I. capensis* (Stinchcombe & Schmitt, 2006). Collectively, these studies suggest that heterospecific plant species, by altering light quality and quantity, and the timing of these cues of competition, may substantially affect the evolution of shade avoidance.

Are marshes poor habitat?

We predicted that *I. capensis* would have lower reproductive output under competition in the marsh than in the removal treatments, but that plants in both of these treatments would have higher reproductive success than plants from monoculture populations. Instead, plants in both marsh treatments had greater average per capita reproductive output than plants from monoculture blocks. This demonstrates that the marsh represents a suitable habitat for *I. capensis*, despite intense interspecific competition. While subsidy from monoculture habitats may occur (due to the floating, ballistically dispersed seeds of *I. capensis*), our results suggest that marsh habitats are qualitatively different, but not necessarily poorer habitats, than monoculture sites. It is important to note that the average initial density of *I. capensis* in the monoculture (213.5 plants m^{-2}) populations was significantly greater than in the marsh (44.2 plants m^{-2}). These densities, which differ in the opposite direction from the one that would be predicted based on our data on total seed production (marsh plants have an approximately sixfold advantage in seed production, but have nearly fivefold lower initial densities) suggests that marsh populations must experience higher seed mortality (perhaps because of differences in seed quality: McCall *et al.*, 1991), a longer seed bank or some combination of the two. Winsor (1983) noted that *I. capensis* dominates some habitats because of early germination and growth under colder temperatures, and the formation of dense canopies that shade interspecific competitors. The lack of monocultures in the marsh habitats despite high seed production suggests that these life-history strategies and features are ineffective in the marsh habitat or against marsh competitors.

Directional selection

Directional selection on shade-avoidance traits changed depending on the presence of heterospecifics. The most obvious difference between selection models was the inclusion of growth rates and hypocotyl length in the interspecific competition model. These data suggest that under interspecific competition growth rates became important for determining fitness. Hypocotyl length was likely under positive selection in the interspecific competition treatment because very early growth would allow *I. capensis* to remain taller than competitors early in the season.

Similar results for hypocotyl length have been described for *I. capensis* germinating under forest leaf litter (Stinchcombe & Schmitt, 2006).

Our use of naturally occurring plants may have led to biased estimates of phenotypic selection due to environmentally induced covariances between traits and fitness (Rausher, 1992; Stinchcombe *et al.*, 2002). We attempted to minimize this influence by randomly selecting blocks in the marsh, randomly allocating treatments to each half of the block, and accounting for block effects in our statistical models. While the initial densities of *I. capensis* did not differ between treatments, our treatment itself could have produced environmentally induced covariances. For example, in the removal treatment, plants would have received more light and, as a consequence, reach larger size and have higher fitness, potentially leading to an inflated estimate of the true relationship between size and fitness. Environmental covariances induced by the treatment itself are, however, less likely to explain selection on novel traits and trait combinations in the interspecific competition treatment – these sections of the marsh were left unaltered and simply observed, so the treatment itself was unlikely to alter environmental conditions.

Available evidence suggests environmentally induced covariances between traits and fitness mainly bias the magnitude of selection gradients and not the sign (Stinchcombe *et al.*, 2002). Many of the selection gradients in the removal treatment (which should be more susceptible to environmentally induced covariances caused by the treatment itself) show larger selection gradients than the control (see Tables 4 and 5, especially for maximum height and first internode length) and may be biased estimates. While our data are consistent with this type of bias, it is also possible that environmental covariances could have reversed the sign of individual selection gradients (either across the experiment, or how selection was occurring within individual plots) or had the effect of masking selection and leading us to conclude that it was absent. Ultimately, addressing any of these possibilities, however, requires transplanting quantitative genetic families into the marsh to perform genotypic selection analysis (Stinchcombe *et al.*, 2002).

Nonlinear selection

Maximum height was under both positive directional and curvilinear selection in both the treatments (Tables 4, 5). In the removal treatment, a greater height would allow an *I. capensis* plant more access to light than its neighbours, giving it a competitive edge. In the interspecific competition treatment, increased height would allow plants to emerge from as much vegetation as possible. Comparison of the γ matrices suggests a handful of differences in nonlinear selection (Table 5a,b). In the removal treatment, there was saturating curvilinear selection on late growth (i.e. the fitness benefits of late growth levelled off) and there was negative correlational selection on early and late growth; neither of these were significant in interspecific competition. Opposite combinations of early and late growth being favoured suggest that plants that grow slowly throughout season are at a disadvantage (they are at the bottom of the size hierarchy), as are plants that grow quickly the entire season, possibly because later in the season, selection favours allocation to reproduction or branching rather than vertical growth.

The canonical analysis facilitated an overall assessment of the mode of nonlinear selection in the experiment, and how similar it was between treatments. These analyses revealed that *c.* 95–99% of the variation in the γ matrix was associated with disruptive selection (axes m_1–m_2 in each treatment). Partial residual blocks of relative fitness against the synthetic variables suggested true disruptive selection on axes m_1 and m_2 in each treatment, with a hint of stabilizing selection on axis m_7 in each treatment. Overall, the contribution of stabilizing selection on the synthetic traits appears to be weak. Subspace similarity methods suggested that the two γ matrices shared similar, but not identical orientations in multivariate space.

Using canonical analysis, we found more evidence for statistically significant nonlinear selection on the synthetic traits than we did on the original traits, and the median values of the selection gradients (γ_{ii} vs λ_i) were larger for synthetic traits. Both of these patterns have been previously demonstrated (Blows & Brooks, 2003; also see Blows, 2007a,b). While it is an algebraic necessity that at least one λ_i will be larger than a γ_{ii} (provided that there is any correlational selection; Kruuk & Garant, 2007), we found selection on the canonical traits to be appreciably stronger than on the original traits. These data suggest that combinations of traits were under stronger nonlinear selection in the experiment than the individual traits themselves. Put another way, there is more curvature in the fitness surface along the axes described by the synthetic traits than along the axes described by the original traits. This result is notable for shade-avoidance traits, for which we have ample prior evidence of the phenotypic traits under selection, and for which phenotypic differences can be both quite apparent and dramatic (Table 2). It also counters the hypothesis that canonical analysis is unlikely to be revealing in field studies (Preziosi & Harris, 2007). As emphasized by Blows (2007b), if combinations of traits are under selection, methods such as canonical analysis that can identify those trait combinations will be necessary.

Conclusions

Our study indicates that shade-avoidance traits can be under very different types of selection depending on the level and type of competition, which could lead to the evolution of increased plasticity. This is contingent on gene flow between patches in different competitive habitats, which is known to occur in *I. capensis* (von Wettberg *et al.*, 2008). Our results suggest that shade-avoidance responses may facilitate the coexistence of *I. capensis* with dominant heterospecific competitors, by providing for flexible elongation timing based on the R : FR light cue.

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