

Interacting effects of microsite quality, plasticity and dispersal distance from the parental site on fitness in a natural population of *Impatiens capensis*

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ABSTRACT

Hypothesis: Induced plastic responses, environmental heterogeneity and local adaptation may have interacting and counteracting effects on the performance of organisms.

Organism: The North American herbaceous annual *Impatiens capensis*.

Site of experiment: This experiment was performed in a forest understory site in Bristol, RI, USA, where previous experiments have shown declines in fitness with transplanting up to 12 m from parental sites.

Methods: Eight replicated genotypes were pre-treated in a glasshouse to induce or suppress shade avoidance responses and then transplanted into 50 randomly chosen microsites within 50 m of the site from which their parents were originally collected.

Results: Overall plant fitness was significantly autocorrelated at distances less than 4 m, the primary dispersal distance of *Impatiens* seeds and the distance with greatest environmental spatial autocorrelation. The fitness of transplants was affected by site quality but not by distance from the site of original collection. In addition, genotypes were more sensitive to environmental factors when induced to elongate in response to neighbour shading. Finally, the genotypes most responsive to increasing site quality were the most fit.

Keywords: environmental quality, phenotypic plasticity, shade avoidance, spatial autocorrelation.

INTRODUCTION

Sessile organisms, such as plants and filter feeders, experience environmental heterogeneity on a fine spatial scale of centimetres and metres (e.g. Harper, 1977; Mitsch and Gooselink, 1993). If different phenotypes have higher fitness in particular conditions, adaptive plasticity, local adaptation, or both at once may be favoured (e.g. Van Tienderen, 1991; Kawecki and Stearns, 1993; Sultan, 1995; Schlichting and Pigliucci, 1998; Sultan and Spencer, 2002; Donohue, 2003). With adaptive plasticity a single

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genotype can produce multiple phenotypes depending on conditions; a locally adapted genotype has a phenotype better suited to the conditions in which it occurs than to conditions elsewhere. Plasticity and local adaptation are not exclusive, as a locally adapted form may be locally adapted through having more or less plasticity to a certain factor. Both adaptive plasticity and local adaptation can be characterized by reaction norms, changes in a genotype's phenotype and performance across a range of habitats.

The evolution of reaction norms depends in part on the genetic basis of phenotypes, gene flow, and site predictability and quality (e.g. Via and Lande, 1985; Van Tienderen, 1991; Kawecki and Stearns, 1993; Sultan, 1995; De Jong, 1995, 1999; Schlichting and Pigliucci, 1998; Sultan and Spencer, 2002; Donohue, 2003). Plastic reaction norms can evolve if there is a predictive cue to future conditions and if there is genetic variation for the ability to produce an appropriate phenotype in response to the cue. Gene flow from genotypes that perform well in certain sites to sites where these genotypes do not perform as well can prevent evolution towards optimal phenotypes in the latter sites (Kirkpatrick and Barton, 1997; Stanton and Galen, 1997; Sultan and Spencer, 2002). Unpredictable future conditions or site quality can disrupt selection for plasticity, and instead maintain variation in traits (Tufto, 2000; Sultan and Spencer, 2002). Thus, understanding the spatial scale at which organisms respond to environmental heterogeneity is essential for an understanding of the evolution of reaction norms.

The scale of response to environmental heterogeneity, and therefore the capacity for the evolution of either locally adapted phenotypes or genotypes expressing high levels of phenotypic plasticity, may differ among species or genotypes depending upon the factors to which they are sensitive (Antonovics *et al.*, 1987). One way to determine the scale on which plants experience environmental heterogeneity is to measure the spatial autocorrelation of fitness and phenotype of a set of replicated genotypes, or 'phytometers'. A number of experiments employing phytometers have found spatial autocorrelation of phenotypes on small scales of a few metres, with greater variability at greater distances (e.g. Antonovics *et al.*, 1987; Stratton and Bennington, 1998; Juenger and Bergelson, 2002). Different environmental factors vary and covary at different scales (Caldwell and Pearcy, 1994). Sensitivity to environmental heterogeneity may also depend upon the expression of plastic phenotypes. For example, plastic shade avoidance may increase sensitivity to drought or nutrient stress (Maliakal *et al.*, 1999; Huber *et al.*, 2004). Furthermore, early elongation, in response to neighbouring plants, overhead canopy or leaf litter, which casts a low ratio of red to far red light similar to foliage shade (Bliss and Smith, 1985), may limit the subsequent ability of plants to elongate (Weinig and Delph, 2001; von Wettberg and Schmitt, 2005; E. von Wettberg and J. Schmitt, unpublished data). Thus plasticity to one environmental factor may influence the capacity for local adaptation to another factor, and early plastic responses to one cue may affect subsequent plastic responses to the same cue.

The well-documented phenotypic plasticity and local adaptation in *Impatiens capensis* and its congener *Impatiens pallida*, annual forest understory herbs native to eastern North America, make it possible to study the interaction between plasticity and fitness under varying environmental conditions experimentally with field transplantation. There is considerable evidence for adaptive plasticity, particularly elongation and suppressed branching in response to neighbour density and foliage shade ('shade avoidance') in *Impatiens* (Wulff, 1989; Weiner *et al.*, 1990; Dudley and Schmitt, 1995, 1996; Donohue and Schmitt, 1999; Donohue *et al.*, 2000a,b, 2001; Schmitt *et al.*, 2003). Plastic shoot elongation in response to foliage shade increases sensitivity to water and nutrient stress (Maliakal *et al.*, 1999; Huber *et al.*, 2004), factors that are also important determinants of plant fitness (Schoen *et al.*, 1986; Bell and Lechowicz, 1991; Lechowicz and Bell, 1991). All three of these factors vary on small microgeographical scales of centimetres to

metres (Schoen *et al.*, 1986; Bell and Lechowicz, 1991; Lechowicz and Bell, 1991), as do the strength and direction of selection on plant traits (e.g. Stewart and Schoen, 1987; Brassard and Schoen, 1990; Bell *et al.*, 1991; Huber *et al.*, 2004).

There is also evidence for adaptive population differentiation, or local adaptation, in *Impatiens* between open and closed canopy sites (Schemske, 1984; Schmitt, 1993; Dudley and Schmitt, 1995; Donohue and Schmitt, 1999; Donohue *et al.*, 2001; Schmitt *et al.*, 2003), and between dry and wet sites (Bennington and McGraw, 1995; Heschel and Hausmann, 2001; Heschel *et al.*, 2002). Trade-offs in performance result from the differential fitness of plant architectures in different shade and water availability regimes (Weiner *et al.*, 1990; Dudley and Schmitt, 1996) and a cost to elongating in response to shading (Dudley and Schmitt, 1996; Donohue *et al.*, 2001; Huber *et al.*, 2004). There is also evidence for microgeographic genetic differentiation (Schoen and Latta, 1989; Argyres and Schmitt, 1991) and limited gene flow in *Impatiens* (Schmitt *et al.*, 1985; Knight and Waller, 1987), which suggests the potential for fine-scale microgeographic local adaptation. In addition, there is some evidence for a decline in fitness of offspring with dispersal from the parental site (Schmitt and Gamble, 1990), but this experiment did not distinguish local adaptation from site quality because genotypes were not replicated across all sites. The observed declining fitness with distance from the parental site could have occurred due to a decline in site quality with dispersal distance if the parents were sampled from above-average sites.

Here we ask at what scale *Impatiens* experiences local environmental heterogeneity. By estimating spatial autocorrelation in environmental factors and the performance of replicated phytometers (transplanted genotypes), it is possible to determine the scale at which phytometer performance varies. Within the limits of an experiment designed to assess the frequency of selection for different elongation phenotypes (Huber *et al.*, 2004), we ask whether the spatial scale of similar performance differs among a subset of genotypes and whether it depends on the expression of shade avoidance phenotypes. Secondly, we ask whether fitness declines with dispersal distance from the parent as observed by Schmitt and Gamble (1990). If fitness does decline with distance from the parent, does it do so because of local adaptation or because of decreasing site quality with increased dispersal distance? Moreover, does the relationship of offspring fitness with dispersal distance vary depending on the shade avoidance phenotype expressed by the offspring?

METHODS AND MATERIALS

Study species

Impatiens capensis Meerb. (Balsaminaceae) is an annual, self-compatible herb of North American deciduous forests and wetlands (Gleason and Cronquist, 1963; Leck, 1979, 1996). It has a mixed mating system, commonly producing self-fertilizing cleistogamous flowers as well as outcrossing chasmogamous flowers (Waller, 1979). In the forest inbreeding is prevalent, and there are only occasional outbreeding events under especially favourable conditions. Seeds usually disperse less than 1.5 m from parent plants (Schmitt *et al.*, 1985), and pollinators typically transfer pollen within 3 m (Dube, 1988). In summer 1996, seedlings were collected from a 40 × 40 m permanent grid (Schmitt and Gamble, 1990; Argyres and Schmitt, 1991) in the centre of a population in the understory of an oak–hickory forest at Brown University's Haffenreffer Reserve (Bristol, Rhode Island). These seedlings were grown in the greenhouse to start a collection of inbred lines. These lines were maintained in the greenhouse by single-seed descent for six generations.

Precultivation of seedlings

From December 1999 to January 2000, seventh-generation selfed seeds were collected from eight genotypes (Huber *et al.*, 2004). These seeds were stored in microtitre trays filled with distilled water at 4°C. On 11 April 2000, the seeds, most of which had emerging radicles, were planted into eight plug trays filled with Metromix 350 (Scotts-Sierra Horticultural Products Co., Marysville, OH, USA). The 28 × 55 cm plug trays had 128 cells (in 8 rows and 16 columns), each yielding an average distance between seedlings of 3 cm. One seed per genotype was planted in a randomized position into each row. The total number of seeds planted was 880 (8 lines, 2 pre-treatments, 55 replicates). The trays were placed in the Brown University greenhouse under natural light conditions.

After 10 days, when almost all seedlings had emerged and produced their first true leaf pair, we imposed a red to far red light ratio manipulation. The red:far red manipulation pre-treatments simulated two different levels of initial shading from neighbours that seedlings might experience in natural populations. Four trays were allocated to each of the two pre-treatments, to induce two different phenotypes. Half of the seedlings were grown under a plastic panel designed to remove far red light [Mitsui Chemical Inc. Tokyo, Japan (Huber *et al.*, 2004)], thus artificially maintaining a high red:far red ratio, and keeping elongation from being induced in the plants (e.g. Ballaré *et al.*, 1991; Donohue and Schmitt, 1999). The panel reduced photosynthetically active radiation by 30%. The remaining trays were placed under a clear plastic panel covered with cheesecloth to equalize the total amount of radiation intercepted by the plants in the two pre-treatments. In this treatment, the normal reduction of red:far red light at high seedling density induced elongation. Plants subjected to the high and low red:far red treatments will henceforth be referred to as 'non-elongated' and 'elongated', respectively. We surrounded trays in both pre-treatments with sheets of tin foil to reflect lateral radiation and reduce edge effects. The trays were rotated within pre-treatments every 3–4 days. On 1 May, one day before transplanting, we measured seedling height, length of the longest leaf and number of nodes. Adjusted for germination date, which has a significant effect on early size, the mean height of elongation-induced seedlings was 10.79 cm (± 0.09) and that of non-elongated seedlings was 4.67 cm (± 0.09).

Field experiment

On 2 and 3 May 2000, we planted seedlings into 50 microsites at the site of Schmitt and Gamble's (1990) experiment at randomly chosen positions within the initial collection grid (Huber *et al.*, 2004). The dimensions of each microsite were 60 × 60 cm. In each microsite, one elongated and one non-elongated seedling of each of the eight genotypes was planted into a randomized position within a 4 × 4 checkerboard array and each was marked with a plastic-coated wire ring around its base. Background vegetation, including naturally occurring *Impatiens* seedlings, was not removed. Spacing experimental plants at 20 cm minimized interaction between the plants. Plants dying within the first 3 days after planting were replaced with seedlings from the same genotype and pre-treatment whenever possible. Due to variable germination among lines, elongated and non-elongated pairs of each genotype could not be replicated in all 50 microsites. For each genotype, one plant from each pre-treatment was placed into at least 40 of the 50 microsites, giving a total of 770 experimental plants. Assignment of genotypes to microsites was random and each microsite contained

pre-treatment pairs from at least six lines. Throughout the experiment we counted number of flowers, immature fruits, mature fruits and pedicels every 10 days to obtain an estimate of lifetime fitness (Donohue and Schmitt, 1999). Absolute fitness was measured as the total number of mature fruits produced through the season. We corrected absolute fitness for differences in germination time by taking the residual of a regression of fitness on germination date, and used this value for all subsequent analyses (Huber *et al.*, 2004). Mortality was scored twice a week until 10 August and thereafter once a week until the last plant had died.

Environmental measurements

We measured environmental variables found to affect fitness in previous experiments with *Impatiens* (i.e. Schoen *et al.*, 1986; Stewart and Schoen, 1987; Brassard and Schoen, 1990; Lechowicz and Bell, 1991; Bell *et al.*, 1991). On 26 April, the number of naturally occurring seedlings of *I. capensis* was counted in each of the 50 microsites to estimate local density. Water availability for each microsite was measured as water vapour pressure deficit on 21 June, 22 June, 7 July and 24 July using a soil tensiometer (2900 FI, Soil Moisture Equipment Corp., Santa Barbara, CA). Water vapour pressure readings were performed in the uppermost 6 cm of the soil because *I. capensis* has a shallow root system (Heschel and Hausmann, 2001). These tensiometer readings covered a wide range of water availabilities experienced by plants during the growing period, ranging from ample water supply in early summer to water deficit in late July. We used the first principal component of the four readings to summarize them into a single measure of water availability.

On a clear and sunny day in the middle of June, we measured light availability in the herbaceous understory using an AccuPAR ceptometer (Decagon, Washington, USA). For each microsite we measured the light profiles in the plots with the ceptometer held parallel to the soil surface, and integrated the PAR measurements done by the 80 sensors of the 80 cm long probe to one average value. Light measurements were taken every 10 cm up to 1 m above soil level. We took the first principal component of these measurements to describe the understory light levels.

We quantified overhead canopy openness above the herbaceous understory by means of canopy images. A hemispheric image was taken above the centre of each microsite after all experimental *I. capensis* plants had died in the middle of September. Taking the picture in September allowed us to measure microsite canopy openness after canopy closure without disturbing the experimental plants during the experiment. The picture was taken with a Nikon Coolpix 950 Digital camera to which a Nikon FC-E* Fisheye Converter was attached. Images were taken late in the afternoon to avoid interference by direct sunlight. Canopy openness was quantified as the percentage of total light transmitted through the canopy (calculated as the sum of direct and indirect light) using Winphoto, v 5.0 (ter Steege, 1996).

To estimate nutrient availability, we took soil samples from the uppermost 5–10 cm at five to eight different locations (depending on soil depth) in each microsite. It was impossible to take soil samples within the microsites during the experiment, because this would have caused too much disturbance and could have affected plant growth and performance. Therefore, we took the soil samples in June 2001. We took the soil samples at the peak of the *Impatiens* growing period to avoid over- or underestimation of nutrient availability due to seasonal changes in nutrients. The samples of each microsite were air dried and sent to the Soil Testing Laboratory of the University of Massachusetts (Amherst, MA, USA)

for analysis. Availability of the macronutrients P, K, Ca, Mg, NH₄ and NO₃ was measured in parts per million.

Statistical methods

We used the R package (Casgrain and Legendre, 2001, 2002) for analyses of spatial autocorrelation. To determine on what scale parameters are spatially autocorrelated, one compares a variable (Z) against a distance matrix with a number of distance classes determined by Sturge's and Yule's rules (Casgrain and Legendre, 2001). We used a Euclidean distance matrix of the coordinates of each plot, while the Z variable was the parameter of interest. Following Sturge's and Yule's rules, we used 12 equidistant distance classes. The extent of spatial autocorrelation can be summarized by Moran's I , a number that falls between -1 and $+1$. Negative spatial autocorrelation occurs towards -1 and positive autocorrelation towards $+1$. Because of the number of distance classes being used, we applied a Bonferroni correction to the significance of spatial autocorrelation. Because Bonferroni corrections may be unnecessarily conservative, we also report significant spatial autocorrelations that did not remain so after correction. We performed this analysis with the AUTOCOR function of the R package. For regular correlation analysis of environmental variables, we used PROC CORR in SAS 8.0.

We analysed the relationship between the environmental factors and absolute fitness with a mixed model (PROC MIXED in SAS). Denominator degrees of freedom for F -tests were determined by Satterthwaite approximation (the 'DDFM = SATTERTH' statement in Proc Mixed). To determine whether the relationship of distance from the parental site, site quality, or any of the environmental factors measured varied between genotypes, we performed mixed models on these factors with genotype as a class variable and fitness corrected for initial size as the dependent variable. The multiple environmental factors measured (see above) were condensed into a single variable by taking the first principal component, which accounted for 95.79% of the variation in the factors (Table 1). For cases where we detected statistically significant interaction terms between pre-treatment and an environmental factor, we output and compared solutions for slopes (beta terms) to the factors split by pre-treatment.

Table 1. Loadings of environmental factors on the first principal component used as a summary measure of environmental quality

Environmental factor	Loading on first principal component
Natural <i>Impatiens</i> seedling density	0.494334
Nitrates	0.492677
Water vapour deficit	-0.476452
Light	-0.373799
Soil depth	-0.275718
Percent overhead canopy openness	0.209327
Phosphorus	0.162272

Note: The first principal component accounted for 95.79% of the variation.

To test for the relationship of fitness with distance from original collection site (i.e. local adaptation), we regressed absolute fitness on the distance of each individual genotype within each microsite from the site where that genotype was originally collected. Because some sites might be better *Impatiens* sites due to factors we did not measure, we calculated the average number of fruits produced per plant in each site across the experiment, and calculated the deviation of each site's mean fruit production from this grand mean as the 'site quality'. This phytometer-based measure of site quality was inspired by joint regression analysis, although it is not the same because we used phenotypic values rather than genotypic means in each microsite (e.g. Yates and Cochran, 1938; Finlay and Wilkinson, 1963; Perkins and Jinks, 1968a,b; Zuberi and Gale, 1976; Mather and Jinks, 1982; Falconer and Mackay, 1996, pp. 133–134). Using the deviation of each site's average fruit production from the grand mean of per site fruit production as a covariate in our analysis of the effect of site quality on local adaptation avoids the problem of using fitness (i.e. fruit production) as both a response variable and a covariate. We examined the correlation between distance from parental site and site quality with PROC CORR. We also looked at the correlation between site quality from this experiment and the collection sites used by Schmitt and Gamble (1990) to determine if a correlation between distance from collection sites and site quality could be responsible for the fine-scale local adaptation observed in that study.

To determine if our design had the power to detect the signal of local adaptation reported by Schmitt and Gamble (1990), we performed a power test using G*Power software (Buchner *et al.*, 1997). Instead of performing a *post-hoc* power test, we used the means and standard deviations reported by Schmitt and Gamble (1990) to determine our effect size, and reclassified our distance classes to correspond to the distance classes used in their experiment.

To establish whether the effects of site quality on local adaptation were due to specialization on sites of certain quality or particular environmental factors, we used a technique from plant breeding to identify genotypic responsiveness to different sites (Finlay and Wilkinson, 1963; Falconer and Mackay, 1996). We plotted the slope of the linear regression of a genotype's fitness in each site on site quality against a genotype's mean absolute fitness across sites. Genotypes with a high mean absolute fitness may be well adapted across sites or do extremely well in certain sites. Those with low mean absolute fitness may do poorly across all sites, or well in some sites but very poorly in others. Genotypes with positive regression coefficients respond to increasing site quality with increased reproduction indicating plasticity to environmental quality, and are therefore specialists on good sites. Genotypes with negative coefficients may be adapted to poor quality sites. Genotypes with little responsiveness to site quality would be generalists. We plotted regression coefficients versus average genotype fitness for both elongated and non-elongated plants. We calculated Pearson's and Spearman's correlation coefficients of the relationship of the regression coefficients between light pre-treatments in SAS PROC CORR.

Because the variance of the genotypic fitness averaged across environments is dominated by the environments with largest variance, we tested for a correlation between variance and mean fitness in a site with PROC CORR. A significant positive correlation would suggest that genotypes with highest fitness across sites will be most fit in the environment where the variance is greatest, and will tend to have the highest average fitness, even if they perform poorly elsewhere. Such a result indicates that variance of fitness is greatest in the good environments.

RESULTS

Scale of environmental heterogeneity

Taken individually, water availability (Moran's $I = 0.687$, $P < 0.001$; Fig. 1), light availability in the understory (Moran's $I = 0.546$, $P = 0.001$; Fig. 1), canopy openness (Moran's $I = 0.347$, $P = 0.018$; Fig. 1) and nitrates (Moran's $I = 0.8084$, $P < 0.001$; Fig. 1) were positively spatially autocorrelated at a distance of up to 3.9 m, with water (Moran's $I = 0.275$, $P = 0.003$; Fig. 1) and canopy openness (Moran's $I = 0.2607$, $P = 0.003$; Fig. 1) also being significantly autocorrelated on a larger scale of up to 7.8 m. The density of naturally occurring *Impatiens capensis* seedlings (Moran's $I = 0.594$, $P < 0.001$) was also significantly autocorrelated in the smallest distance class of up to 3.9 m. Spatial autocorrelation sharply decreased at greater distances. For water availability we found a significant negative autocorrelation at a distance of 35.1–39.0 m (Moran's $I = -0.355$, $P = 0.001$; Fig. 1), suggesting a spatial gradient across the site. Calcium (Moran's $I = 0.253$, $P = 0.045$), magnesium (Moran's $I = 0.243$, $P = 0.052$) and mean soil depth (Moran's $I = 0.277$, $P = 0.045$) were marginally positively spatially autocorrelated in the first distance class, but not significantly so after Bonferroni correction. For ammonium and potassium, we found no evidence for spatial autocorrelation in any distance class.

When resource levels were summarized by a single principal component, with high loadings from surrounding natural *Impatiens* seedling density, nitrates, water vapour deficit, light, soil depth, overhead canopy openness and phosphorus (Table 1), resource levels were highly spatially autocorrelated in the distance class of up to 3.9 m (Moran's $I = 0.959$, $P < 0.001$; Fig. 1), with significant spatial autocorrelation at distances of up to 11.7 m (4.0–7.8 m, Moran's $I = 0.253$, $P = 0.005$; 7.9–11.7 m, Moran's $I = 0.231$, $P = 0.001$). However, the strength of autocorrelation was less in larger distance classes. There was also

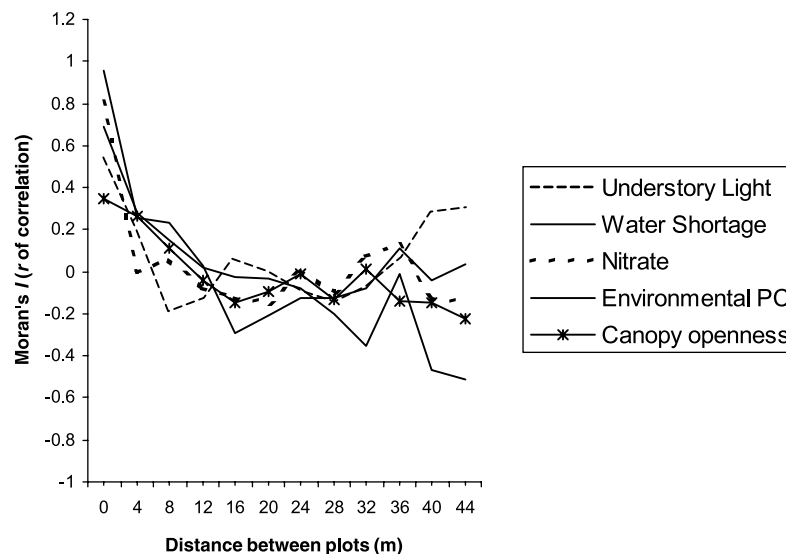


Fig. 1. Pattern of autocorrelation of environmental factors. Distances are between microsites, and autocorrelation values are calculated as Moran's I . Positive values of Moran's I indicate positive spatial autocorrelation, whereas negative values indicate negative spatial autocorrelation.

weak but significant negative spatial autocorrelation at a distance of 14.6–22.4 m (14.6–17.5 m, Moran's $I = -0.295$, $P = 0.001$; 17.6–22.4 m, Moran's $I = -0.205$, $P = 0.003$), although the value of the correlation was not very high despite being significant. These results show that, at least for factors likely to be most important in influencing *Impatiens* fitness, resources were patchily distributed on a scale similar to seed and pollen dispersal distances. Conditions were predictably comparable at short distances up to about 4 m, while conditions either became unpredictable or dissimilar at greater distances.

Water availability and nitrates were both positively correlated with natural *Impatiens* seedling density, as was canopy openness and nitrates (Table 2). Water availability and light levels were negatively correlated (Table 2). No other factors were significantly correlated (Table 2).

The overall fitness of all lines in both light pre-treatments was spatially autocorrelated in the smallest distance class (0–3.9 m, Moran's $I = 0.3215$, $P = 0.020$; Fig. 2). Across genotypes, both the fitness of non-elongated (Moran's $I = 0.2656$, $P = 0.042$) and elongated plants (Moran's $I = 0.3774$, $P = 0.009$) was spatially autocorrelated in the smallest distance class. Spatial autocorrelations declined in greater distance classes. The pattern of spatial autocorrelation, however, differed by genotype and depended on the initial phenotypes of the plants: depending on the respective genotype, different spatial autocorrelation occurred throughout the range of distance classes (Fig. 2a,b). Site quality, measured with a phytometer approach, was not spatially autocorrelated in any distance class.

Relationship of plant fitness and environmental parameters

Genotypes did not respond equally to changes in resource levels. There were significant interactions between genotype and the first environmental principal component (Table 3), genotype and water vapour deficit, genotype and nitrates, and a marginally significant interaction between genotype and light (Table 3). Genotypes differed significantly in fitness responses to resource level (first environmental factor principal component), although mean fitness increased significantly with resource level (Table 3). Increased water availability,

Table 2. Pearson correlation coefficients and their significances between environmental parameters

Environmental factor	Water vapour deficit	Light level	Nitrates	Natural <i>Impatiens</i> seedling density
Water availability	1			
Light level	$r = -0.365$ $P = 0.01$	1		
Nitrates	$r = 0.213$ $P = 0.142$	$r = -0.078$ $P = 0.589$	1	
Natural <i>Impatiens</i> seedling density	$r = 0.464$ $P < 0.0001$	$r = -0.205$ $P = 0.154$	$r = 0.540$ $P < 0.0001$	1
Canopy openness	$r = 0.172$ $P = 0.237$	$r = 0.098$ $P = 0.502$	$r = 0.319$ $P = 0.026$	$r = 0.204$ $P = 0.160$

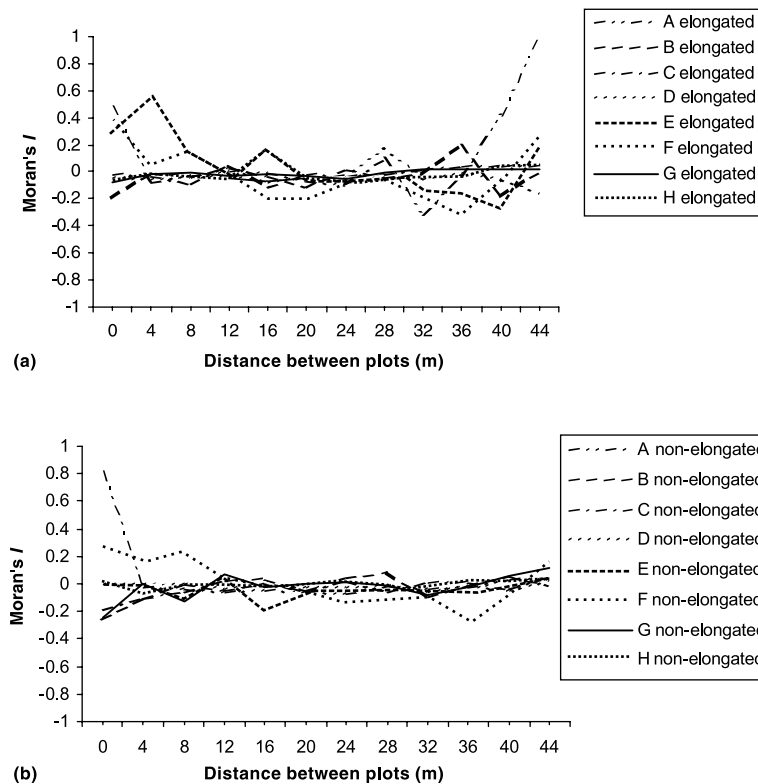


Fig. 2. Scale of autocorrelation of absolute fitness for the eight genotypes separately. Distances are between microsites, and autocorrelation values are calculated as Moran's I . We show both (a) non-elongated plants and (b) plants induced by pre-treatment to elongate.

nitrate and light all increased fitness, but did not do so equally for all genotypes (Table 3). We did not find significant genotype by environment interactions for the other factors.

We determined that plants in both the elongation-induced and non-induced pre-treatments performed better in high resource sites, particularly sites with high water availability, by comparing estimated slopes between the pre-treatments with a mixed model. Plants in the elongation-induced treatment had a greater response to resource level (slope = 1.583, $P < 0.0001$) than plants in the non-elongated treatment (slope = 0.8573, $P = 0.0075$). Plants in the elongated pre-treatment responded more to soil moisture availability (slope = 2.159, $P < 0.0001$) than the non-elongated plants (slope = 1.101, $P < 0.0001$). Responses to light were significant and equal between the elongated (slope = -0.5721, $P = 0.0051$) and non-elongated pre-treatments (slope = -0.5964, $P = 0.0042$).

Effect of distance from site of collection and site quality: local adaptation or site effects?

There was no significant main effect of distance from site of parental collection (Table 4), suggesting no overall linear pattern of local adaptation. Site quality, however, had a mar-

Table 3. Environmental factors with differential effects on fitness among the eight genotypes

Effect	Numerator d.f.	Denominator d.f.	<i>F</i> -value	Pr > <i>F</i>
Resource level				
Pre-treatment (P)	1	725	0.84	0.3611
Genotype (G)	7	725	2.56	0.0131
Resource level (R)	1	725	31.07	< 0.0001
G × P	7	725	1.15	0.3269
R × P	1	725	2.73	0.0988
R × G × P	7	725	0.48	0.8527
Water availability				
Pre-treatment	1	725	0.87	0.3500
Genotype	7	725	2.81	0.0067
Water availability (W)	1	725	81.74	< 0.0001
G × P	7	725	1.23	0.2855
W × P	1	725	8.79	0.0031
W × G	7	725	2.38	0.0205
W × G × P	7	725	2.69	0.0093
Light				
Pre-treatment	1	725	0.91	0.3413
Genotype	7	725	2.38	0.0205
Light availability (L)	1	725	16.28	< 0.0001
G × P	7	725	1.13	0.3444
L × P	1	725	0.00	0.9928
L × G	7	725	0.74	0.6364
L × G × P	7	725	0.88	0.5201
Nitrates				
Pre-treatment	1	725	0.03	0.8703
Genotype	7	725	1.89	0.0691
Nitrate level (N)	1	725	0.16	0.6921
G × P	7	725	1.04	0.4015
N × P	1	725	0.14	0.7130
N × G	7	725	0.86	0.5397
N × G × P	7	725	0.74	0.6396

Note: Homogeneity of response was measured by the significance of the genotype × factor term in a type III mixed model in SAS (version 8).

ginally significant main effect on fitness (Table 4). Genotypes did not vary significantly in their responses to distance or site quality, but there was a significant three-way interaction between site, genotype and pre-treatment (Table 4). Plants in both pre-treatments performed better in sites with high site quality (elongated: slope = 2.7275, $P < 0.0001$; non-elongated: slope = 2.509, $P < 0.0001$). Distance from parental site was not correlated with site quality (Pearson's $r = 0.0339$, $P = 0.338$; Spearman's $r = 0.0099$, $P = 0.782$). Distance from parental sites in the Schmitt and Gamble (1990) experiment were also uncorrelated with site quality as measured in this experiment (Pearson's $r = -0.009$, $P = 0.744$; Spearman's $r = -0.0317$, $P = 0.2629$).

Table 4. Effects of site quality and distance from site of parental collection on plant fitness

Effect	Numerator d.f.	Denominator d.f.	F-value	Pr > F
Pre-treatment (P)	1	700	0.16	0.6867
Genotype (G)	7	700	1.26	0.2688
Distance (D)	1	700	0.00	0.9521
Site quality (S)	1	700	3.41	0.0651
G × P	7	700	0.64	0.7260
D × G	7	700	0.86	0.5402
D × P	1	700	0.18	0.6742
S × P	1	700	0.08	0.7709
S × G	7	700	1.51	0.1606
D × S	1	700	0.01	0.9334
D × G × P	7	700	0.44	0.8792
S × G × P	7	700	2.13	0.0387
D × S × P	1	700	0.25	0.6139
D × S × G	7	700	1.38	0.2093

Note: Homogeneity of response was measured by the significance of the genotype × factor term in a type III mixed model in SAS (version 8).

Schmitt and Gamble (1990) reported a mean and standard deviation in absolute fitness of 6.96 ± 0.71 within 3 m and 5.81 ± 0.85 over 12 m, which we used to calculate an effect size of 0.6110 in a power analysis. With 10 data points within 3 m and 30 data points within 2 m of 12 m, our power to detect a comparable fitness decline with increasing distance from the parental site is 0.9558 at $\alpha = 0.05$. With these sample sizes, our critical value is $F_{1,36} = 4.1132$ and our lambda is 14.1862. We therefore had adequate power to detect a signal of local adaptation if it were present.

Patterns of specialization

We found no evidence of genotypes specialized in poor quality sites or of generalists that performed well across sites using the method of Finlay and Wilkinson (1963). Instead, the genotypes with the highest average fitness were those that responded most to high site quality, not those that performed well across all sites or those that performed well in low quality sites (Fig. 3). There was no significant correlation of ranking of genotypes in the different light pre-treatments (Pearson's $r = -0.273$, $P = 0.513$; Spearman's $r = -0.238$, $P = 0.570$). This suggests that no genotypes were more fit under both light pre-treatments and that high fitness under one pre-treatment is not associated with poor performance under the other. Sites with higher fitness had significantly higher variance in fitness (elongated pre-treatment: $r = 0.687$, $P < 0.0001$; non-elongated pre-treatment: $r = 0.902$, $P < 0.0001$).

DISCUSSION

Scale on which environmental heterogeneity is experienced

We found significant spatial autocorrelation of several important environmental factors at a distance of less than 4 m in this population of *Impatiens capensis*. Although many factors

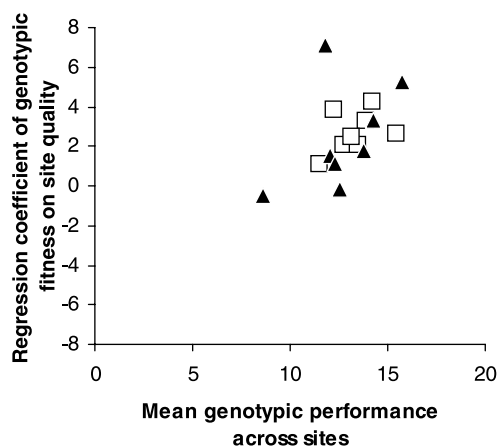


Fig. 3. Relationship of the regression coefficients from the regression of pre-treatment $\times$ genotype absolute fitness on site quality with the mean pre-treatment $\times$ genotype fitness. Regression coefficients come from a regression of 50 points per genotype and pre-treatment combination. $\square$, elongated pre-treatment; $\blacktriangle$, non-elongated pre-treatment.

were predictable at small spatial scales, most factors were not predictable at larger distances and did not covary strongly with each other. Long-term microsite monitoring over 7 years (M.S. Heschel, unpublished data) suggests that the ranking of sites by quantity of light is more variable between years than by soil quality, particularly water availability. Although water levels vary with years of drought, the relative rankings of sites remain constant (M.S. Heschel, unpublished data). Light levels, conversely, may vary between years due to largely unpredictable patterns of branch and tree falls. Gaps open stochastically, creating new high light patches, then predictably close over time.

We found significant spatial autocorrelation of fitness at the same small distances that environmental factors were spatially autocorrelated. There was variation among genotypes, and between elongated and non-elongated phenotypes, in the extent of spatial autocorrelation. This observation suggests that genotypes respond differently to environmental factors, and that these responses vary depending on the expression of shade-induced elongation at an early developmental stage. Similar to previous experiments (Maliakal *et al.*, 1999) and other analyses of this data set (Huber *et al.*, 2004), we found that plants that have allocated more to above-ground elongation are more sensitive to water shortage. This may stem from reduced allocation to roots in elongated plants. Regardless of the mechanism, the different patterns of selection for local adaptation to soil conditions and for adaptive plasticity to crowding could lead to counteracting selective forces for elongation and for increased allocation to roots.

Counteracting selective forces on early life elongation responses may be common, as differences in initial elongation probably occur regularly in *Impatiens*. Differences in early elongation may occur in response to either shading by neighbours or the forming of overhead canopy, or in response to varying levels of leaf litter on the forest floor. Leaf litter lowers the ratio of red to far red light similar to vegetative foliage (Bliss and Smith, 1985), can cause elongation of *Impatiens* hypocotyls (J.R. Stinchcombe and J. Schmitt, unpublished; E. von Wettberg and J. Schmitt, unpublished), and can affect the ability of plants to avoid shade later in life (E. von Wettberg and J. Schmitt, unpublished). Furthermore, some of the genotypes used in this experiment were

found to respond to supplemental far red light (simulating a low red to far red shaded environment) added to their first internodes or first leaves with suppressed elongation (i.e. a high irradiance response) rather than a shade avoidance elongation response (von Wettberg and Schmitt, 2005).

Fitness and distance from parental site: site quality and local adaptation

Schmitt and Gamble (1990) previously observed that fitness decreases with dispersal distance from parental sites in the same *Impatiens* population used in the present experiment. Fitness of transplants was high within 3 m of the parent, and decreased at 12 m from the parental site. In that experiment, seedlings of 25 parents were transplanted to different distances from the original parental microsite, but genotypes were not replicated across sites, so local adaptation could not be distinguished from spatial variation in site quality as possible explanations for the observed decline in fitness with dispersal distance. Although the present experiment was not designed explicitly to test for local adaptation, the replication of eight genotypes across microsites in the study of Schmitt and Gamble (1990) allowed us to explore the possible mechanisms underlying their result. If the observed decline in fitness with dispersal distance from the parent were due to a decline in average microsite quality (which might occur if parents were originally sampled from above-average sites), we would expect to see a positive correlation between dispersal distance and average microsite quality as measured in our experiment. However, we found no correlation between site quality and distance from the parental sites in our experiment or from the parental sites in the Schmitt and Gamble (1990) experiment. As site quality is largely determined by water level, which does not change in rank from year to year (Schmitt *et al.*, 2003; M.S. Heschel, unpublished data), site quality in the present experiment is likely to be similar to site quality in the experiment 15 years ago. Thus, our data suggest that an unmeasured correlation of distance with site quality cannot explain the results of Schmitt and Gamble (1990), which leaves local adaptation as a likely explanation.

However, we found little direct evidence for local adaptation in this experiment, despite conditions that should favour local adaptation: the presence of fine-scale microgeographic variation in important environmental factors, genotypic differences in response to micro-environment, and limited dispersal. In contrast with Schmitt and Gamble (1990), we found no significant fitness effect of distance from the parental site. Our power analysis indicates that we had sufficient power to detect the general decrease in fitness with transplants beyond 12 m observed by Schmitt and Gamble (1990). Nevertheless, the design of the experiment is sufficiently different from that of Schmitt and Gamble (1990) that we cannot entirely reject local adaptation as a possible explanation for their result. It is possible that the eight genotypes used in this experiment had inadvertently been selected for adaptation to greenhouse conditions over several generations of propagation, with concomitant loss of adaptation to the original parental microsite. Our experiment was also performed on a slightly larger spatial scale, with many microsites over 12 m from the parental sites. This may indicate that sites become less predictable with increasing distance from the parental site, and that patches with a specific combination of microenvironmental conditions occur largely at random at great distances. It is also possible that local adaptation does not take a linear function, and that instead local adaptation occurs to patches with particular resource profiles that occur with complex patterns over a landscape. Sampling on small scales, such as done by Schmitt and Gamble (1990), may give local adaptation the appearance of linearity

that was not detected on the slightly larger scale of the present experiment. Our experimental design also necessarily bypassed the seed and seedling establishment stages, which may be critical components of local adaptation.

Swamping or specialization?

We did not find evidence of specialization to specific environmental factors by the genotypes used in this study. Although there were significant environmental factor by genotype interactions for water, light and nitrates for plants induced to elongate by a low red to far red light ratio, we were unable to find clear evidence that some genotypes were always doing better at particular levels of specific factors. Using the method of Finlay and Wilkinson (1963), we found little evidence for specialization on poor sites, as well as little evidence for generalist genotypes. Instead, it appears that the genotypes most responsive to increasing site quality are the most fit. If flooding or caching by animals spreads these seeds beyond their 3 m primary dispersal distance (Schmitt *et al.*, 1985), these genotypes could swamp the rest of a population by producing very high numbers of seeds. This would interfere with the formation of locally adapted genotypes specialized on levels of particular factors (Kirkpatrick and Barton, 1997). Measuring the average distance of secondary seed dispersal in *Impatiens* populations should reveal the potential for swamping to occur.

The positive correlation between fitness and variance in fitness shows that fitness is most variable in good sites. This observation could be due to more intense formation of size hierarchies (Weiner, 1990) in high quality sites due to greater early season growth and higher densities of surrounding *Impatiens* seedlings, or could result from there being more opportunity for variance in growth rate to be expressed.

CONCLUSIONS

Our results suggest that local adaptation in *Impatiens* is a complex phenomenon modulated both by adaptive phenotypic plasticity to predictable environmental heterogeneity for which there are clear phenotypic responses (e.g. Dudley and Schmitt, 1996; Schmitt *et al.*, 2003), and by complex environmental factors that vary on different spatial scales contributing differentially to site quality. Although genetic differentiation (Argyres and Schmitt, 1991; E. von Wettberg, D. Remington and J. Schmitt, unpublished data) and local adaptation (Schmitt and Gamble, 1990) may occur on small spatial scales up to 3 m in this population of *Impatiens capensis*, on larger scales (i.e. up to 50 m) this pattern becomes swamped by variability in site quality. On greater spatial scales, such as sunny, open, river bed populations, and forest understory populations, there is abundant evidence for genetic differentiation in this species (Schmitt, 1993; Bennington and McGraw, 1995; Dudley and Schmitt, 1995; Donohue and Schmitt, 1999; Donohue *et al.*, 2000a,b, 2001; Heschel and Hausmann, 2001; Heschel *et al.*, 2002). The relationship between ecological differentiation and isolation by distance needs to be clarified to place ecotype formation and within-habitat local adaptation into the context of evolutionary change in ecological time.

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