

POPULATION DIFFERENTIATION FOR PLASTICITY TO LIGHT IN AN ANNUAL HERB: ADAPTATION AND COST¹

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Phenotypic plasticity allows plants to cope with environmental heterogeneity. Environmental variation among populations may select for differentiation in plasticity. To test this idea, we used the annual plant *Geranium carolinianum*, which inhabits old fields that are densely vegetated and lack canopy cover and wood margins with tree shade but less neighbor shade. Individuals from three populations of each habitat were planted in natural low and high light environments, and morphological traits important for light acquisition were measured. Old-field plants were more plastic, with greater elongation of petioles and internodes in low light than those from wood margins. This larger shade avoidance response suggests evolution of greater plasticity to neighbor shade than to the tree canopy. Fitness of old-field plants was high across both light environments, whereas fitness of wood-margin plants was reduced in low light. Selection favored longer internodes in low than high light. Finally, plasticity for internode length was negatively associated with fitness in high light, suggesting a cost of plasticity for this trait. Together these results indicate that shade-avoidance plasticity of petiole and internode length is adaptive. However, greater elongation of internode length may be constrained by the cost of plasticity expressed in high light. The evolution of plasticity appears to reflect a balance between its adaptive nature and its cost to fitness.

Key words: cost of plasticity; *Geranium carolinianum*; Geraniaceae; internode length; petiole length; phenotypic plasticity; population differentiation; shade.

Phenotypic plasticity, that is, environmentally induced changes in trait expression, is ubiquitous in both plant and animal systems (Bradshaw, 1965; Schlichting, 1986; Sultan, 1987; Pigliucci, 2001). Plasticity has been proposed as an adaptation for organisms to cope with the fact that environments vary in space and time. To distinguish adaptive plasticity from passive responses to resource limitation (Sultan, 1987, 1995), explicit tests of the adaptive nature of plasticity have increasingly been the focus of rigorous study (e.g., Dudley and Schmitt, 1996; Winn, 1999; Weinig, 2000b; Caruso et al., 2006). One approach for determining whether plasticity is adaptive is to measure selection on phenotypic traits within specific environments (e.g., Kingsolver, 1995; Donohue et al., 2000; Steinger et al., 2003; Van Kleunen and Fischer, 2005). If selection differs between environments and the plastic response expressed within an environment is in the same direction as selection, the plasticity is adaptive.

Although plasticity may enhance fitness, organisms are not infinitely plastic. Therefore, it stands to reason that some factor(s) must constrain the evolution of plasticity (Via and Lande, 1985; Van Tienderen, 1991, 1997; Tufto, 2000; Sultan and Spencer, 2002). A likely candidate is a cost of plasticity (Van Tienderen, 1991; DeWitt et al., 1998), defined as the reduction in fitness of an organism capable of plasticity relative to an organism that produces the same phenotype via fixed development (Van Tienderen, 1991; DeWitt et al., 1998; Van Kleunen

and Fischer, 2005). Mechanisms that underlie the cost of plasticity include maintenance costs and production costs (reviewed in DeWitt et al., 1998). Despite recent intensive study, limited evidence for the cost of plasticity has been found (DeWitt, 1998; Tuccillo et al., 1998; Dorn et al., 2000; Agrawal et al., 2002; Relyea, 2002; Steinger et al., 2003; Caruso et al., 2006; reviewed in Van Kleunen and Fischer, 2005). However, only two studies have been conducted under natural field conditions (Donohue et al., 2000; Weinig et al., 2006), where the cost of plasticity is ecologically relevant and limited resources enhance the probability of detection (Van Kleunen and Fischer, 2005).

Costs and adaptive benefits of plastic responses may be most easily investigated for traits where the mechanism (e.g., phytochromes) of response to the environment is understood (Agrawal et al., 2002; Weinig et al., 2006). Plasticity in response to light availability and quality has been well studied (e.g., Ballaré et al., 1991; Schmitt, 1993; Sultan and Bazzaz, 1993; Andersson and Shaw, 1994; Ballaré et al., 1995; Pigliucci and Schlichting, 1995; Dudley and Schmitt, 1996; Huber, 1996; Tuccillo and Avramov, 1996; Dorn et al., 2000; Steinger et al., 2003). Elongation of petioles and stems are commonly observed responses to shading (“shade-avoidance”; Schmitt, 1993; de Kroon and Hutchings, 1995; Gautier et al., 2001). In erect and semierect plants, such elongation responses arguably allow for the optimal placement of leaves in horizontal (in the case of petioles) and vertical (internodes of stem) space for enhanced light interception (e.g., de Kroon and Hutchings, 1995). The expression of shade-avoidance responses enhances fitness under shaded conditions, but results in decreased performance when light is not limited (Dudley and Schmitt, 1996), indicating that shade-avoidance plasticity is adaptive.

However, the fitness benefits of plastic shade-avoidance responses are likely to be habitat specific. Elongation of petioles and internodes in response to shade from comparably sized neighbors will likely yield greater light interception, whereas similar responses in habitats with canopy shade will result in little change in light interception. Therefore, adaptive responses to reduced light may vary among populations, depending upon

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the habitat (e.g., Petit and Thompson, 1997; Weinig, 2000a; Donohue et al., 2001; Gianoli, 2004). The complexity of the light environment in natural habitats means that plant responses to these conditions may be different or more nuanced than those found under controlled conditions. However, there are relatively few tests of adaptive plasticity under natural conditions (but see studies of plasticity to neighbor shade: Winn, 1999; Donohue et al., 2000; Weinig 2000a). Only by studying populations in nature can we understand the effect of the complex environmental factors that influence adaptive evolution.

Here we focused on whether there is an association between plasticity and natural variation in light environments using *Geranium carolinianum*, an herbaceous annual that inhabits sites differing in light availability. We use a common-environment approach to address the following questions: (1) Do plants from sites with contrasting light availability differ in patterns of plasticity for traits important to light interception? (2) Does selection differ between open and under-canopy light environments? (3) Is plasticity to light adaptive? (4) Is there a cost to plasticity that may constrain its evolution?

MATERIALS AND METHODS

Study system and study populations—*Geranium carolinianum* L. (Geraniaceae) is a winter annual native to North America and is considered a weed of old fields, disturbed sites, and wood margins (Gleason and Cronquist, 1991). In Virginia, *G. carolinianum* germinates in October–November and overwinters as a rosette with about five leaves. It bolts in April and then is an erect plant with at least one main stem of several nodes and relatively compact inflorescences. Plants produce flowers and fruits from late April through June; flowers typically produce five seeds (Strausbaugh and Core, 1997). Although flowering is indeterminate, plant size changes little after the initiation of flowering. *Geranium carolinianum* readily self-fertilizes in the greenhouse and appears to be highly selfing under natural conditions (Roach, 1986; D. L. Bell, personal observation). Seeds disperse explosively and dispersal distances average 1.4 m (± 0.7 m; Bell, 2004).

Geranium carolinianum habitats differ in light availability. Six populations with contrasting light availability were chosen for study. Three populations were located in old fields and had relatively high light levels with no overhead canopy but a dense herbaceous cover composed primarily of grasses. Three populations were at wood margins that had patchy canopy shade, resulting in less available light, and a lower density of herbaceous plants than the open sites. At both wood-margin and old-field sites, the nearest neighbors of *G. carolinianum* were rarely conspecific (D. L. Bell, personal observation). For quantifying light availability in each site, photosynthetically active radiation (PAR) was measured monthly at ground level with a LICOR LI-250 light meter (LI-COR, Lincoln, Nebraska, USA) at 20 locations throughout each of the six sites over the course of the growing season for *G. carolinianum*. The wood-margin sites had lower average PAR than the old-field sites ($F_{1,4} = 333.88$, $P < 0.0001$). All six populations were located in Albemarle County, Virginia, within 30 km of each other, with no population closer than 2 km from another.

Experimental design—Seeds were collected in May–June from naturally occurring plants in each of the six populations. Plants were haphazardly chosen from throughout each population to maximize collection of seeds from different genetic individuals, with a total of approximately 20 families per population.

Field-collected seeds were grown in the greenhouse at the University of Virginia for two generations to ensure a uniform maternal environment. All families germinated and grew successfully, indicating relatively low amounts of inbreeding depression from the two generations of self-fertilization. Autogamous seeds from several maternal plants of the same family were pooled to create 10 self-sib families from each of the six populations, for a total of 60 families. Thirty-six seeds from each family were germinated. For inducing germination, each seed was individually scarified in early October with a wood file. Scarification breaks the hard seed coat (Baskin and Baskin, 1974), which decreases the variance in timing of germination such that phenology is fairly constant among families (D. L. Bell and E. Saunders (University of Virginia), unpublished data). Scarified seeds were individually placed in plug trays filled

with Pro-Mix (Premier Horticulture, Dorval, Quebec, Canada). Plug trays were placed on a misting bench in the greenhouse through cotyledon emergence and after 1 wk were moved to a greenhouse bench. After 1.5 wk, the seedlings were placed outside to acclimate to autumnal temperatures and were watered as needed.

To understand the effects of light availability on plant traits, we established “high” and “low” light plots in a site with variable light that is home to a natural *G. carolinianum* population (Mint Springs Valley Park, Albemarle County). In the autumn and winter, overall light is uniform across the site because there are no leaves on the trees (PAR $F_{1,38} = 1.99$, $P = 0.1660$). In the spring, after trees leaf out, an uneven canopy cover creates high and low light areas throughout the site (PAR low light 146 ± 99 , high light $1660 \pm 114 \mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$; $F_{1,38} = 2023.12$, $P < 0.0001$). We used the differences in PAR to establish low and high light plots and focus on spring and summer traits. Neighbor density was slightly greater in high light than low light plots (D. L. Bell, personal observation). Whereas the high light plots were similar to old-field habitats in terms of both canopy cover and neighbor shade, the low light plots had comparable canopy cover but a greater density of neighbors than most wood-margin populations (D. L. Bell, personal observation). In both low and high light plots, neighbors were primarily grasses mixed with occasional poison ivy vines (*Toxicodendron radicans*).

Plants were grown in a split-plot design in the field where a block included both high and low light plots. From mid-October to early November, seedlings (1–2 true leaves) were transplanted into six paired high and low light plots. Three seedlings from each family were transplanted into random positions in each plot at each light level, for a total of 2160 seedlings (six populations \times 10 families/population \times three plants/family/light treatment \times six blocks \times two light treatment/block). Each experimental seedling was 10 cm from any other seedling in a regular grid pattern such that each plot measured 1.0×1.9 m. Seedlings were watered when transplanted. Thereafter, plants were watered once a week for a month because of severe drought conditions. Seedlings that died from transplant shock were replaced 2 weeks after initial planting. No further manipulation of experimental seedlings or extant vegetation was done.

Morphological traits were measured on bolted plants. Leaf number was recorded at flowering. At the end of flowering (late June), the length of the longest petiole at the first and second nodes, the length of the first two internodes on the main stem, and the number of fruits produced (to estimate fitness) were measured for each plant. Most plants (76.4%) survived, with most mortality before bolting; analyses only included surviving individuals.

Statistical analysis—*Patterns of plasticity*—Multivariate analysis of variance (MANOVA) was used to test for differentiation in plasticity between plants from old-field and wood-margin sites for traits important for light interception, including petiole length at node 1, petiole length at node 2, and the lengths of internode 1 and internode 2. The main effects of light, site type, population nested in site type, family nested in population and site type, block and the interactions light \times site type, light \times population(site type), and block \times light were tested (Proc GLM, SAS version 9.1; SAS Institute, Cary, North Carolina, USA). Population, family, and block were considered random variables; therefore, site type was tested over population(site type), population(site type) was tested over family(population \times site type), and light \times site type was tested over light \times population(site type). All other effects were fixed. Because of the split-plot design, light and block were tested over the main plot error term (block \times light). Additional interactions were only tested between light and both site type and population because of the highly nested design. A canonical analysis was conducted to illustrate the contribution of each of the variables to the light \times site type interaction. The canonical structure reflects the correlation between each variable and the canonical scores. We also tested for differences in fitness (fruit number, $\ln+1$ transformed) between the site types and evaluated whether any differences were environment-dependent using an analogous univariate ANOVA.

Because many of the traits important for light interception are size-related, multivariate analysis was done on residuals following regression between the trait and number of leaves (cf. Relyea, 2002). This procedure reduces the possibility that differences among the experimental factors were due to plant size and its correlation with the trait of interest (see also Bell and Galloway, 2007). In *G. carolinianum*, number of leaves is highly correlated with total dried plant biomass ($r = 0.96$; D. L. Bell, unpublished data). In addition, traits were averaged over family members within plots to remove microenvironmental effects (cf. Donohue et al., 2000). Petiole lengths at nodes 1 and 2 were square-root transformed, and number of leaves and lengths of internodes 1 and 2 were log transformed to meet the assumptions of ANOVA.

Light-dependent selection—Selection analyses were conducted within each light environment to measure phenotypic selection on traits important for light capture (Lande and Arnold, 1983). All characters, except fitness, were standardized within each light environment (mean of 0 and standard deviation of 1), and traits were not otherwise transformed. Relative fitness was calculated within light environments by dividing the fitness of each individual by the mean fitness within each light environment; beyond this calculation, fitness was not transformed (Lande and Arnold, 1983). To avoid the effects of multicollinearity, we removed petiole length at node 2 (highly correlated with other traits, $r \geq 0.70$) from all selection analyses (as suggested by Lande and Arnold, 1983; Mitchell-Olds and Shaw, 1987). Leaf number was included in the selection analyses to determine the relationship between traits and fitness independent of plant size. Previous work in this system demonstrated that the allometric changes represented by shade avoidance response can be more readily detected when variation in plant size is accounted for (Bell and Galloway, 2007). Block and population were also included in the model. We performed both phenotypic (individual) and genotypic (family mean) selection analyses (Rausher, 1992) but only report the results of the phenotypic analyses because the patterns of selection were qualitatively similar between the two analyses. Phenotypic and genotypic analyses are likely similar because including block in the analysis accounts for spatial patterns of variation and thus reduces environmental covariance between the phenotype and fitness (Mitchell-Olds and Shaw, 1987; Wade and Kalisz, 1990). Inclusion of plant size in the model has a similar effect at the scale of an individual.

Selection differentials (S) and gradients (β and γ) were estimated for each light environment. Selection differentials measure the direction and strength of linear selection on a phenotypic trait, including direct selection on the trait and indirect selection acting on correlated traits (Lande and Arnold, 1983). Differentials were estimated as the covariance between relative fitness and the standardized morphological trait. Selection gradients indicate the direction and strength of selection on a trait while holding all other traits constant (Lande and Arnold, 1983). Directional selection gradients (β) were estimated as the partial regression coefficients from a multiple regression model for which standardized traits were regressed on relative fitness within each environment. Nonlinear selection gradients (γ) were estimated with the partial regression coefficients of nonlinear terms from multivariate regression analysis with all linear and nonlinear terms in the model following standard procedure (Wade and Kalisz, 1990).

Analysis of covariance (ANCOVA) was performed to determine whether selection differed between light environments. In the ANCOVA, we tested for the main effects of light, block, and population on relative fitness. The covariates were the standardized plant characters (number of leaves, petiole length, and internode lengths). Interactions between covariates and light were also included; a significant interaction indicates that the pattern of selection differed between the light environments.

Cost of plasticity—Potential costs of plasticity were assessed by examining the relationship between trait plasticity for each family and relative fitness within each of the environments while accounting for variation in trait means (DeWitt et al., 1998; Scheiner and Berrigan, 1998). The plasticity of a trait was calculated by taking the mean trait value of a family in the low light environment and subtracting it from the mean trait value of the same family in the high light environment. This procedure was repeated for each of the 60 families. The relationship between trait plasticity and fitness was determined using a multiple regression where family means within an environment and the plasticity of the traits were regressed on relative fitness within the environment (DeWitt, 1998; Scheiner and Berrigan, 1998). Separate regressions were conducted for each environment. A cost of plasticity would be indicated by a significant negative partial regression coefficient for the plasticity term(s) in the model. Based on initial analyses, there were no effects of nonlinear selection on the cost of plasticity, so these terms were eliminated from the analyses (cf. Scheiner and Berrigan, 1998).

RESULTS

Patterns of plasticity—Plants in the low light environment were elongated relative to plants grown in high light, although the power to detect a significant light effect is limited (Table 1; Fig. 1). There was no overall difference in multivariate phenotypes between plants from the two site types (Table 1). However, old-field plants were more plastic for light interception

traits than wood-margin individuals (light \times site type, Table 1; Fig. 1). Petiole length at node 1 and length of internode 2 contributed substantially to the light by site type interaction (canonical analysis, Table 1). Old-field plants were more plastic for petiole length at node 1 per unit plant size, increasing from high light to low light by 32.4% as compared to a 21.6% increase for wood-margin plants (Fig. 1). Similarly, old-field plants were more plastic for length of internode 2 per unit plant size (Fig. 1). Populations within site-types were differentiated for average phenotypes (Table 1).

Old-field plants had marginally greater fitness than wood-margin plants (Table 2, Fig. 2). Although plants grown in low and high light had very similar average fitness, plants from the two site types had different patterns of response to light (Table 2). Old-field plants had relatively constant fitness over the two light environments, while wood-margin plants had reduced fitness in low light relative to high light (Fig. 2).

Light-dependent selection—Plants with longer petioles, longer internodes, and more leaves had higher fitness under both high and low light (Table 3). However, selection on plants with longer second internodes in high light acted through correlated traits and was not significant in the multivariate analysis (S vs. β , Table 3). In both light environments, selection was stronger on petiole length than internode length. Direct selection on internode 1 length was stronger under low light than high light, indicating longer internodes were favored under low light conditions (Table 3; light \times trait for β).

There was positive nonlinear selection on petiole length and internode 1 length under high light. Inspection of the data indicated accelerating selection where the strength of selection increased with petiole and internode length.

Cost of plasticity—Families that were more plastic for internode 1 length had reduced fitness under high light than less plastic families (Table 4). This same pattern, although not significant, was also found for plasticity of petiole length in high light. This analysis reveals a cost of plasticity for internode 1 length. There was no association between plasticity and fitness for any trait under low light conditions (Table 4).

DISCUSSION

Geranium carolinianum plants elongate both petioles and internodes in response to shading from an overhead canopy. The elongation of these traits when plants are grown under low light conditions or high neighbor densities is termed a “shade-avoidance” response. By spreading out in space, plants may ameliorate the reduced availability of light and enhance light capture by leaves (Sultan, 1995; Dudley and Schmitt, 1996; Huber and Wiggerman, 1997; Leeftang et al., 1998). Similar shade avoidance responses have been observed for a number of plant species (e.g.; Ballaré et al., 1991; Leeftang et al., 1998; Annicchiarico and Piano, 2000; Gautier et al., 2001; Weijschedé et al., 2006). We found that the pattern of shade avoidance differed between *G. carolinianum* plants from old-field and wood-margin habitats. Plants from old-field sites elongated their petioles and second internodes more under low light conditions than plants from wood-margin populations. This differentiation in shade-avoidance response between habitats may be due to differences in the pattern of light availability in old-field and wood-margin sites.

TABLE 1. Multivariate analysis of variance of light and site type on traits of *Geranium carolinianum*. Plants from 10 families from each of three populations per site-type (wood-margin and old-field) were grown in the field under two light environments (low and high light). Petiole length at node 1, petiole length at node 2, length of internode 1, and length of internode 2 (all adjusted for plant size, see Methods) were included in the analysis. The canonical structure is given to illustrate the contribution of individual traits to the light \times site type effect.

Source	df ^a	Wilks' λ	F ratio	P
Light	4, 2	0.03	13.80	0.0687
Site type	4, 1	0.31	0.55	0.7508
Population(site type)	16, 156	0.52	2.33	0.0040
Family(population \times site type)	216, 2338	0.68	1.07	0.2291
Light \times site type	4, 1	0.0001	2830.46	0.0141
Light \times population(site type)	16, 1788	0.98	0.89	0.5843
Block	20, 8	0.00003	8.33	0.0029
Block \times light	20, 1941	0.91	2.85	<0.0001

Trait	Canonical structure of light \times site type
Petiole length, node 1	0.67
Petiole length, node 2	0.08
Internode 1 length	0.14
Internode 2 length	0.71

^adf for the numerator, denominator

Neighboring plants and overhead tree canopy both influence light availability in *G. carolinianum* habitats. Old-field sites are characterized by no canopy and dense growth of grasses, whereas wood-margin sites have partial canopy cover and a reduced neighbor density. The shade from a tree canopy decreases the quantity as well as the quality of light, as defined by the ratio of red to far red light (R:FR; Schmitt and Wulff, 1993; Dudley and Schmitt, 1996; Franklin and Whitelam, 2005). In contrast, shade from neighboring plants results in a gradient of

light within an individual that is associated with a decreasing R:FR ratio. In the old-field habitat, light reduction is an environmental cue that indicates the presence of neighbors (Ballaré et al., 1991, 1990; Huber and Wiggerman, 1997; Weinig, 2000b), and a shade-avoidance response will typically enhance light interception. In contrast, a shade-avoidance response is not expected to increase light capture dramatically in wooded areas. Indeed, elongation of petioles and internodes under the canopy may be maladaptive because the investment in carbon

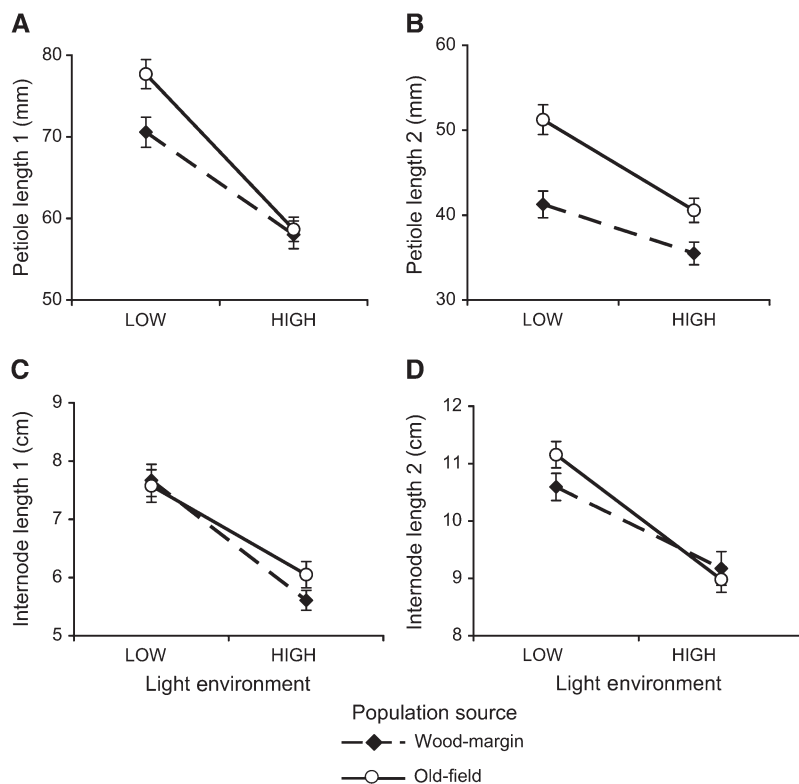


Fig. 1. Norms of reaction of traits important for light capture for *Geranium carolinianum* plants from old-field and wood-margin sites grown in natural low and high light conditions (mean \pm SE). (A) Petiole length at node 1. (B) Petiole length at node 2. (C) Internode length, node 1. (D) Internode length, node 2.

TABLE 2. Analysis of variance on fitness of *Geranium carolinianum* plants representing sites that differed in light availability that were planted in low and high light field environments. Ten families from each of three populations per site type (wood-margin and old-field) were used.

Source	df ^a	F ratio	P
Light	1, 5	0.29	0.616
Site type	1, 4	5.48	0.076
Population(site type)	4, 8	1.51	0.289
Family(population × site type)	55, 646	1.30	0.079
Light × site type	1, 4	8.51	0.043
Light × population(site type)	4, 646	0.70	0.594
Block	5, 5	7.32	0.024
Block × light	5, 646	6.88	0.001

^adf for numerator, denominator

that results in elongation does not increase light capture. Consistent with our expectations, we found a greater shade-avoidance response to low light in plants from old-field populations, which face shading from neighbors, than in plants from wood margins, which are shaded by a canopy. Similar to our results here, a previous study in *G. carolinianum* indicated that habitat differences in light availability in old-field and wood-margin sites influence plant size, whereas the presence of neighbors induces a shade-avoidance response (Bell and Galloway, 2007).

Furthermore, our results suggest the divergence in shade-avoidance response between populations from different habitats is adaptive. Low light plots had both canopy and neighbor crowding, and plants from the old-field populations, which were more elongated under these low light conditions than wood-margin populations, had a constant fitness across the light environments. In contrast, plants from wood-margin populations that had less of a shade-avoidance response had reduced fitness under low light conditions. These contrasting patterns suggest that greater plasticity to low light enabled the old-field populations to maintain fitness in the lower resource environment. Plasticity of morphological traits often underlies the constancy of fitness components across environments (Bradshaw, 1965; Sultan, 1987; Pigliucci, 2001). Fitness of the wood-margin populations under low light may be reduced because of the neighbor crowding in addition to canopy shade in the experimental plots. Wood-margin populations typically experience low neighbor density and therefore weaker selection for a shade-avoidance response to neighbors than old-field plants. The reduced shade avoidance response of wood-margin populations in the presence of neighbors in the low light plots resulted in lower fitness. Adaptive divergence in plasticity in response to specific environmental cues has also been found in other studies (e.g., Cook and Johnson, 1968; Dudley and

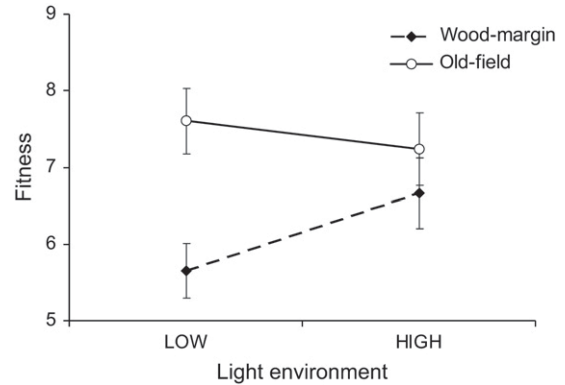


Fig. 2. Norms of reaction of fitness, as measured by the number of fruits, for *Geranium carolinianum* plants from old-field and wood-margin sites grown in natural low and high light conditions (mean ± SE).

Schmitt, 1996; Weinig, 2000b; Sultan, 2001; Gianoli and González-Teuber, 2005). However, few of these studies have tested replicate populations from different habitat types under natural environmental conditions (but see Donohue et al., 2001). By using natural environmental variation to evaluate differences between populations, we can identify differentiation in response to common selective patterns (i.e., habitat type) and the fitness consequences associated with specific patterns of plasticity, providing insight into the diverse patterns of plasticity in nature.

Selection on petiole and internode length in the two light environments also suggests that the shade-avoidance response underlies fitness differences between populations from different habitats. The estimates of selection revealed that when holding plant size constant, plants with longer petioles and internodes had greater fitness under low and high light conditions. However, the strength of this selection differed for the length of internode 1. For this trait, selection for elongated internodes was stronger for plants growing under low light than high light. The evolutionary response to this selection represents a stronger shade-avoidance phenotype. Note that internode 1 length, the only trait where the pattern of selection differed between environments, was also the one trait associated with light capture in which old-field plants did not respond with enhanced elongation relative to the wood-margin plants. If selection across environments on internode 1 results in an increased shade-avoidance response, then the pattern of plasticity for internode 1 will be more similar to that of the petioles and internode 2, which appear to be adaptive. In summary, internode length selection is in the same direction as the plastic response, supporting the interpretation that plasticity of this trait is adaptive.

TABLE 3. Phenotypic selection on traits important for light interception in *Geranium carolinianum* grown under high and low light conditions in the field. Linear selection differentials (*S*), linear selection gradients (β), and nonlinear selection gradients (γ) are shown. *F* ratio is from ANCOVA tests for differences in linear and nonlinear selection between light environments.

Trait	High light			Low light			<i>F</i> ratio Light × trait (β)	<i>F</i> ratio Light × trait (γ)
	<i>S</i>	β	γ	<i>S</i>	β	γ		
Petiole length, node 1	0.59***	0.54***	0.46*	0.52***	0.40***	-0.31	3.36 [†]	3.51 [†]
Internode 1 length	0.14***	0.07**	0.18*	0.14***	0.12***	0.03	9.71**	1.11
Internode 2 length	0.29***	0.04	0.11 [†]	0.32***	0.10**	0.26 [†]	0.96	1.01
Number of leaves	0.49***	0.32***	-0.58*	0.47***	0.36***	-0.26	2.45	0.04

[†] *P* < 0.10, * *P* < 0.05, ** *P* < 0.01, *** *P* < 0.001.

TABLE 4. The relationship between trait plasticity and relative fitness within each light environment. The standardized selection gradients (β) reflect selection on plasticity after accounting for variation in the trait mean in each light environment.

Trait	High light β	Low light β
Petiole length, node 1	-0.211 ⁺	0.062
Internode 1 length	-0.324*	0.072
Internode 2 length	0.103	0.141
Number of leaves	0.078	-0.236

⁺ $P < 0.10$; * $P < 0.05$.

We detected a cost of plasticity for internode 1 length under high light conditions. Families that changed their internode length in response to the light environment had lower fitness than families with more similar internode lengths across the two environments. This cost of plasticity was expressed in the very same trait that had the strongest selection for plasticity. Our test of the association of genotype plasticity with fitness suggests that increased plasticity, although favored by selection, is constrained by a cost. However, this cost is only expressed when plants are grown under high light environments. Therefore, shade avoidance plasticity for internode 1 may evolve more rapidly in populations that largely inhabit lower light regions. This prediction requires genetic variation within populations in internode 1 length under low light conditions. Such variation was found in a greenhouse study of *G. carolinianum* grown in high and low light environments (Bell, 2004).

A number of previous studies under controlled conditions have either found limited costs of plasticity or no cost at all (DeWitt et al., 1998; Scheiner and Berrigan, 1998; Tucic et al., 1998; Donohue et al., 2000; Dorn et al., 2000; Van Kleunen et al., 2000; Agrawal et al., 2002; Relyea, 2002; Steinger et al., 2003; Stinchcombe et al., 2004; Caruso et al., 2006). It has been suggested that there is little evidence of a cost of plasticity because few studies have been conducted under natural conditions where resources are limited and costs therefore more likely to be expressed (Steinger et al., 2003; Van Kleunen and Fischer, 2005). In two field studies to test the costs of plasticity to density, a cost for stem elongation was found in one case (Weinig et al., 2006), but no cost of plasticity in the other (Donohue et al., 2000). When these two studies are considered along with the current study, it remains unclear whether costs of plasticity are more likely to be expressed in field studies than in studies done under controlled conditions. Costs of plasticity may be difficult to detect, either under field or controlled environments because natural selection may have already removed individuals expressing costs (DeWitt et al., 1998; Weinig et al., 2006). Families of a largely selfing species from natural populations were used in the current study, and past selection has probably reduced the frequency of lineages with larger genetic costs, limiting the potential for detecting of costs of plasticity (cf. Weinig et al., 2006).

In summary, elongation of petioles and internodes appears to be an adaptive response to reduced light availability in *G. carolinianum*. This conclusion is supported by several lines of evidence. Enhanced shade avoidance response in populations from sites where neighbor shade is common and therefore elongation is beneficial, relative to those from sites where elongation is not likely to substantially enhance light capture, indicate that past habitat-specific selection has differentiated the populations. The elongation of structures important for light capture under

low light results in greater fitness at the population level and the individual level (although for the latter, for internode elongation only). A cost of plasticity was detected for internode elongation but only under high light. This cost is expected to constrain the evolution of plasticity across environments. Together with the findings of other studies, these results suggest that there is adaptive differentiation in response to light between habitats, and cost may limit the evolution of plasticity.

LITERATURE CITED

- AGRAWAL, A. A., J. K. CONNER, M. T. J. JOHNSON, AND R. WALLSGROVE. 2002. Ecological genetics of an induced plant defense against herbivores: additive genetic variance and costs of phenotypic plasticity. *Evolution; International Journal of Organic Evolution* 56: 2206–2213.
- ANDERSSON, S., AND R. G. SHAW. 1994. Phenotypic plasticity in *Crepis decorum* (Asteraceae): genetic correlations across light regimens. *Heredity* 72: 113–125.
- ANNICCHIARICO, P., AND E. PIANO. 2000. Response of white clover genotypes to evaluation environments of dense and spaced planting, and implications for selection. *Euphytica* 111: 111–120.
- BALLARÉ, C. L., A. L. SCOPEL, M. L. ROUSH, AND S. R. RADOSEVICH. 1995. How plants find light in patchy canopies. A comparison between wild-type and phytochrome-B-deficient mutant plants of cucumber. *Functional Ecology* 9: 859–868.
- BALLARÉ, C. L., A. L. SCOPEL, AND R. A. SÁNCHEZ. 1991. Photocontrol of stem elongation in plant neighborhoods—Effects of photon fluence rate under natural conditions of radiation. *Plant, Cell & Environment* 14: 57–69.
- BALLARÉ, C. L., A. L. SCOPEL, AND R. A. SÁNCHEZ. 1990. Far-red radiation reflected from adjacent leaves: An early signal of competition in plant canopies. *Science* 247: 329–332.
- BASKIN, J. M., AND C. C. BASKIN. 1974. Some eco-physiological aspects of seed dormancy in *Geranium carolinianum* L. from central Tennessee. *Oecologia* 16: 209–219.
- BELL, D. L. 2004. The maintenance of genetic variation for patterns of phenotypic plasticity in *Geranium carolinianum*: The roles of cost and adaptation. Ph.D. dissertation, University of Virginia, Charlottesville, Virginia, USA.
- BELL, D. L., AND L. F. GALLOWAY. 2007. Plasticity to neighbor shade: fitness consequences and allometry. *Functional Ecology* 21: 1145–1152.
- BRADSHAW, A. D. 1965. Evolutionary significance of phenotypic plasticity in plants. *Advances in Genetics* 13: 115–155.
- CARUSO, C. M., H. MAHERALI, AND M. SHERRARDA. 2006. Plasticity of physiology in *Lobelia*: testing for adaptation and constraint. *Evolution; International Journal of Organic Evolution* 60: 980–990.
- COOK, S. A., AND M. P. JOHNSON. 1968. Adaptation to heterogeneous environments. I. Variation in heterophylly in *Ranunculus fammula* L. *Evolution; International Journal of Organic Evolution* 22: 496–516.
- DE KROON, H., AND M. J. HUTCHINGS. 1995. Morphological plasticity in clonal plants—the foraging concept reconsidered. *Journal of Ecology* 83: 143–152.
- DEWITT, T. J. 1998. Costs and limits of phenotypic plasticity: tests with predator-induced morphology and life history in a freshwater snail. *Journal of Evolutionary Biology* 11: 465–480.
- DEWITT, T. J., A. SIH, AND D. S. WILSON. 1998. Costs and limits of phenotypic plasticity. *Trends in Ecology & Evolution* 13: 77–81.
- DONOHUE, K., E. HAMMOND PYLE, D. MESSIQUA, M. S. HESCHEL, AND J. SCHMITT. 2001. Adaptive divergence in plasticity in natural populations of *Impatiens capensis* and its consequences for performance in novel habitats. *Evolution; International Journal of Organic Evolution* 55: 692–702.
- DONOHUE, K., D. MESSIQUA, E. HAMMOND PYLE, M. S. HESCHEL, AND J. SCHMITT. 2000. Evidence of adaptive divergence in plasticity: density- and site-dependent selection on shade avoidance responses in *Impatiens capensis*. *Evolution; International Journal of Organic Evolution* 54: 1956–1968.

- DORN, L. A., E. H. PYLE, AND J. SCHMITT. 2000. Plasticity to light cues and resources in *Arabidopsis thaliana*: testing for adaptive value and costs. *Evolution; International Journal of Organic Evolution* 54: 1982–1994.
- DUDLEY, S. A., AND J. SCHMITT. 1996. Testing the adaptive plasticity hypothesis: density-dependent selection on manipulated stem length in *Impatiens capensis*. *The American Naturalist* 147: 445–465.
- FRANKLIN, K. A., AND G. C. WHITELAM. 2005. Phytochromes and shade-avoidance responses in plants. *Annals of Botany* 96: 169–175.
- GAUTIER, H., C. VARLET-GRANCHER, AND J. M. MEMBRE. 2001. Plasticity of petioles of white clover (*Trifolium repens*) to blue light. *Physiologia Plantarum* 112: 293–300.
- GIANOLI, E. 2004. Plasticity of traits and correlations in two populations of *Convolvulus arvensis* (Convolvulaceae) differing in environmental heterogeneity. *International Journal of Plant Sciences* 165: 825–832.
- GIANOLI, E., AND M. GONZÁLEZ-TEUBER. 2005. Environmental heterogeneity and population differentiation in plasticity to drought in *Convolvulus chilensis* (Convolvulaceae). *Evolutionary Ecology* 19: 603–613.
- GLEASON, H. A., AND A. CRONQUIST. 1991. Manual of vascular plants of northeastern United States and adjacent Canada, 2nd ed. New York Botanical Garden, Bronx, New York, USA.
- HUBER, H. 1996. Plasticity of internodes and petioles in prostrate and erect *Potentilla* species. *Functional Ecology* 10: 401–409.
- HUBER, H., AND L. WIGGERMAN. 1997. Shade avoidance in the clonal herb *Trifolium fragiferum*: A field study with experimentally manipulated vegetation height. *Plant Ecology* 130: 53–62.
- KINGSOLVER, J. G. 1995. Fitness consequences of seasonal polyphenism in western white butterflies. *Evolution; International Journal of Organic Evolution* 49: 942–954.
- LANDE, R., AND S. J. ARNOLD. 1983. The measurement of selection on correlated characters. *Evolution; International Journal of Organic Evolution* 37: 1210–1226.
- LEEFLANG, L., H. J. DURING, AND M. J. A. WERGER. 1998. The role of petioles in light acquisition by *Hydrocotyle vulgaris* L. in a vertical light gradient. *Oecologia* 117: 235–238.
- MITCHELL-OLDS, T., AND R. G. SHAW. 1987. Regression analysis of natural selection—Statistical inference and biological interpretation. *Evolution; International Journal of Organic Evolution* 41: 1149–1161.
- PETTIT, C., AND J. D. THOMPSON. 1997. Variation in phenotypic response to light availability between diploid and tetraploid populations of the perennial grass *Arrhenatherum elatius* from open and woodland sites. *Journal of Ecology* 85: 657–667.
- PIGLIUCCI, M. 2001. Phenotypic plasticity: beyond nature and nurture. Johns Hopkins University Press, Baltimore, Maryland, USA.
- PIGLIUCCI, M., AND C. D. SCHLICHTING. 1995. Ontogenetic reaction norms in *Lobelia siphilitica* (Lobeliaceae): response to shading. *Ecology* 76: 2134–2144.
- RAUSHER, M. D. 1992. The measurement of selection on quantitative traits: Biases due to environmental covariances between traits and fitness. *Evolution; International Journal of Organic Evolution* 46: 616–626.
- RELYEA, R. A. 2002. Costs of phenotypic plasticity. *The American Naturalist* 159: 272–282.
- ROACH, D. A. 1986. Life history variation in *Geranium carolinianum*. 1. Covariation between characters at different stages of life cycle. *The American Naturalist* 128: 47–57.
- SCHNEIDER, S. M., AND D. BERRIGAN. 1998. The genetics of phenotypic plasticity. VIII. The cost of plasticity in *Daphnia pulex*. *Evolution; International Journal of Organic Evolution* 52: 368–378.
- SCHLICHTING, C. D. 1986. The evolution of phenotypic plasticity in plants. *Annual Review of Ecology and Systematics* 17: 667–693.
- SCHMITT, J. 1993. Reaction norms of morphological and life-history traits to light availability in *Impatiens capensis*. *Evolution; International Journal of Organic Evolution* 47: 1654–1668.
- SCHMITT, J., AND R. D. WULFF. 1993. Light spectral quality, phytochrome and plant competition. *Trends in Ecology & Evolution* 8: 47–50.
- STEINGER, T., B. A. ROY, AND M. L. STANTON. 2003. Evolution in stressful environments II: Adaptive value and costs of plasticity in response to low light in *Sinapis arvensis*. *Journal of Evolutionary Biology* 16: 313–323.
- STINCHCOMBE, J. R., L. A. DORN, AND J. SCHMITT. 2004. Flowering time plasticity in *Arabidopsis thaliana*: A reanalysis of Westerman and Lawrence (1970). *Journal of Evolutionary Biology* 17: 197–207.
- STRAUSBAUGH, P. D., AND E. L. CORE. 1978. Flora of West Virginia, 2nd ed. Seneca Books, Inc., Morgantown, West Virginia, USA.
- SULTAN, S. E. 1987. Evolutionary implications of phenotypic plasticity in plants. *Evolutionary Biology* 21: 127–176.
- SULTAN, S. E. 1995. Phenotypic plasticity and plant adaptation. *Acta Botanica Neerlandica* 44: 363–383.
- SULTAN, S. E. 2001. Phenotypic plasticity for fitness components in *Polygonum* species of contrasting ecological breadth. *Ecology* 82: 328–343.
- SULTAN, S. E., AND F. A. BAZZAZ. 1993. Phenotypic plasticity in *Polygonum persicaria*. I. Diversity and uniformity in genotypic norms of reaction to light. *Evolution; International Journal of Organic Evolution* 47: 1009–1031.
- SULTAN, S. E., AND H. G. SPENCER. 2002. Metapopulation structure favors plasticity over local adaptation. *The American Naturalist* 160: 271–283.
- TUCIC, B., AND S. AVRAMOV. 1996. Maternal effects on early juvenile traits in *Iris pumila* (Iridaceae). *Plant Systematics and Evolution* 201: 179–197.
- TUCIC, B., V. TOMIC, S. AVRAMOV, AND D. PEMAC. 1998. Testing the adaptive plasticity of *Iris pumila* leaf traits to natural light conditions using phenotypic selection analysis. *Acta Oecologica* 19: 473–481.
- TUFTO, J. 2000. The evolution of plasticity and nonplastic spatial and temporal adaptations in the presence of imperfect environmental cues. *The American Naturalist* 156: 121–130.
- VAN KLEUNEN, M., AND M. FISCHER. 2005. Constraints of the evolution of adaptive phenotypic plasticity in plants. *The New Phytologist* 166: 49–60.
- VAN KLEUNEN, M., M. FISCHER, AND B. SCHMID. 2000. Costs of plasticity in foraging characteristics of the clonal plant *Ranunculus reptans*. *Evolution; International Journal of Organic Evolution* 54: 1947–1955.
- VAN TIENDEREN, P. H. 1991. Evolution of generalists and specialists in spatially heterogeneous environments. *Evolution; International Journal of Organic Evolution* 45: 1317–1331.
- VAN TIENDEREN, P. H. 1997. Generalists, specialists, and the evolution of phenotypic plasticity in sympatric populations of distinct species. *Evolution; International Journal of Organic Evolution* 51: 1372–1380.
- VIA, S., AND R. LANDE. 1985. Genotype-environment interaction and the evolution of phenotypic plasticity. *Evolution; International Journal of Organic Evolution* 39: 505–522.
- WADE, M. J., AND S. KALISZ. 1990. The causes of natural selection. *Evolution; International Journal of Organic Evolution* 44: 1947–1955.
- WEISCHÉDÉ, J., J. MARTÍNKOVÁ, H. DE KROON, AND H. HUBER. 2006. Shade avoidance in *Trifolium repens*: costs and benefits of plasticity in petiole length and leaf size. *The New Phytologist* 172: 655–666.
- WEINIG, C. 2000a. Differing selection in alternative competitive environments: shade-avoidance responses and germination timing. *Evolution; International Journal of Organic Evolution* 54: 124–136.
- WEINIG, C. 2000b. Plasticity versus canalization: population differences in the timing of shade-avoidance. *Evolution; International Journal of Organic Evolution* 54: 441–451.
- WEINIG, C., J. JOHNSTON, Z. M. GERMAN, AND L. M. DEMINK. 2006. Local and global costs of adaptive plasticity to density in *Arabidopsis thaliana*. *The American Naturalist* 167: 826–836.
- WINN, A. A. 1999. Is seasonal variation in leaf traits adaptive for the annual plant *Dicerandra linearifolia*? *Journal of Evolutionary Biology* 12: 306–313.