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EVIDENCE OF ADAPTIVE DIVERGENCE IN PLASTICITY: DENSITY- AND SITE-DEPENDENT SELECTION ON SHADE-AVOIDANCE RESPONSES IN *IMPATIENS CAPENSIS*

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Abstract.—We investigated the conditions under which plastic responses to density are adaptive in natural populations of *Impatiens capensis* and determined whether plasticity has evolved differently in different selective environments. Previous studies showed that a population that evolved in a sunny site exhibited greater plasticity in response to density than did a population that evolved in a woodland site. Using replicate inbred lines in a reciprocal transplant that included a density manipulation, we asked whether such population differentiation was consistent with the hypothesis of adaptive divergence. We hypothesized that plasticity would be more strongly favored in the sunny site than in the woodland site; consequently, we predicted that selection would be more strongly density dependent in the sunny site, favoring the phenotype that was expressed at each density. Selection on internode length and flowering date was consistent with the hypothesis of adaptive divergence in plasticity. Few costs or benefits of plasticity were detected independently from the expressed phenotype, so plasticity was selected primarily through selection on the phenotype. Correlations between phenotypes and their plasticity varied with the environment and would cause indirect selection on plasticity to be environment dependent. We showed that an appropriate plastic response even to a rare environment can greatly increase genotypic fitness when that environment is favorable. Selection on the measured characters contributed to local adaptation and fully accounted for fitness differences between populations in all treatments except the woodland site at natural density.

Key words.—Adaptive plasticity, density-dependent selection, environmental heterogeneity, local adaptation, natural selection, phenotypic plasticity.

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The relationship between population differentiation and local adaptation has been the subject of considerable research (e.g., Schemske 1984; Silander 1985; Galen et al. 1991; Bennington and McGraw 1995; Linhart and Grant 1996). In particular, population differentiation in phenotypic plasticity has been documented in a variety of systems (e.g., Cook and Johnson 1968; Schwaegerle and Bazzaz 1987; Lotz and Spoormakers 1988; Schlichting and Levin 1990; Miller and Fowler 1993; Dudley and Schmitt 1995; also reviewed in Schlichting 1986). Such differentiation has often been hypothesized to be the result of adaptive divergence. For plasticity to be adaptive, selection must be environment dependent, favoring the phenotype expressed in each environment relative to alternative phenotypes (Via and Lande 1985; Schmitt et al. 2001). Plasticity is expected to be favored in more variable environments (Via and Lande 1985; Weis and Gorman 1990; Van Tienderen 1991), and there is some evidence that populations or species that experience more heterogeneous environments also exhibit greater plasticity (e.g., Mitchell 1976; Wilken 1977; Lotz and Spoormakers 1988). However, environment-dependent selection on plastic characters has rarely been measured to test explicitly the adaptive value of plasticity in different sites. Moreover, few studies have investigated how the frequency of selective environments influences the adaptive value of plasticity under natural conditions (but see Weis and Gorman 1990; Scheiner and Callahan 1999).

Recently, it has been proposed that selection can act directly on plasticity itself, independent from environment-specific selection on the trait that exhibits plasticity. In particular, if the capacity for a plastic response is physiologically costly, plastic genotypes will have lower fitness within an environment than nonplastic genotypes expressing the same mean phenotype within that environment (Van Tienderen 1991). That is, selection will act directly to decrease plasticity. Conversely, if phenotypic constancy (homeostasis) of a trait is costly, plastic genotypes will have higher fitness than nonplastic genotypes expressing the same trait mean; direct selection will favor increased plasticity (Winn 1997). Costs of plasticity have been suggested as potential constraints on the evolution of adaptive plasticity (Van Tienderen 1991; DeWitt 1998; DeWitt et al. 1998; Scheiner and Berrigan 1998). It is therefore important to determine whether such costs exist in natural populations. It is also of interest to ask whether populations in different natural environments experience differences in the strength or direction of direct selection on plasticity that might contribute to population differentiation. Only a few studies have tested for costs of plasticity or homeostasis (DeWitt 1998; Scheiner and Berrigan 1998), and even fewer studies have been conducted in natural environments.

The outcome of selection on reaction norms depends not only on environment-specific selection on trait means and direct selection on plasticity, but also on the nature of the correlation between genotypic plasticity and genotypic mean phenotype (Via and Lande 1985; Falconer 1990; Scheiner 1993). Genetic correlations between the trait mean and its

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plasticity can vary with the environment (Appendix). The degree to which the strength and direction of the correlation changes with the environment is a function of the differences in the additive genetic variance of the character between the two environments; stronger correlations are present in the environment in which additive genetic variation is greater (Appendix). The magnitude and direction of the correlations between phenotypic values and plasticities can determine whether selection on the phenotype will result in correlated selection for increased or decreased plasticity (Scheiner and Lyman 1989, 1991; Scheiner 1993). Consequently, observed differentiation in plasticity between populations may be the result of environmental differences in selection on phenotypes, environment-dependent differences in costs of plasticity, and/or environment-dependent genetic correlations between phenotypic means and plasticities.

To test hypotheses concerning local adaptation and adaptive divergence in plastic responses to density, we studied two genetically differentiated populations of *Impatiens capensis* from contrasting canopy environments. Many plants respond to crowding and vegetation shade by elongating internodes and accelerating flowering (Smith 1982; Schmitt et al. 1986, 1987; Casal et al. 1987; Geber 1989; Weiner and Thomas 1992; Thomas and Bazzaz 1993; Davis and Simmons 1994). These plastic responses are mediated both by reduced irradiance and by the low ratio of red:far red wavelengths (R : FR) characteristic of vegetation shade. Such shade-avoidance responses are hypothesized to be adaptive (Casal and Smith 1989; Schmitt and Wulff 1993; Smith 1995). By elongating, plants may be able to escape competition for light beneath a vegetation canopy (Morgan and Smith 1979; Schmitt et al. 1995; Dudley and Schmitt 1996). By flowering earlier, a plant can increase the probability of reproduction under conditions associated with early mortality (Schemske 1984; Lacey 1986a,b; Biere 1995). Experimental demonstrations of density-dependent selection on plastic traits support the hypothesis that photomorphogenic shade avoidance responses are adaptive (Schmitt et al. 1995; Dudley and Schmitt 1996). However, the adaptive value of these plastic responses may depend on the overhead canopy environment. In open habitats, irradiance and R:FR are reliable cues of neighbor proximity and the relative position of a plant within a stand of competitors (Ballaré et al. 1990; Smith and Whitelam 1990; Smith et al. 1990). In woodland habitats, however, these light cues are unreliable and responding to them may be maladaptive, because the overhead canopy can produce the same changes in the light environment as neighboring competitors. It has therefore been hypothesized that photomorphogenic shade-avoidance responses are adaptive under open-canopy conditions but not under closed-canopy conditions in woodland habitats (Morgan and Smith 1979). Comparative studies of species or populations from open- and closed-canopy habitats are consistent with this hypothesis (Morgan and Smith 1979; Corré 1983; Van Hinsberg 1996; Van Tienderen and Van Hinsberg 1996).

Our study populations of *I. capensis* exhibit genetic differentiation in plasticity consistent with the adaptive hypothesis of Morgan and Smith (1979). Genotypes from a natural population in an open-canopy site exhibit greater plasticity in elongation and/or flowering date in response to light availability (Schmitt 1993), R:FR (Dudley and Schmitt 1995), and density (unpubl. data) than genotypes from a nearby woodland population, as predicted. However, to test directly the hypothesis that this population differentiation is adaptive, it is necessary to measure density-dependent selection on plastic shade-avoidance traits in both sites. The adaptive divergence hypothesis predicts that selection will be more strongly density dependent in the site of the open-canopy population than in the woodland site.

To test this prediction, and to examine the nature of environment-specific selection on plasticity to density, we planted inbred lines from the open-canopy and woodland populations into both sites in a reciprocal transplant design coupled with a density manipulation. We asked the following questions. Is the pattern of density-dependent selection on stem elongation and flowering date in the different sites consistent with the hypothesis of adaptive divergence? Is there evidence for direct selective costs of plasticity? How does selection on phenotypes within environments influence correlated selection on plasticity? How does the frequency of density environments influence the adaptive value of plasticity in each site? Finally, to what extent does selection on shade-avoidance traits and their plasticity contribute to local adaptation?

Methods

Experimental Design

Seeds were originally collected from two sites at Brown University's Haffenreffer Reserve in Bristol, Rhode Island. Inbred lines were maintained through single-seed descent of self-pollinated seeds for six generations. The ''sun'' population (Dudley and Schmitt 1995; Donohue and Schmitt 1999) grows in an open-canopy area near a seep, and seedlings grow in densities up to 3000 per m^2. The ''woodland'' population (Schmitt and Gamble 1990; Argyres and Schmitt 1991; Dudley and Schmitt 1995, 1996; Donohue and Schmitt 1999) grows beneath a canopy of oak and hickory, and maximum seedling densities are 450 per m^2 (M. S. Heschel, unpubl. data). The two populations are separated by less than 1 km.

Seeds collected from 18 lines from the sun population and 17 lines from the woodland population were weighed and stored in distilled water in plastic microtiter trays at 4°C for four months. In late April 1997, seeds were randomly assigned to treatments (see below) and planted into plug trays filled with Metromix 350 (Scotts-Sierra Horticultural Products Co., Marysville, OH). The timing of planting corresponded as closely as possible to germination timing in the field, but it was approximately two weeks later than the beginning of field germination. However, seedlings were still emerging in the field when the experimental seeds were planted. The plug trays were kept in a cold frame for two weeks, until the majority of the seedlings had emerged.

In early May 1997, the seedlings were transplanted into the sunny and the woodland sites in randomized positions into three low-density blocks and three natural-density blocks in each site. All blocks were cleared of native vegetation so that the effect of conspecific density would not be obscured by variable interspecific competition among blocks. Up to

three individuals from each line were planted into each block in both sites, giving a total of 1115 seedlings. Seedlings in the low-density blocks were planted in a 7 × 16 array, 15 cm apart, giving a density of 53 seedlings/m^2. Natural density treatments differed between the two sites because the natural seedling densities differed between sites. Natural density in the woodland site approximated the maximum density that seedlings experience in that site. Seedlings were therefore planted 5 cm apart in a 12 × 14 array, giving a density of 470 seedlings/m^2. In the sunny site, seedlings were planted 3 cm apart in a 12 × 14 array, giving a density of 1305 seedlings/m^2. This density was less than the maximum density observed at that site, but was the highest feasible density for transplanting and measuring seedlings without damage. Consequently, the difference in experimental density between the two sites was less than that under natural conditions. Two border rows were planted around the natural-density treatments in both sites to reduce edge effects. The natural-density treatment most closely approximated the natural environment experienced by seedlings in the sunny site, and the low-density treatment most closely approximated natural conditions in the woodland site.

Two weeks after the seedlings were planted into the treatments, we measured the length of the first internode, the number of nodes, and the length of the largest leaf. The length of the first internode was chosen as an index of stem elongation because this internode had fully elongated by the time of measurement and because population differentiation had previously been observed for this trait (Dudley and Schmitt 1995). Number of nodes and leaf length were indicators of seedling size. Twice a week throughout the season, plants were censused for the presence of cleistogamous or chasmogamous flowers, and the date of first flowering was estimated from these censuses.

To estimate total lifetime fitness, twice a month the total numbers of cleistogamous and chasmogamous flowers and the total numbers of immature and mature cleistogamous and chasmogamous fruits were recorded for each plant. Twice during the season, the average number of seeds produced by cleistogamous and chasmogamous fruits was determined by counting seeds in the surrounding nonexperimental plants. Fitness was estimated as the total number of cleistogamous and chasmogamous fruits produced over the lifetime of the plant, weighted by the average number of seeds within cleistogamous and chasmogamous fruits. The number of seeds per fruit estimated from the sample taken closest to the census period of fruits was used for the weighting. Total lifetime fitness was thereby estimated as the total number of seeds produced by each plant over its lifetime.

Data Analysis

SAS (SAS Institute 1990) was used for all statistical analyses. To measure selection on morphological characters and flowering date, phenotypic (Lande and Arnold 1983) and genotypic (Rausher 1992) selection analyses were conducted in each site and density separately (PROC GLM). Genotypic selection analysis eliminates difficulties of interpretation caused by microenvironmentally induced correlations between phenotypes and fitness because it averages phenotype and fitness across replicates of a genotype that is distributed at random across microenvironments. All individuals were included in estimates of genotypic mean values for traits and fitness, whether they successfully reproduced or not. Characters were natural-log transformed to normality when appropriate and were standardized within each treatment. Fitness was relativized within each treatment by dividing by the mean fitness within that treatment. Residuals from all analyses were normally distributed. Block was included in the analyses because large block effects on fitness were observed. For the genotypic selection analysis, genotypic means were calculated for each block and treatment combination. Interactions with block were pooled with the error term because we were interested in whether phenotype influenced fitness when distributed over all blocks, not whether it influenced fitness differently within each block (Newman et al. 1997). The source population was also included in the analyses to control for possible differences in fitness between populations due to population differences in unmeasured characters. Selection differentials (s), which measure the strength of total selection (both direct selection and indirect selection through correlations with other characters under selection), were estimated as the regression coefficient of relative fitness against standardized morphological traits (corrected for block and population effects) in a simple regression. Selection gradients (β), which measure the strength of direct selection, were estimated as the regression coefficient obtained from a multiple regression of relative fitness on all characters (corrected for block and population effects). Stabilizing or disruptive selection was estimated as the regression coefficients of quadratic terms from univariate and multivariate regression analysis. In general, the phenotypic selection analysis gave similar results to the genotypic selection analysis. Therefore, results of the phenotypic selection analysis are not reported unless they differed from the genotypic selection analysis. No correlational selection was detected in the genotypic analysis, so interactions among morphological characters were not included in the genotypic selection analyses reported. Significant correlational selection detected in the phenotypic analysis is reported in the text.

To determine whether selection was density dependent in each site, analysis of covariance was performed within each site, with density, population, and block as main effects, the standardized plant traits as covariates, and relative fitness as the dependent variable. Significant interactions between the traits and density would indicate that selection varied significantly with density. To determine site-dependent selection, a similar analysis of covariance was conducted at each density, with significant interactions between traits and sites indicating that selection differed between sites. Because the natural density treatments varied between sites, the effect of site at natural density includes effects of both site differences and of differences in density, whereas the effect of site at low density indicates effects of site differences while controlling for differences in natural density.

Additional ad hoc selection analyses were performed in the sun site, natural-density treatment because of heavy mortality that drastically altered the density environment experienced by the survivors. These analyses, described below, investigated selection on individuals that died before flow-

TABLE 1. Results of genotypic selection analysis. Selection differentials (s) and selection gradients (β) are shown for each site and density treatment.

Trait	Sun site, low density		Sun site, natural density		Woods site, low density		Woods site, natural density	
	s	β	s	β	s	β	s	β
Internode 1 (cm)	−0.14*	−0.07	0.39†	0.61*	0.32***	0.10	0.29***	0.21**
Flowering date	0.20***	0.16*	0.26	0.22	−0.53***	−0.49***	−0.52***	−0.46***
No. of nodes	0.02	0.04	−0.43*	−0.57*	0.12	0.05	0.06	−0.05
Leaf length (cm)	0.05	0.11*	−0.11	0.08	0.12	0.05	−0.07	−0.12†

† $P < 0.1$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

ering. Survivorship curves were compared between sites, densities, and populations using Kaplan-Meier survival analysis (PROC LIFETEST).

An additional selection analysis investigated selection on plasticity independently from selection on phenotypes, that is, tested for costs of plasticity. Plasticity to density was calculated as the genotypic mean phenotype expressed in low density minus that expressed in high density for all traits except internode length. Because internode length displayed higher mean values at high density, plasticity was calculated by subtracting the genotype mean at low density from that at high density. Genotypic plasticities were calculated separately for site and for each block, as before. Within each site and density environment, we conducted a genotypic selection analysis in which relative fitness was regressed against genotype plasticity and the genotype mean, correcting for block and population effects. We also calculated Pearson correlations between genotypic mean phenotype and genotypic plasticity within each site and density.

The influence of the frequency of density environments (low vs. natural density) on the adaptive value of plastic responses to density in each site was investigated by simulation in two ways. The mean fitness of each genotype was calculated, assuming the genotype experienced different frequencies of environments. This was done by multiplying the fitness expressed in one environment by the frequency of that environment and summing over both environments, for a range of hypothetical environmental frequencies from zero to one. In addition, the association between plasticity and genotypic mean fitness was determined by separate selection analyses at each simulated environmental frequency. Genotypic mean fitness at each environmental frequency was regressed against genotypic values of plasticity for all the traits.

To test for local adaptation, an analysis of variance was conducted with site, density, and population as fixed effects, block (nested in density and site) and genotype as random effects, and absolute or relative fitness as dependent variables. A significant population-by-site interaction would indicate that the expression of fitness differences between the populations depended on the site; local adaptation exists when a population has higher fitness in its native site. To determine the extent to which measured characters and their plasticities contributed to local adaptation, least square means of relative fitness of the two populations were calculated and compared from an analysis of variance that included no plant traits and from an analysis that included plant traits and their plasticities. As in the selection analyses, genotypic mean values were calculated by block, and block interactions were pooled with the error.

RESULTS

Density-Dependent Selection

The direction of selection on the length of the first internode differed with density in the sun site, but not in the woodland site (Tables 1, 2). In the sun site, plants with shorter internodes had higher fitness at low density, and plants with longer internodes had higher fitness at high density. Because the plastic response of internode length caused a more selectively favored phenotype to be expressed in both densities, the observed plasticity was considered adaptive. In the woodland site, selection favored longer internodes at both densities, indicating that plasticity in internode length—specifically, shorter internodes at low density—was maladaptive in the woodland site. Thus, population differentiation in internode elongation in response to density is consistent with the hypothesis of adaptive divergence. The nonsignificant selection gradients at low density, in both sites indicate that internode length was not under direct selection at low density, but was selected only through correlated characters. Direct selection for increased internode length at high density occurred in both sites. Phenotypic selection analysis gave similar results, except that direct selection for decreased internode elongation was detected at low density in the sun site ($\beta = -0.12$, $P = 0.026$).

Later flowering was favored at low density in the sun site (Table 1), but flowering date was apparently selectively neutral at natural density. However, this sample of plants at natural density includes only those plants that survived to flower and therefore includes either early-flowering individuals or individuals that experienced low-density conditions later in their life (see below). Earlier flowering was favored at both densities in the woodland site, and a significant nonlinear relationship was found between flowering date and fitness (disruptive multivariate selection coefficients: sun site $\gamma = 0.17$, $P < 0.01$; woodland site $\gamma = 0.12$, $P < 0.01$). Consequently, plasticity in flowering date is not adaptive in the woodland site. Selection on flowering date was not density dependent, although it differed significantly between sites and was stronger in the woodland site (Table 2). Phenotypic selection analysis gave similar results.

Surprisingly, decreased node production was favored at natural density in the sun site, but selection was neutral regarding node production in the other treatments (Tables 1, 2). Phenotypic selection analysis differed somewhat from the genotypic selection analysis in the sun site; increased node production was favored at low density ($s = 0.16$, $P < 0.001$; $\beta = 0.14$, $P < 0.001$), but the number of nodes was not significantly associated with fitness at natural density. The

TABLE 2. *F*-ratios to test for density-dependent selection within the sun and woodland sites (four columns on left) and site-dependent selection at low and natural densities (four columns on right). *F(s)*, tests for differences between selection differentials; *F*(β), tests for differences between selection gradients. *F*-ratio is based on Type III sums of squares. Analysis was based on genotypic mean values. $N = 188–199$, df = 1.

	F-ratios for trait × density				*F*-ratios for trait × site			
	Sun site		Woodland site		Low density		Natural density	
Trait	*F(s)*	*F*(β)	*F(s)*	*F*(β)	*F(s)*	*F*(β)	*F(s)*	*F*(β)
Internode 1 (cm)	5.98*	4.51*	6.38*	0.01	36.06***	3.22†	2.43	2.10
Flowering date	0.53	1.56	0.45	0.53	97.01***	72.63***	13.73***	8.22**
No. of node	1.64	5.21*	5.28*	0.60	7.62**	0.68	2.70	5.13*
Leaf length (cm)	0.01	0.03	13.64***	6.72*	6.62*	0.33	0.81	0.91

† $P < 0.1$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

significant relationship between fitness and the number of nodes at low density can probably be explained by microenvironmentally induced covariances between node number and fitness (Rausher 1992); plants in favorable microenvironments had more nodes and higher fitness.

Larger leaf size was favored by direct selection at low density in the sun site (Table 1). However, selection on correlated characters canceled out direct selection on leaf length, leading to no total selection on this character. No significant selection was detected in the other treatments, although the direction of selection differed with density in the woodland site (Table 2). With phenotypic selection analysis, the selection at low density in the sun site became only marginally significant ($\beta = 0.08$, $P = 0.065$). In addition, increased leaf length was associated with higher fitness at low density in the woodland site ($s = 0.14$, $P = 0.039$) and at natural density in the sun site ($s = 0.38$, $P = 0.034$; $\beta = 0.47$, $P = 0.060$). Again, the positive associations observed in the phenotypic selection analysis suggest microenvironmentally induced covariances between leaf size and fitness.

No significant correlational selection was observed in the genotypic selection analysis. However, in the phenotypic selection analysis, selection on flowering date covaried with selection on other characters. In the woodland site at both densities, selection favored earlier flowering on individuals with smaller leaves (low density: $\beta = 0.18$, $P = 0.038$; natural density: $\beta = 0.15$, $P = 0.037$). In the sun site at natural density, selection favored earlier flowering in individuals with fewer nodes ($\beta = 0.47$, $P = 0.029$). At the two natural-density treatments, selection favored earlier flowering on individuals with increased internode elongation (sun site: $\beta = -0.71$, $P = 0.002$; woodland site: $\beta = -0.17$, $P = 0.016$).

Patterns of Mortality and the Unmeasured Fraction

Approximately two months after the beginning of the experiment, a drought occurred that resulted in substantial mortality. Plant densities dropped precipitously in the woodland site at both densities (Fig. 1). By 98 days into the experiment, all plants were dead in the natural-density treatment and a

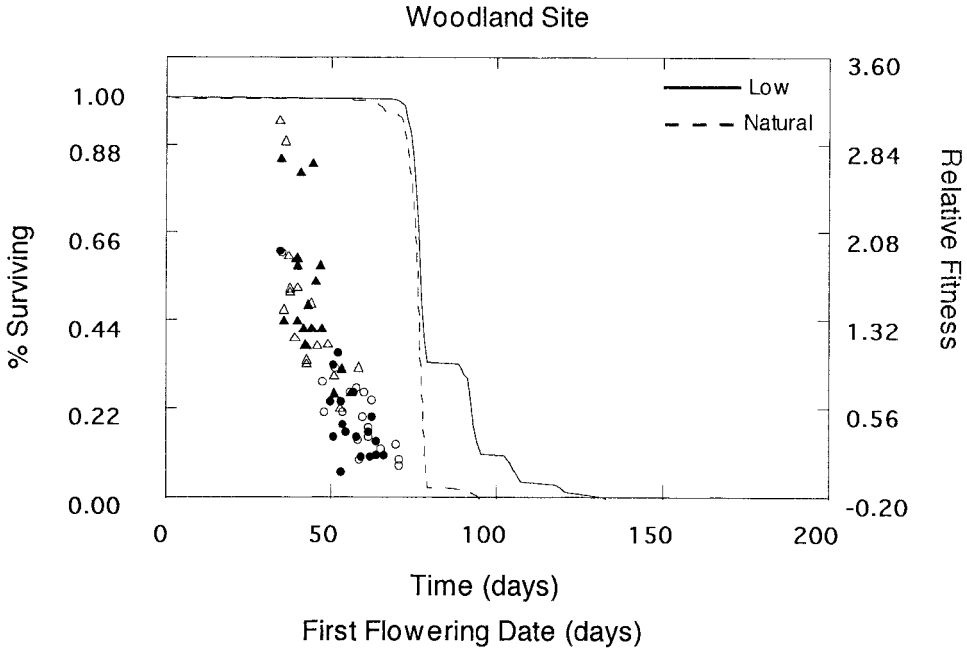

FIG. 1. Survivorship curves of plants at low (solid line) and natural (dashed line) densities in the woodland site and genotypic mean relative fitness (symbols, by block) as a function of flowering date. Circles, population originating from the sun site (sun population); triangles, population originating from the woodland site (woodland population); white, low density; black, natural density.

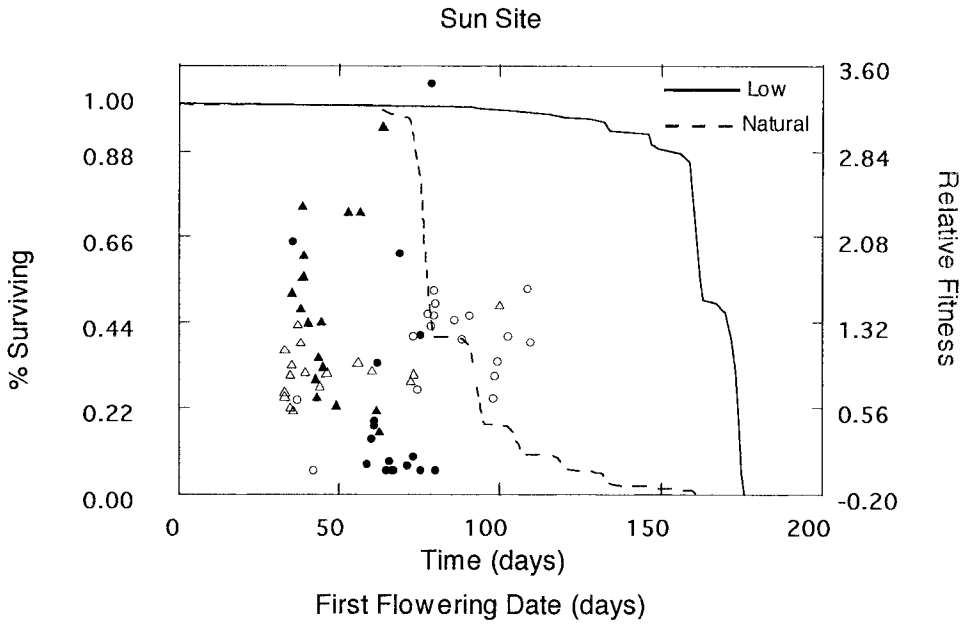

FIG. 2. Survivorship curves of plants at low and natural densities in the sun site (lines) and genotypic mean relative fitness (symbols, by block) as a function of flowering date. Symbols are as in Figure 1.

few plants, apparently stressed, lingered up to 125 days in the low-density treatment. In the sun site, mortality was strongly density dependent ($\chi^2 = 27.57$, $P < 0.001$). Plants at natural density suffered much mortality during the drought, whereas plants at low density did not (Fig. 2). Mortality was significantly earlier in the woodland site than in the sun site for both density treatments (low density: $\chi^2 = 44.33$, $P < 0.001$; natural density: $\chi^2 = 20.28$, $P < 0.001$). Plants from the sun and woodland populations had similar survivorship curves in most treatments, but plants from the woodland population senesced significantly earlier than those from the sun population in the sun site, low-density treatment ($\chi^2 = 26.15$, $P < 0.001$).

Early mortality caused some plants to die before flowering, so flowering date was unmeasured in this fraction of the sample. In the sun site, natural-density treatment, 23% of the plants died before flowering due to the drought, whereas prereproductive mortality was less than or equal to 4% in the other treatments. Consequently, the fraction of measured plants was substantially smaller in the sun site, natural-density treatment, and the flowering dates measured include only individuals that flowered early before they died and/or those that survived to experience lower densities. Plants that survived the drought in that treatment experienced densities of 1305 seedlings/m^2 before the drought, but only 990 seedlings/m^2 immediately after the drought. Seedling densities continued to decrease rapidly after the drought in that treatment. Later in the season, surviving plants experienced densities that approached that of the low-density treatment. Consequently, if selection on characters were density dependent, then selection may have changed during the season in this treatment. Such variable selection and the censored flowering date estimates may in part explain why so little of the variation in fitness was explained by the measured characters in this treatment (R^2-values based on genotypic selection: sun, low = 0.27; sun, natural = 0.16; woodland, low = 0.63; woodland, natural = 0.63).

A separate phenotypic selection analysis was conducted on the sun site, natural-density treatment to determine whether selection on characters changed over the course of the season and whether selection on flowering date may have occurred but could not be measured in the above analysis because the phenotype was not expressed by many individuals in that treatment. In this analysis, plants were classified as surviving the drought or not. For those plants that did not survive the drought and that had not flowered at the time of the drought, we assigned their flowering date as the day on which they died. In this manner, we were able to include all plants in the analysis rather than only those that survived to flower. The assigned flowering dates are underestimates of the flowering date that plants would have expressed had they survived. These assignments therefore provide a conservative test of the hypothesis that late flowering was disadvantageous in that treatment.

Selection on most characters did not differ between plants that survived the drought and those that did not (Table 3). However, early flowering was strongly favored in plants that died during the drought. Plants that survived the drought experienced variable densities thereafter, and selection on flowering date was not significant in that sample. No significant phenotypic selection was detected on the measured characters in the sample that survived the drought in that treatment.

By examining the survivorship curves in the sun site and comparing them to patterns of flowering date (Fig. 2), it can be seen that plants that had the highest fitness at low density flowered after most of the plants at natural density had died. Only 27% (SD = 45%) of the plants in natural density survived until the mean flowering date of their genotype at low density. Logistic regression revealed that genotypes flower-

TABLE 3. Phenotypic selection analysis in the sun site, natural-density treatment. The first sample consists of plants that did not survive the drought. The second sample consists of plants that survived the drought. Least square (LS) means of fitness are shown for the sun and woodland populations.

Trait	Not surviving	Surviving
Internode 1 (cm)	0.08	−0.06
Flowering date	−0.80***	−0.19
No. of nodes	−0.02	−0.22
Leaf length (cm)	0.03	0.40
LS mean fitness:		
sun	0.74	0.92
woodland	1.09	0.88
R^2	0.44	0.05
N	191	131

*** $P < 0.001$.

ing later at low density had significantly lower survival to the date at which they flowered at low density when they were growing at natural density (PROC CATMOD; $\chi^2 = 19.02$, $P < 0.0001$), indicating that few plants would have survived to flower had plants not accelerated their flowering date in the natural-density treatment. The average flowering date expressed at low density by the sun population (86 days) was almost simultaneous with the episode of mortality experienced at natural density. The average flowering date expressed at natural density by the sun population (64 days), however, was before the drought. If the sun population had not accelerated its flowering date at high density, most individuals would not have matured any fruits by the time they died. Consequently, the observed plasticity in flowering date by the sun population was, in fact, adaptive plasticity. Because mortality was early at both densities in the woodland site, delayed flowering at low density, as observed in the sun population, would be maladaptive. Thus, the population differentiation in plasticity in flowering date is consistent with the hypothesis of adaptive divergence.

Costs of Plasticity

No costs or benefits of plasticity were detected for any traits in any treatment when genotypic mean phenotypes were used in a multivariate selection analysis that included plasticities, except for a significant cost of plasticity in leaf length in the woodland site at natural density (Table 4). Plasticity of this trait was not adaptive in the woodland site, so no direct cost of adaptive plasticity was found. When each character was analyzed separately, a significant benefit of plasticity in internode length was found at low density in the sun site, and significant costs were found at both densities in the woodland site. However, this selection on plasticity is likely to be due to selection on correlated characters. Therefore, evidence for costs or benefits of plasticity independent from phenotypic values is weak.

TABLE 5. Environment-dependent Pearson correlations between genotypic mean phenotypes and genotypic plasticity in response to density.

	Sun site		Woodland site	
Trait	Low	Natural	Low	Natural
Internode 1	−0.57***	0.83***	−0.47**	0.14
Flowering date	0.89***	0.45**	0.65***	0.30
No. of nodes	0.51**	−0.64***	0.44**	−0.47**
Leaf length	0.51**	−0.44**	0.24	−0.48**

** $P < 0.01$; *** $P < 0.001$.

Correlations between Phenotypes and Plasticity

Genotypic mean phenotypes and genotypic plasticities were significantly correlated (Table 5). Moreover, the direction and magnitude of the correlation between mean phenotypes and plasticities changed with the density environment. Plasticity of internode length to density was significantly negatively correlated with the genotypic mean at low density, but positively correlated with the mean at natural density in both sites. Plasticity of flowering date was associated with later flowering in all treatments, although this correlation was not significant in the woodland site at natural density. In contrast, plasticities of node number and leaf length were positively correlated with the genotypic means for those traits at low density and negatively correlated at natural density. Thus, direct selection on trait means within environments is expected to result in correlated selection on plasticity, and the direction and magnitude of this correlated selection is environment specific.

The Influence of the Frequency of Environments on the Adaptive Value of Plasticity

A genotypic selection analysis shows the relationship between the phenotypes expressed at low and natural density and genotypic mean fitness when the genotype experienced different simulated frequencies of low density (Fig. 3). The slopes of the lines indicate the strength of the relationship between the phenotype expressed at natural density (along

TABLE 4. Cost of plasticity in response to density. Selection gradients for plasticity are based on genotypic selection analysis. They show the magnitude of direct selection on plasticity independently of selection on the expressed phenotype Single trait, the selection gradient when only one trait and its plasticity were included in the analysis; multitrait, the selection gradient when all traits and their plasticities were included in the analysis.

	Sun site, low density		Sun site, natural density		Woods site, low density		Woods site, natural density	
Trait	Single trait	Multitrait	Single trait	Multitrait	Single trait	Multitrait	Single trait	Multitrait
Internode 1 (cm)	0.10*	0.02	−0.14	−0.31	−0.15*	−0.10	−0.16*	−0.12
Flowering date	0.06	0.03	0.24	0.16	0.01	0.03	−0.09	−0.06
No. of nodes	−0.06	0.03	0.09	0.06	0.06	0.02	0.02	−0.01
Leaf length (cm)	−0.09	−0.09	0.02	0.09	−0.04	−0.08	−0.05	−0.15*

* $P < 0.05$.

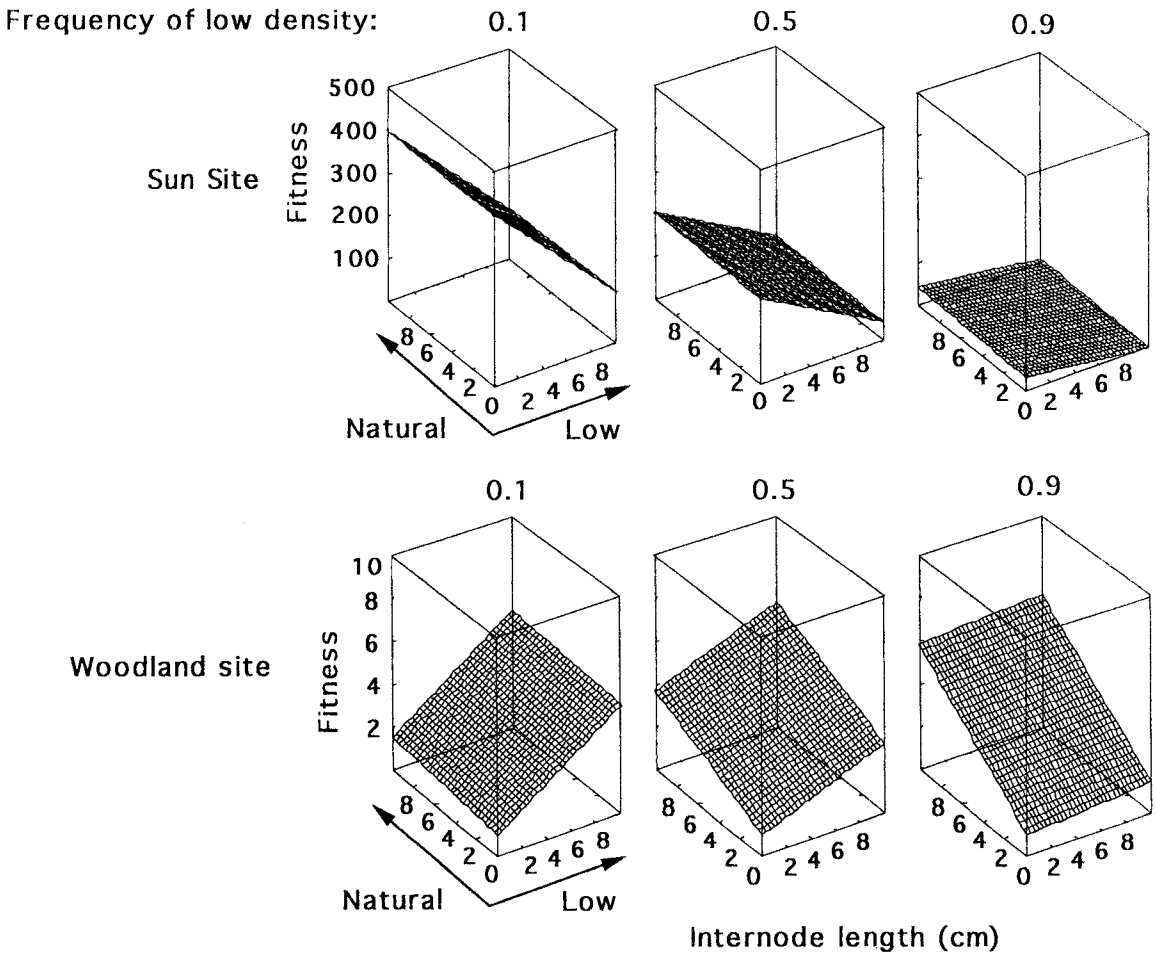

FIG. 3. Relationship between genotypic mean fitness (total lifetime seed production) and internode length expressed at low and natural density at three frequencies of low density for plants grown in the sun site (upper) and woodland site (lower).

the x-axis) or the phenotype expressed at low density (the z-axis) and genotypic mean fitness (y-axis). For example, in the sun site, when the genotype experiences 90% low density and only 10% natural density (upper left graph), the slope of the relationship between the phenotype expressed at natural density and fitness is shallow, indicating that the phenotype expressed in the rare, natural-density environment has very little influence on genotypic fitness. The slope of the relationship between the phenotype expressed at low density and fitness is steep, however, indicating that the phenotype expressed at low density strongly influences genotypic fitness. This is expected, because most of the individuals (90%) of that genotype are experiencing the low-density environment. What is more remarkable is that even when only 10% of the individuals experience low density (upper right graph), the phenotype expressed at low density still influences fitness more strongly than the phenotype expressed in the much more common natural-density environment. This is the case for all characters that showed density-dependent selection in the sun site. In the woodland site, in contrast, the phenotypes expressed in low and natural density have nearly equivalent influences on genotypic mean fitness when the environments are equally frequent (center graph).

This asymmetric contribution of the low- and natural-density phenotypes to fitness is due to strong density-dependent reproduction in the sun site (Fig. 4). The average number of seeds produced at low density in the sun site was nearly 100 times that produced at natural density, whereas in the woodland site, plants in low and natural density produced similar numbers of seeds. Consequently, in the sun site, even when low density is rare, a plant can gain an enormous fitness advantage by responding to it with the appropriate phenotype. In short, with hard selection (Van Tienderen 1991)—or density-dependent reproduction, in this case—even a rare environment can strongly influence the adaptive value of plasticity.

The total adaptive value of phenotypic plasticity was strongly site dependent and changed with the simulated frequency of environments experienced by a genotype within each site (Fig. 5). For internode length, plasticity was adaptive across all frequencies of environments in the sun site, and a very small increase in the frequency of low density resulted in a substantial increase in the adaptive value of plasticity. In the woodland site, however, plasticity in internode length was maladaptive across all frequencies of environments, and the strength of selection on plasticity did not change dramatically with the frequency of environments. Plasticity in flowering date was adaptive in the sun site across

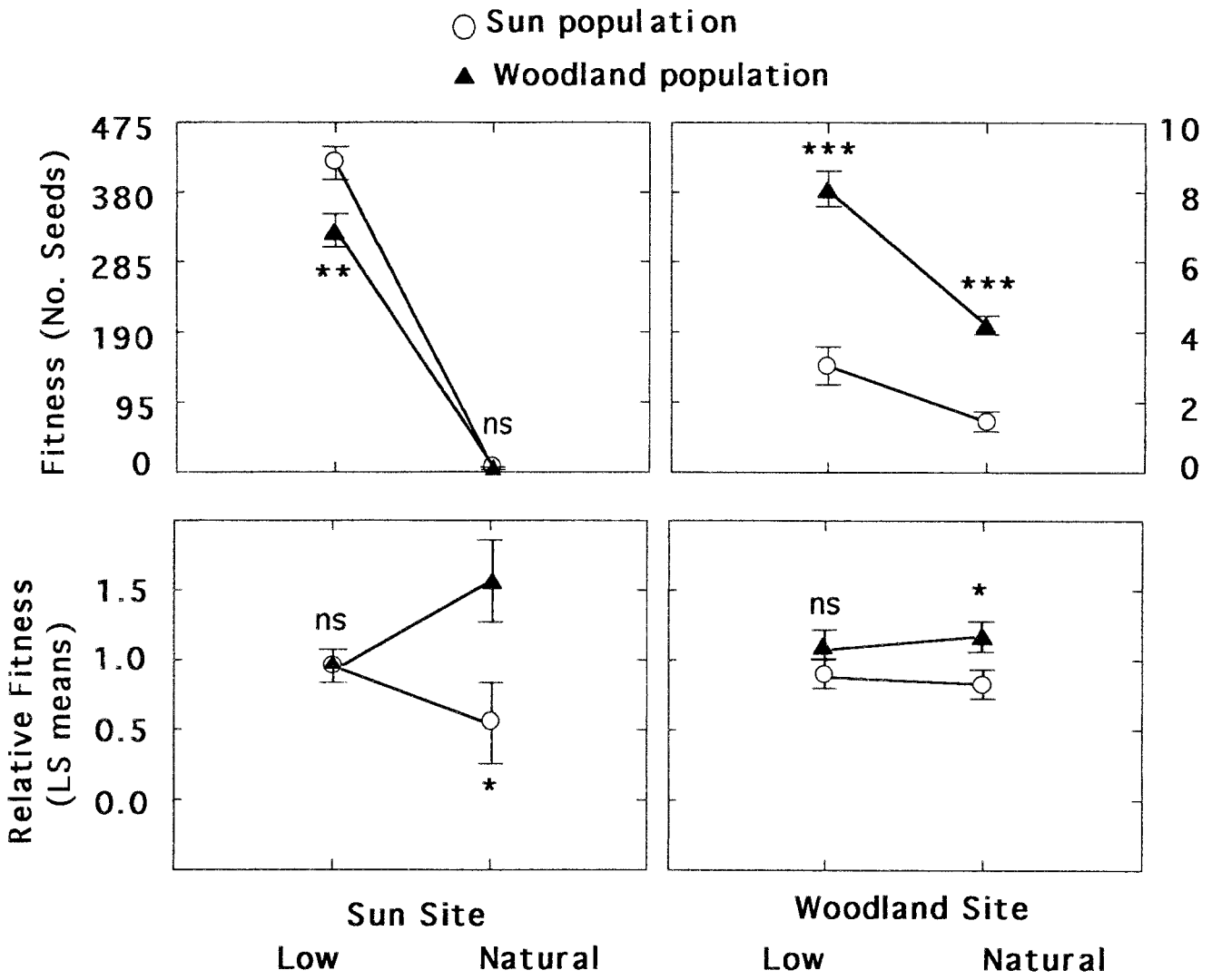

FIG. 4. Means and standard errors of total seed production by each population in each treatment (upper) and least square means of relative fitness after controlling for differences in phenotype (lower). Upper graph shows absolute fitness, but the genotypic analysis of relative fitness gave the same results. Significance refers to the difference between population. ns, not significant; $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

all frequencies of environments, and its adaptive value increased greatly with a small increase in the frequency of low density. In the woodland site, plasticity in flowering date was maladaptive across all frequencies of density environments, and its adaptive value did not change much with the frequency of environments. For node production, plasticity was adaptive only when the natural-density environment was the only environment in the sun site, and plasticity was maladaptive over all frequencies of environments in the woodland site. Plasticity in leaf length was adaptive in the sun site only when the frequency of low density was less than 20%. It was maladaptive over all frequencies of environment in the woodland site.

The adaptive value of plasticity shown in Figure 5 includes selection on the phenotypes and any associated costs or benefits of plasticity independently of the phenotype. However, because of a lack of strong costs or benefits of plasticity per se, the adaptive value of plasticity is determined primarily by correlated selection on the phenotype. Consequently, the direction of selection on the phenotype (Table 1) and the direction of the genetic correlation between the phenotype and plasticity (Table 5) determine the total selection on plasticity (Fig. 5).

Local Adaptation

Local adaptation was observed in this system, with plants having higher fitness in their home site (Fig. 4, upper). However, in the sun site, the fitness difference between populations was apparent only at low density. To what extent does density-dependent selection on plastic characters explain these fitness differences between the populations?

By including all the measured characters and their plasticities in an analysis of covariance that also included population, we asked whether fitness differences between populations still existed after adjusting fitness for their differ-

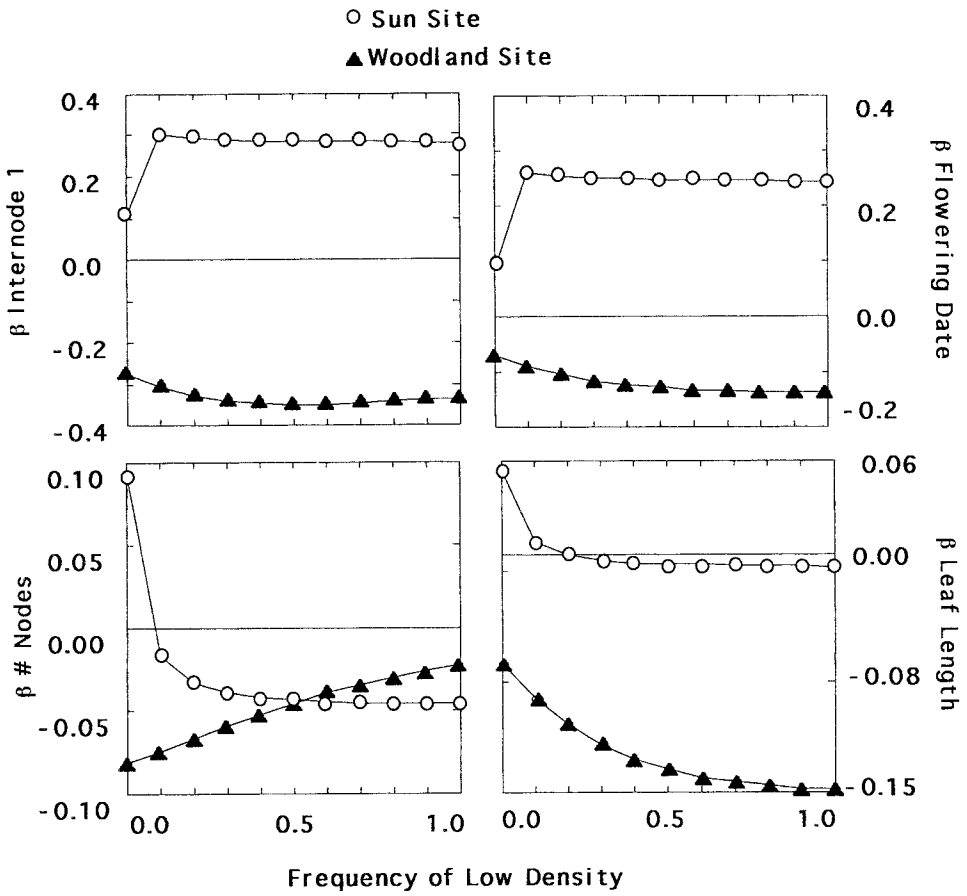

FIG. 5. Selection gradients (β) as a function of the frequency of low density. Selection gradients indicate the strength of the relationship between relative genotypic fitness and plasticity to density. Values above the zero line indicate that plasticity is adaptive.

ences in phenotypes (Fig. 4, lower). No fitness differences between populations were found in the sun site, low-density treatment after adjusting for phenotypes, indicating that these characters, their plasticity, and any unmeasured correlated characters fully accounted for the fitness differences between the populations in this treatment. In the natural-density treatment in the sun site, the woodland population actually had significantly higher fitness than did the sun population when variation in the measured characters was controlled. In the woodland site, the measured characters fully accounted for fitness differences between the populations at low density but not at natural density. Therefore, other unmeasured characters that differ between the populations also contributed to local adaptation at natural density.

DISCUSSION

Our results support the hypothesis of adaptive divergence in plastic responses to density. The sun population displays greater plasticity in internode length and flowering date than does the woodland population (Dudley and Schmitt 1995). This study showed that selection on internode length and flowering date would favor plasticity of both of these characters in the sunny site but not in the woodland site, as predicted from comparative studies (Morgan and Smith 1979). Although the observed patterns of differentiation between these two populations may be due to drift rather than selection, they are consistent with the hypothesis of adaptive divergence. No evidence of direct costs or benefits of adaptive plasticity was found. The strength and direction of genetic correlations between phenotype and plasticity differed with density. Consequently, total selection on plasticity within each site was determined both by the pattern of density-dependent selection on expressed phenotypes and by density-dependent correlated selection on the plasticity of those phenotypes. The selective advantage of plasticity within each site also depended on the frequency of density environments. Local adaptation in the sun site was largely attributable to selection on shade avoidance traits and their plasticities. However, selection on these traits did not fully explain the local adaptation observed in the woodland site, suggesting that selection on other unmeasured characters was important for the observed adaptive divergence.

As predicted by Dudley and Schmitt (1995), selection favored increased internode elongation at natural density, but decreased elongation at low density in the sunny site. This pattern of density-dependent selection was in the same direction as the plastic response to density observed in genotypes from the sun population. In contrast, internode plasticity was maladaptive in the woodland site and woodland genotypes display reduced plasticity. Thus, local adaptation

for shade avoidance responses appears to have occurred on a microgeographic scale in this system. This divergence is in the direction predicted from functional arguments and comparative studies that led to the hypothesis that the shade avoidance response is adaptive only in open habitats (Morgan and Smith 1979; Dudley and Schmitt 1995). However, contrary to those predictions, longer internodes were favored under closed-canopy conditions. At low density, longer internodes appear to be favored through indirect selection on unmeasured characters. At natural density, direct selection favored longer internodes, suggesting that elongation can be adaptive at high densities even under closed-canopy conditions.

We also detected local adaptation for plasticity of flowering date, as predicted by Schmitt (1995). Early flowering was advantageous at both densities in the woodland site because of early mortality in that site; consequently, plasticity of flowering date was maladaptive. In the sunny site, the timing of mortality was strongly density dependent. Delayed flowering was favored at low density, where plants lived much longer. In this favorable environment, we observed a cost of early flower production, probably due to a trade-off in allocation of axillary meristems to early flowers versus branch production and consequent later reproduction (Geber 1990; Schmitt 1995). Measuring selection on flowering date at natural density in the sunny site was complicated by the fact that the density changed during the experiment and because many genotypes responded to high density by flowering earlier. This plasticity altered the opportunity for direct selection on flowering time, and thus our ability to detect it (Kingsolver 1995; Dudley and Schmitt 1996). If plants had flowered at the same time as they did at low density, however, then most of the plants would have died before they had flowered. Moreover, genotypic selection analysis demonstrated that plasticity of flowering time was advantageous in the sun site over a wide range of hypothetical frequencies of density environments. We therefore conclude that the observed population differentiation in plasticity of flowering time is adaptive. Early flowering has been shown to be advantageous in habitats in which mortality is likely to be early (Lacey 1986a,b; Fox 1990; Biere 1995). Schemske (1984) observed fine-scale adaptive divergence in flowering time in *Impatiens pallida*; in woodland areas, early mortality caused selection to favor genotypes that flowered earlier than those growing on forest edges. Bennington and McGraw (1995) also observed adaptive differentiation in flowering time in this species in response to variation among sites in timing of mortality. Our study demonstrates that when a reliable cue of expected longevity exists (such as density in the sun site), plasticity of flowering date to that cue can also evolve.

In this system, local adaptation in shade-avoidance traits was manifest as either increased or decreased plasticity, depending on the site. In contrast, the observed plasticity in the size-related characters, node production and leaf size, was not adaptive in either site.

We found little evidence of direct costs of plasticity as potential constraints on adaptive differentiation (Van Tienderen 1991; DeWitt et al. 1998) in *I. capensis.* These observations are consistent with other recent studies that also failed to detect such costs (e.g., Scheiner and Berrigan 1998) or found that such costs were attributable to selection on correlated characters (DeWitt 1998). Such results are not entirely unexpected (DeWitt et al. 1998). Costs of plasticity may be subtle compared to selection on phenotypes per se. Moreover, selection may have acted to minimize such costs (DeWitt et al. 1998), although genetic or developmental constraints would limit the degree to which selection can do so.

One genetic constraint of particular interest in this study is the relationship between the phenotype expressed by a genotype in an environment and the plasticity of that genotype (which is related to the across-environment correlation of Via and Lande 1985; de Jong 1995; Roff 1997). In this system, selection acted primarily on the phenotype, rather than directly on plasticity. However, the phenotype was correlated with plasticity, so environment-dependent selection on the phenotype resulted in environment-dependent correlated selection on plasticity. For example, selection on internode length would cause correlated selection for increased plasticity in the sunny site, but decreased plasticity in the woodland site. Consequently, when we consider total selection on plasticity, working primarily through selection on the phenotypes expressed in the different environments, we see that plasticity in internode length was favored in the sunny site, but not in the woodland site (Fig. 5). Similarly, selection on flowering date led to plasticity being favored in the sunny site, but disfavored in the woodland site.

Differences in shade avoidance traits and their plasticities between these two populations contributed significantly to local adaptation in this system. Selection on the measured traits and their plasticities fully accounted for local adaptation in the sunny site. Moreover, selection on the measured characters prevented the woodland population from having a fitness advantage in the natural-density treatment, which, like the woodland site, experienced severe drought stress and early mortality. Other studies have shown both local adaptation and differentiation in important morphological and phenological traits (e.g., Schemske 1984; Galen et al. 1991; Bennington and McGraw 1995). For example, Bennington and McGraw (1995) demonstrated local adaptation in *I. pallida* populations. Early flowering was favored in the drier site, but selection was neutral regarding flowering time in the more mesic site. Like Schemske's study (1984), their results suggest that the population differentiation in flowering time they observed was due to adaptive divergence and that such divergence contributed to local adaptation.

In the woodland site at natural density, differentiation in the measured characters did not fully account for the differences in fitness between the two populations. For example, in this site, the sun population plants expressed internode lengths comparable to those of the woodland population plants, but this response by the sun population did not result in equal fitness, suggesting that adaptive differentiation in other unmeasured traits was also important. Plasticity of the measured traits could not completely eliminate the expression of population differences within sites. Although the sun population was able to accelerate its flowering date in the woodland site, it did not flower as early as the woodland population and therefore suffered a fitness loss. Thus, although plasticity may increase ecological tolerance (Bradshaw 1965; Sultan

1987; Andersson and Widén 1993), it does not result in higher fitness everywhere within its ecological range.

The results of this study demonstrate that even when an environment is rare, an appropriate response to it can give an enormous fitness advantage when the rare environment is more favorable than the common one. In the sunny site, plant densities are normally high. However, there may be favorable patches of low density or the density may decrease throughout the season, as seen in this study. These results corroborate well-known theoretical results that show that the adaptive value of plasticity depends on both the frequency of environments and the fitness differences between environments under hard selection (Van Tienderen 1991). Plasticity may evolve more easily in environments in which the rare environment causes a large increase in fitness than when the rare environment is associated with low fitness.

In summary, our results provide evidence for adaptive divergence in plastic shade-avoidance responses under natural conditions. The predictions concerning adaptive shade-avoidance responses that were realized in cross-species comparisons (Morgan and Smith 1979) are also realized at the interpopulation level. Such differentiation significantly contributed to local adaptation, although it could not completely explain it in all experimental environments. The role of environmental variability in determining the adaptive value of phenotypic plasticity is shown to be important and to depend on whether selection is hard or soft. In addition, this study shows that genetic constraints can determine how plasticity will be selected when selection acts primarily on the phenotypes rather than on plasticity per se.

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Appendix

The genetic correlation between the trait mean and its plasticity is mathematically related to the across-environment genetic correlation (Roff 1997). The correlation between the trait mean in environment 1 and its plasticity is defined as:

$$r(\text{mean 1, plasticity}) = \text{cov}(x_1, x_2 - x_1)/[V_A(x_1)V_A(x_2 - x_1)]^{1/2}, \quad \text{(A1)}$$

where x_1 is the trait mean in environment 1, x_2 is the trait mean in environment 2, $V_A(x_1)$ is the additive genetic variance of the trait in environment 1, and $V_A(x_2 - x_1)$ is the additive genetic variance of the plasticity of the trait. The genetic correlation between the trait mean and plasticity is a part-whole correlation of x_1 and $x_2 - x_1$, and can be written as (Sokal and Rohlf 1981):

$$r(\text{mean 1, plasticity}) = [V_A(-x_1)]^{1/2} + r(x_1, x_2)[V_A(x_2)]^{1/2} \div [V_A(-x_1) + 2r(x_1, x_2)[V_A(-x_1)V_A(x_2)]^{1/2} + V_A(x_2)]^{1/2}, \quad \text{(A2)}$$

where $r(x_1, x_2)$ is the correlation between the trait across environments. Likewise, the correlation between the trait mean in environment 2 and its plasticity is defined as:

$$r(\text{mean 2, plasticity}) = [V_A(x_2)]^{1/2} + r(x_1, x_2)[V_A(-x_1)]^{1/2} \div \{V_A(x_2) + 2rx_1, x_2[V_A(x_2)V_A(-x_1)]^{1/2} + V_A(-x_1)\}^{1/2}. \quad \text{(A3)}$$

The denominators are equivalent, but the numerators are not. That is,

$$[V_A(-x_1)]^{1/2} + r(x_1, x_2)[V_A(x_2)]^{1/2} \neq [V_A(x_2)]^{1/2} + r(x_1, x_2)[V_A(-x_1)]^{1/2}, \quad \text{(A4)}$$

unless $V_A(-x_1) = V_A(x_2)$. Therefore, the magnitude of the correlation between the trait mean and its plasticity will vary with the environment if the additive genetic variance of the trait varies with environment. The correlation, moreover, will be stronger in the environment in which the additive genetic variance is greater.