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ADAPTIVE DIVERGENCE IN PLASTICITY IN NATURAL POPULATIONS OF *IMPATIENS CAPENSIS* AND ITS CONSEQUENCES FOR PERFORMANCE IN NOVEL HABITATS

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Abstract.—We tested for adaptive differentiation between two natural populations of *Impatiens capensis* from sites known to differ in selection on plasticity to density. We also determined the degree to which plasticity to density within a site was correlated with plastic responses of experimental immigrants to foreign sites. Inbred lines, derived from natural populations in an open-canopy site and a woodland site, were planted reciprocally in both original sites at naturally occurring high densities and at low density. The density manipulation represents environmental variation typically experienced within the site of a given population, and the transplant manipulation represents environmental differences between sites of different populations. Internode elongation, meristem allocation, leaf length, flowering date, and total lifetime fitness were measured. Genotypes originating in the open site, where selection favored plasticity of first internode length and flowering time (Donohue et al. 2000a), were more plastic in those characters than genotypes originating from the woodland site, where plasticity was maladaptive. Therefore, these two populations appear to have responded to divergent selection on plasticity. Plasticity to density strongly resembled plasticity to site differences for many characters, suggesting that similar environmental factors elicit plasticity both to density and to overhead canopy. Thus, plasticity that evolved in response to density variation within a site influenced phenotypic expression in the foreign site. Plastic responses to site caused immigrants from foreign populations to resemble native genotypes more closely. In particular, immigrants from the open site converged toward the selectively favored early-flowering phenotype of native genotypes in the woodland site, thereby reducing potential fitness differences between foreign and native genotypes. However, because genotypes from the woods population were less plastic than genotypes from the sun population, phenotypic differences between populations were greatest in the open site at low density. Therefore, population differences in plasticity can cause genotypes from foreign populations to be more strongly selected against in some environments than in others. However, genetic constraints and limits to plasticity prevented complete convergence of immigrants to the native phenotype in any environment.

Key words.—Environmental heterogeneity, environment-dependent genetic parameters, genetic constraints, niche breadth, phenotypic plasticity, population differentiation, reciprocal transplant.

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Plasticity evolves in response to environmental variation experienced by a population within its native site. Plasticity can be selectively favored in some habitats (Dudley and Schmitt 1996; DeWitt 1998), but maladaptive in others (Donohue et al. 2000a). Such divergent selection can cause evolutionary divergence in plasticity among populations (Dudley and Schmitt 1995; Galloway 1995; Schmitt et al. 1995) or species (Morgan and Smith 1979). Population differentiation in plasticity has been documented in several species (e.g., Wilken 1977; MacDonald and Chinnappa 1989; Ohlson 1989; Winn and Evans 1991; Oyama 1994a,b). However, evidence for evolutionary responses to natural selection on plasticity requires the demonstration of both variable selection on plasticity in natural environments and population divergence in plasticity in a direction consistent with selection. Such evidence of evolutionary responses to divergent selection on plasticity is uncommon.

Plasticity is hypothesized to influence ecological niche breadth (Bradshaw 1965; Sultan and Bazzaz 1993a,b,c; Sultan 1987, 1995; Oyama 1994b; Whitlock 1996; Sultan et al. 1998). Appropriate plastic responses to environmental variation allow organisms to express selectively advantageous phenotypes in a broader range of environments. The influence of plasticity on phenotypic expression in widely varying ecological environments depends on the specificity of plastic responses to particular environmental factors and on the ecological context of the factors that elicit plasticity (Moran 1992; Getty 1996). In particular, plasticity that evolved in response to environmental variation within a site may also influence phenotypic expression in novel sites after colonization or migration (Moran 1992; Zerba and Collins 1992; Getty 1996) if similar environmental factors elicit plastic responses to within- and between-site variation (Sultan 1987, 1995; Sultan and Bazzaz 1993c). If plastic responses to environmental variation within a site resemble plastic responses to environmental differences between sites, divergent selection on plasticity experienced within a site could cause correlated selection on plastic responses to environmental differences between sites. Consequently, in addition to divergence in plasticity to within-site variation, some populations may be more responsive than others to environmental differences between sites. Whether or not such responses to environmental conditions of novel sites enhance the performance of immigrants depends on how similar the selective environment of the novel site is to the selective environment that elicits plasticity in the native site.

Similarity between plasticity to environmental variation within a site and plasticity to environmental differences be-

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tween sites can occur in two ways. Most obviously, it can occur if the same environmental factor varies both within and between sites. It can also occur if cues associated with that environmental factor vary between sites, even if the factor itself does not differ between sites. For example, in high density stands of competitors, plants experience an altered light environment that resembles the light environment altered by an overhead tree canopy. This is because green leaves absorb red light and transmit or reflect far red light. Beneath a foliage canopy, whether a dense canopy of competing neighbors or an overhead tree canopy, plants experience both a reduction in photosynthetically active radiation (PAR) and a lower ratio of red:far red (R : FR) wavelengths (Smith 1982, 1986; Smith et al. 1990). In both cases, plants experience a similar light environment even though a low-density, closed-canopy environment is ecologically very different from an open-canopy, high-density environment. Hence, plants may respond similarly to these different ecological environments because of the similarity in the cues that elicit plastic responses to them. Other possible examples in which cues that elicit plasticity could be similar for different ecological environments include similar resource depletion caused by different competitors, common chemical pheromones released by different predators, or similar hormonal cues in response to different environmental stresses.

Population differentiation in plasticity can cause phenotypic differences between populations to change with the environment. Field studies have shown that phenotypic differences between populations are not expressed equally in all environments (e.g., Miller and Fowler 1994; Bennington and McGraw 1996). Phenotypic differences between populations will influence fitness differences between populations if those phenotypes are under selection (Sultan 1987, 1995; Bennington and McGraw 1995). Such environment-dependent fitness differences between populations can influence the strength of selection against immigrants from foreign populations under different natural conditions. Selection against immigrants can influence colonization ability and thus the direction and magnitude of gene flow (Slatkin 1985). Therefore, the environment-dependent expression of phenotypic differences between populations, caused by population divergence in plasticity, has important consequences for ecotypic differentiation, local adaptation, colonization, and patterns of gene flow.

In this study, we tested for adaptive differentiation between two populations of the North American annual *Impatiens capensis* from adjacent sites known to differ in selection on plasticity to density (Donohue et al. 2000a). We further investigated how plasticity to density variation within a site corresponds to plasticity to environmental differences between sites, and how population differences in plasticity influenced phenotypic expression and fitness of experimental immigrants into foreign sites.

Density varies widely under natural conditions in *I. capensis,* and responses to density are known to have fitness consequences (Dudley and Schmitt 1996). In particular, plastic internode elongation at high density and delayed flowering at low density were favored by natural selection in an open site but were maladaptive in a woodland site (Donohue et al. 2000a). Under open-canopy conditions, such responses effectively enable plants growing at high density to escape competitive light environments and commence reproduction before dying early. At low density under open-canopy conditions, plants lived longer, and those that delayed flowering by allocating meristems to branches rather than flowers had higher fitness (see also Geber 1990; Schmitt 1995). Under closed-canopy conditions, longer internodes were favorable at both densities, and early mortality at both densities caused non-plastic, early flowering to be favored. Thus, plasticity in internode length and flowering time was maladaptive in that site. Natural populations also differ genetically in their plastic responses to R:FR (Dudley and Schmitt 1995) and PAR (Schmitt 1993), both of which may vary with density (Ballaré et al. 1987, 1990; Schmitt and Wulff 1993; Donohue and Schmitt 1999). Consequently, plasticity to density in *I. capensis* provides a system in which the quantitative genetic basis of the response is well-characterized and in which the selective consequences of plasticity are known.

We performed a quantitative genetics study using a reciprocal transplant design coupled with a density manipulation in two natural populations. Companion papers report patterns of site-specific and density-dependent natural selection on plastic traits in those populations (Donohue et al. 2000a) and the genetic architecture of these plastic traits (Donohue et al. 2000b). This design also allowed us to examine population differentiation in plastic responses to density variation within a site and plasticity to environmental differences between sites. Here, we ask: (1) Do populations differ in their plastic responses to density and to environmental differences between sites? (2) Does plasticity to density resemble plasticity to environmental differences between sites? (3) How does plasticity influence the expression of phenotypic and fitness differences between populations in different environments? (4) Do genetic constraints limit the potential for plasticity to buffer these environment-dependent population differences?

Methods

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

Seeds collected from 18 genotypes from the sun population and 17 genotypes from the woodland population were weighed and then stored in distilled water in microtiter trays at 4°C for four months. In late April 1997, seeds were planted into plug trays filled with "Metromix 350" (an artificial soil medium, Scotts-Sierra Horticultural Products Co., Marysville, OH). Seeds were left outside in a cold frame to germinate. The emergence date of each seedling was recorded.

Up to three seedlings from each genotype were planted into each of three low-density and three natural-density blocks in the sun and woodland sites, giving a total of 1115 seedlings in a split-plot design. All blocks were first cleared

of native vegetation. 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 approximated the maximal natural seedling density in each site. In the woodland site, seedlings were planted 5 cm apart in a 12 × 14 array, giving a density of 450 seedlings/m^2. In the sun site, seedlings were planted as closely as possible, 3 cm apart in a 12 × 14 array, giving a density of 1305 seedlings/m^2. However, this planting density still underrepresented the maximum natural seedling densities in the sun site. Two border rows were planted around the natural density treatments in both sites to minimize edge effects.

The density manipulation represents the range of density that each native population typically experiences within its site. The transplant component represents the environmental differences that genotypes from a foreign population would experience as immigrants. The comparison of low and natural-density treatments within each site reveals the effect of density particular to each site. The comparison of low-density treatments between sites reveals the effect of the environmental differences between sites while controlling for density.

Two weeks after the seedlings were planted into the treatments, the following traits were measured: length of the first and second internodes; height; the number of nodes; the number of axillary flowers (''primary flowers''), branches, and quiescent buds at each node of the main stem; length of the largest leaf. Twice a week, plants were censused for the presence of cleistogamous (CL) or chasmogamous (CH) flowers, and the date of first flowering was estimated from these censuses. Total lifetime fitness was measured as the estimated total number of seeds produced during the lifetime of the individual. Fruit number was counted during each census, and seed number per fruit was estimated twice during the season for CL and CH fruits. (See Donohue et al. 2000a, for more detail.) Fitness was relativized within each treatment by dividing the total lifetime fitness of each individual by the mean lifetime fitness of all individuals within the same experimental treatment.

Statistical analyses.—The SAS statistical package (1990) was used for all analyses. A mixed-model multivariate analysis of variance (MANOVA) was used to examine patterns of population differences in their plasticity to density and to site. Because of highly significant three-way interactions between site, density, and population, each site was analyzed separately to determine the degree of population differences in plasticity to density. Germination date was used as a covariate in all analyses. Genotype, a random effect, was nested within population. Block, also a random effect, was nested within density. Interactions between genotype and block and between population and block were pooled with the error term. Main effects of density were tested over the interaction between density and genotype and the block effect (nested within density), using the ''test'' option in SAS which employs Satterthwaite approximations. Main effects of population were tested over the genotype effect. Individual analyses of covariance (ANCOVAs) were conducted on each variable using the same model structure as in the MANOVA. To investigate population differentiation for plasticity to site, while controlling for density, the low-density treatment in both sites was analyzed with the same models as above, except that block was nested within site rather than density. Effects of site were tested over the site by genotype interaction and the block effect (nested within site), and all other tests were as described above.

Population by density interactions in the models described above indicated whether the differences between populations differed with density, and population by site interactions indicated whether the differences between populations differed with site. To determine the degree of population differentiation for morphological and phenological characters for plants grown in each environment, each environment was analyzed separately using separate ANCOVAs. A population main effect indicated significant differences between populations for each trait.

To determine the similarity between plasticity to density and plasticity to environmental differences between sites, two measurements of plasticity were estimated. Plasticity to density under open-canopy conditions was calculated as the genotypic mean phenotype expressed in low density minus that expressed in high density for all traits except internode lengths and height. Because these two traits displayed higher mean values at high density, plasticity was calculated by subtracting the genotype mean at low density from that at high density. Plasticity to site, while controlling for density, was calculated as the mean phenotype expressed at low density in the woodland site minus the mean phenotype expressed at low density in the sun site, except for internode lengths and height, as before. The genotypic correlation between plasticity to density in the sun site and plasticity to site was estimated as the Pearson correlation between genotypic plasticities. These two measures of plasticity are expected to be spuriously correlated because they both depend on the phenotype expressed at low density in the sun site. Therefore, to test whether the correlations between plasticities were significantly different from that expected by spurious correlations, probabilities were taken from the genotypic correlation between the phenotype expressed at natural density in the sun site and that expressed at low density in the woodland site (the correlation between the independent components of the two plasticities). To further test the hypothesis that the two plasticities resemble each other, the genetic correlation across natural density in the sun site and low density in the woodland site was estimated using variance and covariance partitioning (Fry 1992; Windig 1997) in a restricted maximum likelihood analysis (Fry 1999). Correlations were estimated separately for each population and for the sample pooled over populations.

To examine genetic constraints on plasticity to environmental differences between sites, and to test for genetically based trade-offs across sites, genetic correlations across sites at both low and natural density were estimated using variance and covariance partitioning in a restricted maximum-likelihood analysis (Fry 1992, 1999; Windig 1997). Correlations were estimated separately for each population and for the sample pooled over populations.

Results

Population Differentiation in Plasticity to Density and Site

The two populations differed in plasticity to density for many characters, especially in the sun site (Fig. 1). The full

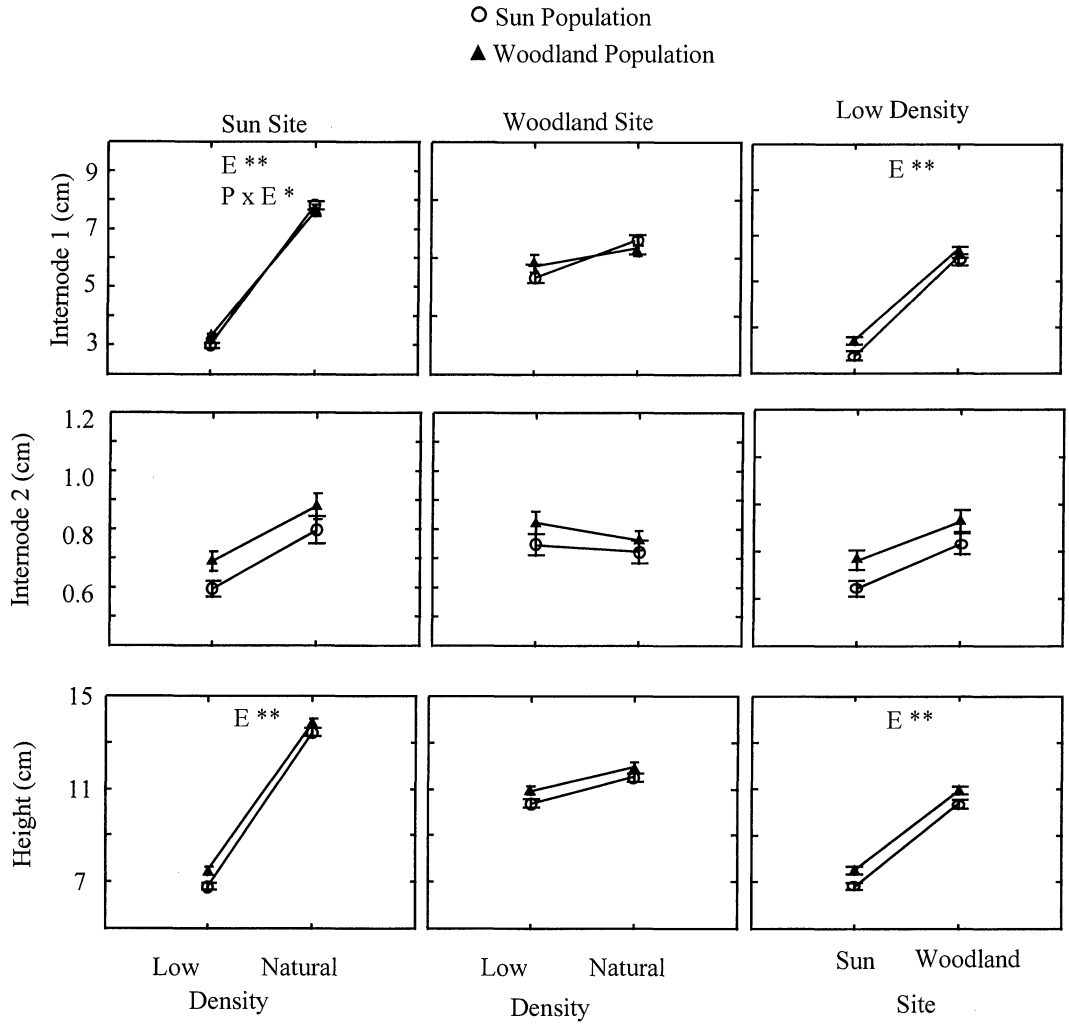

FIG. 1. Population responses to natural densities when grown in sun and woodland sites and population responses to site differences at low density. Population means and standard errors are shown. E, environment (density or site); P × E, population by environment (density or site) interaction. Significance levels are given for effects of environment and for population by environment interactions. Complete analysis of density effects are given in Donohue et al. (2000b). Values of absolute fitness are taken from Donohue et al. (2000a). $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

analysis of population responses to density are presented in Donohue et al. (2000b). In the sun site, genotypes from the sun population were more responsive to density in first internode length than genotypes from the woodland population, exhibiting enhanced elongation at natural density and suppressed elongation at low density. The sun population also showed significantly greater plasticity in flowering date. Flowering of genotypes from the sun population was later at low density than at high density, whereas genotypes from the woodland population flowered early at both densities. The difference in flowering date between populations reflects a difference in plasticity of meristem allocation. Although plants from both populations allocated more meristems to branches at low density, plants from the woodland population compensated for this allocational change by producing significantly more early flowers and fewer dormant buds at low density. Such compensatory plasticity in flower and bud production resulted in the same flowering date as was expressed at natural density. The sun population, in contrast, did not show plasticity in meristem allocation to flowers and dormant buds. Thus, the increased branch production at low density led to delayed flowering as well.

Population differentiation in plasticity to density was also apparent in the woodland site (Fig. 1). Genotypes from the woodland population produced more branches at low density than at high density whereas genotypes from the sun population produced few branches at both densities in this site. Meristem dormancy was more responsive to density in the sun population, with fewer buds remaining quiescent at natural density. Genotypes from the sun population flowered earlier at natural density than at low density, whereas genotypes from the woodland population flowered early at both densities. Population differentiation for plasticity to density was significantly greater in the sun site for primary flower production ($F = 9.55$, $P < 0.005$) and flowering date ($F = 9.35$, $P < 0.005$), as indicated by significant interactions between population, density, and site.

Plasticity to site was apparent as an increase in the length

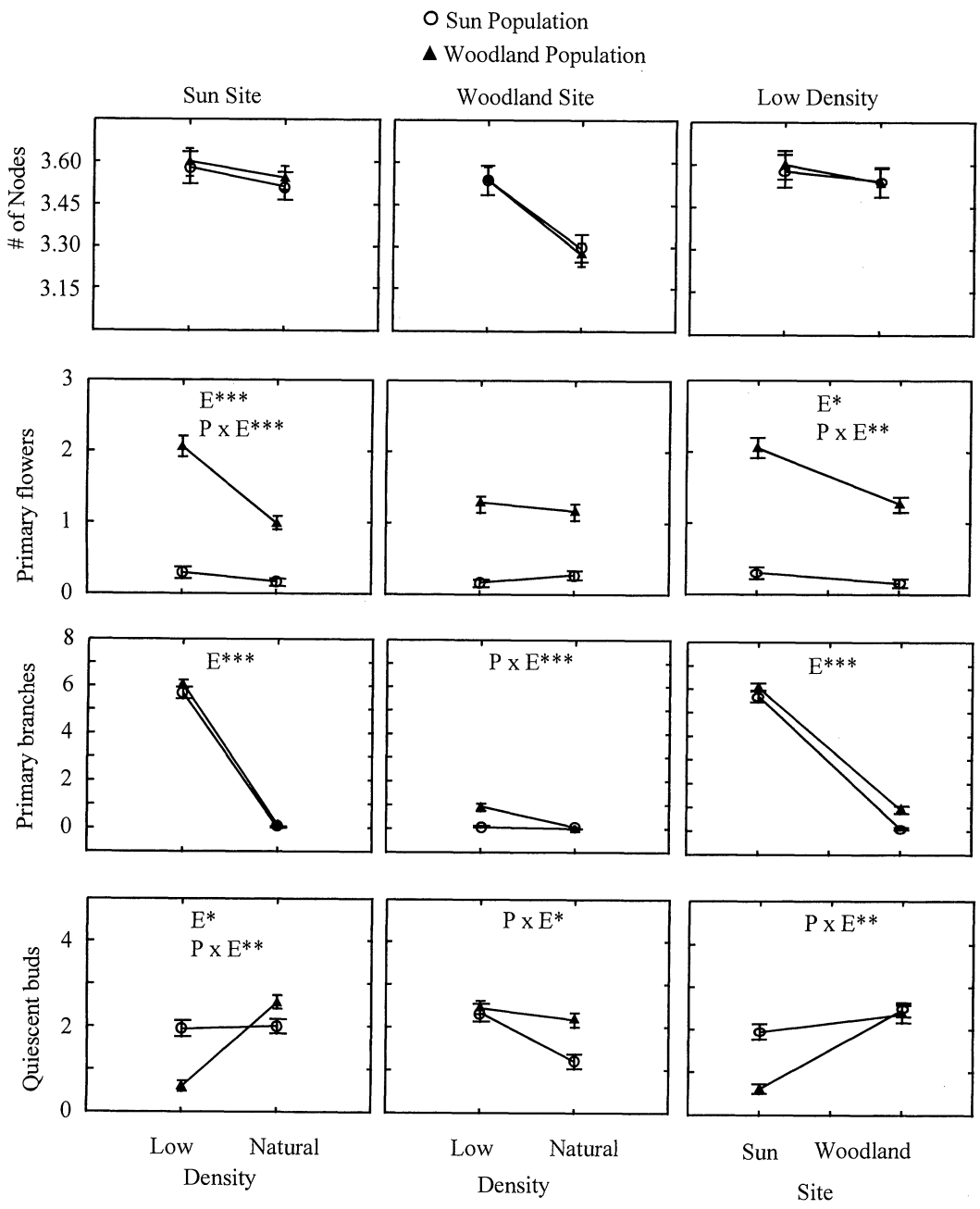

FIG. 1. Continued.

of the first internode and height; decreased flower and branch production; and earlier flowering of plants grown in the woodland site as compared to those grown in the sun site (Fig. 1, Table 1). Genetic variation for plasticity to site was found within populations for all characters except the length of the second internode, height (nearly significant), number of nodes, and leaf length (Table 1).

Although the two populations did not differ significantly in their plastic responses to site according to the MANOVA, individual ANCOVAs indicated that plasticity to site differed between populations for some traits (Fig. 1, Table 1). The sun population exhibited stronger plasticity to site for flowering date by flowering significantly later in the sun site. Greater plasticity in flowering date of sun genotypes corresponded to less plasticity in early flower production and bud dormancy. The woodland population displayed a stronger response to site for primary flower production and bud quiescence by showing increased flower production and reduced bud dormancy in the sun site. All characters that showed population differentiation for plasticity to site differences also were differentiated in their plastic response to density (Fig. 1).

Environment-Dependent Differences between Populations

The differences in plasticity of the two populations caused the expression of phenotypic differences between them to

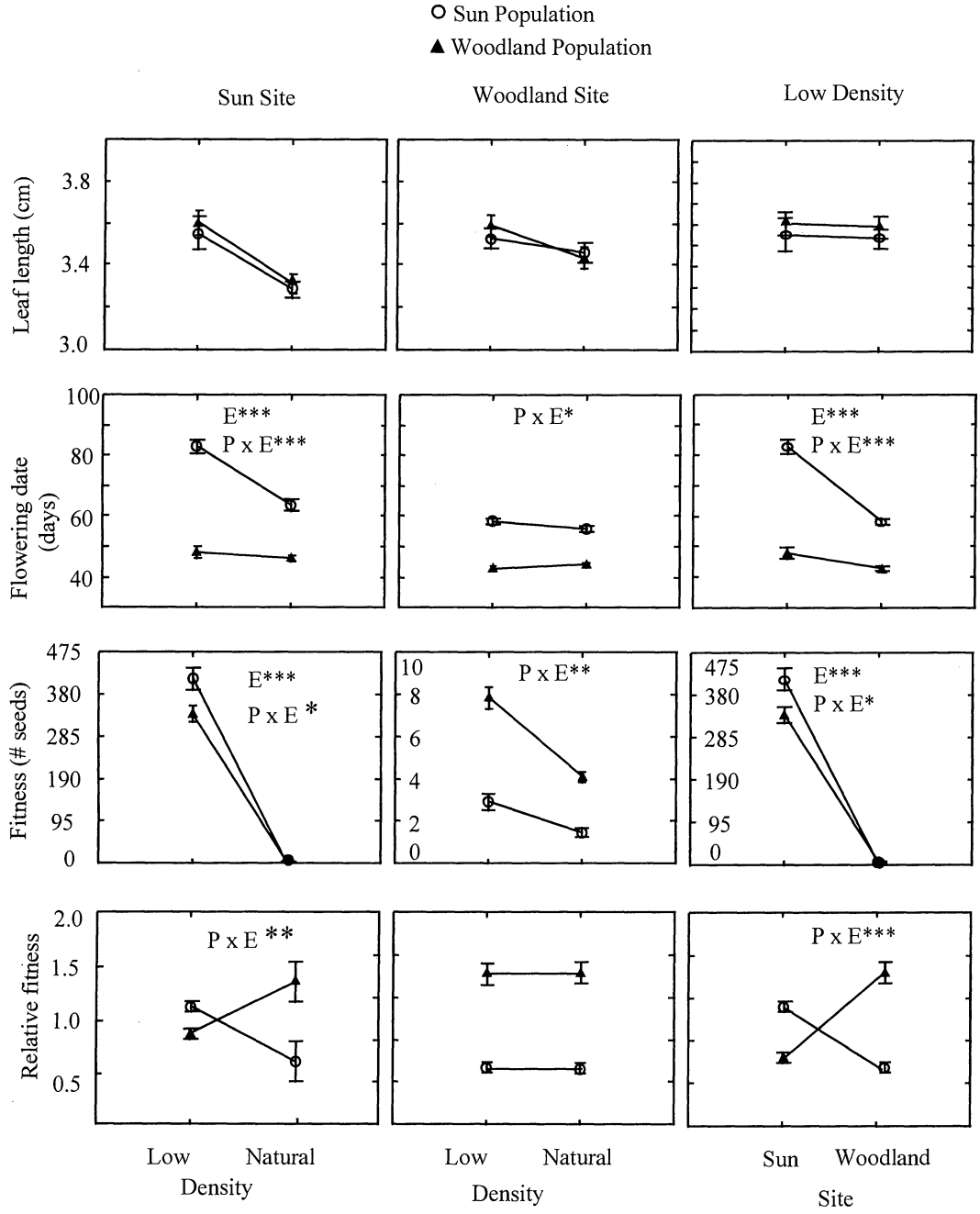

FIG. 1. Continued.

vary with the environment (Table 2). Phenotypic differences between the two populations often varied with density, as indicated by significant population by density interactions (Fig. 1). At low density in the sun site, the two populations differed significantly in all characters except length of the first internode (nearly significantly different), number of nodes, number of branches, and leaf length. In contrast, at natural density only flower production, flowering date, and fitness (see below) differed significantly between the two populations (Table 2). For most characters, therefore, differences between the populations were more pronounced at low density than at natural density in the sun site.

In the woodland site, the differences between populations were not so strongly density-dependent, although population differences in branch and bud production differed with density; differences in branch production were greater at low density, whereas differences in bud production were greater at natural density. Differences in flowering date between populations were slightly greater at low density.

Fitness differences between populations also varied with density. In the sun site, both the magnitude and direction of the fitness difference between populations changed with density (Fig. 1, Table 2). Genotypes from the sun population had higher absolute and relative fitness at low density, in-

TABLE 1. Results of analysis of covariance to test for effects of site differences at low density. *F*-ratios are given. For MANOVA, numerator df = 10, denominator df = 422 for site, population and site × population. Numerator df = 330, denominator df = 4074 for genotype and genotype × site. For ANCOVAs, numerator df = 1 for site, population, and site × population. Numerator df = 33 for genotype and genotype × site. Denominator df ranged from 4 to 454. Full analysis of covariance to examine effects of density are presented in Donohue et al. (2000a). Ψ Significant interactions with density in the full model that included site and density indicate site-specific effects of density. + $P < 0.1$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Trait	Germination	Site	Block	Population	Genotype	Site × pop	Site × geno
MANOVA	20.90***	95.18***	8.89***	4.64***	2.23***	1.68	2.07***
Internode 1 Ψ	101.48***	42.28**	22.28***	2.40	2.99**	0.03	1.87**
Internode 2	106.64***	2.04	19.49***	2.50	3.34***	0.39	1.26
Height Ψ	132.26***	39.64**	21.06***	5.46*	6.95***	0.00	1.42+
No. of nodes	49.71***	0.00	20.63***	0.27	1.55	0.62	1.25
Flowers Ψ	19.43***	11.97*	3.20*	20.94***	13.15***	11.13**	1.60*
Branches Ψ	12.67***	106.32***	10.06***	8.00**	0.83	0.96	2.24***
Buds Ψ	0.51	4.62+	7.83***	10.05**	0.59	10.81**	2.29***
Leaf length	49.15***	0.00	12.97***	0.81	1.78*	0.01	0.89
Flowering date Ψ	11.98***	30.05***	2.48*	35.41***	3.18***	16.44***	6.20***
Fitness Ψ	1.27	176.81***	2.62*	4.06+	0.87	4.49*	1.66*
Relative fitness	5.78*	0.02	18.17***	11.63**	0.75	23.16***	3.72***

dicating local adaptation, but at natural density genotypes from the woodland site had higher absolute and relative fitness than native genotypes. In the woodland site, woodland genotypes had higher absolute and relative fitness than sun genotypes at both densities. This fitness advantage of woodland genotypes was enhanced at low density. This observation corresponds with the greater difference between populations at low density in selectively important characters, notably flowering date (Fig. 1, Table 2).

The degree of difference between populations for some characters depended on site (Fig. 1, Tables 1 and 2). At low density, differences between populations for flower production, bud dormancy, and flowering date were all greater in the sun site. Similarly, differences between the two populations in absolute fitness were greater in the sun site (Fig. 1). However, relative fitness differed more between populations in the woodland site.

TABLE 2. Environment-dependent differences between populations in plastic characters. *F*-ratios to test whether populations differ significantly (population main effect) are shown for plants grown in each environment. Population by density interactions in Figure 1 indicate whether the differences between populations depend significantly on density. Population by site interactions in Figure 1 and Table 1 indicate whether the differences between populations depend significantly on site. Results for "relative fitness" were identical to results for "fitness" because fitness was relativized within each treatment. + $P < 0.1$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

	Sun site		Woodland site	
Trait	Low	Natural	Low	Natural
Internode 1	3.03+	2.45	1.19	0.00
Internode 2	4.42*	0.86	0.84	0.99
Height	4.63*	0.99	4.92*	3.20+
No. of nodes	0.69	0.06	0.02	0.00
Flowers	21.63***	12.17**	17.44***	16.40***
Branches	0.79	1.99	20.24***	1.13
Buds	20.86***	1.45	0.36	14.50***
Leaf length	0.46	0.07	0.56	0.00
Flowering date	31.16***	24.27***	30.93***	25.30***
Fitness	4.36*	4.55*	21.87***	27.62***

Resemblance between Plasticity to Density and Plasticity to Site

Plasticity to density, as expressed in the sun site with no overhead canopy, strongly resembled plasticity to site for many characters (Fig. 1), as indicated by significant genotypic correlations between plasticity to density and plasticity to site (Table 3). Only the length of the first internode and bud dormancy had plastic responses to density that were not significantly correlated to plastic responses to site. These results were verified by the positive genetic correlations across the sun site, natural density and the woodland site, low density environment. The strong resemblance between plasticity to density and site suggests that environmental factors that elicit plastic responses to density under open canopy conditions also elicit responses to the overhead canopy and other environmental factors of the woodland site.

Plasticity to Site, Phenotypic Resemblance, and Consequences for Performance of Immigrants

Foreign genotypes resembled the native genotypes more closely by responding to site differences (Fig. 1). For example, plants from the sun population accelerated flowering in response to the closed canopy conditions of the woodland site. By flowering earlier under closed canopy conditions, sun genotypes flowered closer to the flowering date of the native genotypes than if they had not responded to the closed canopy conditions (although they did not attain the native phenotype). Likewise, plants from the woodland population slightly delayed their flowering in response to open canopy conditions and thereby more closely resembled sun population plants than they would have had they not responded to the open canopy conditions (although this plasticity was much less than that of the native genotypes). Only two characters became more different from the native population by responding to canopy conditions. Primary flower production by the sun population became more dissimilar to that of woodland population for plants grown in the woodland site, and quiescence of meristems in the woodland population be-

TABLE 3. Genotypic correlations between plasticities to density in the sun site and plasticity to site at low density (left column) and genetic correlations across the sun site, natural density treatment and the woods site, low density treatment. Genetic correlations are given for each population separately and for the sample pooled across populations. [a]Correlation is significantly different from 1. NE, Nonestimable. + $P < 0.1$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

		Sun, natural—Woods, low		
Trait	r-Plast.	Sun pop	Wood pop	Pooled
Internode 1	0.22	0.57	0.64+	0.60*[a]
Internode 2	0.51***	1.00***	0.98***	1.00***
Height	0.42**	1.00*	0.84+	1.00**
No. of nodes	0.56***	1.00***	0.77+	1.00***
Flowers	0.90***	1.00***	1.00***	1.00***
Branches	0.88*	NE	0.75	0.68**
Buds	0.68	1.00**	NE	0.60+
Leaf length	0.65***	1.00***	1.00**	1.00***
Flowering date	0.89***	0.69*[a]	0.99***	0.94***
Fitness	1.00**	1.00	1.00	1.00**

came more dissimilar to that of the sun population for plants grown in the sun site.

Despite plasticity to site which reduced phenotypic differences between populations for all but two traits, population differentiation was still observed for many characters (Table 2). Thus, the observed plasticity was insufficient to eliminate the expression of phenotypic differences between populations.

Genetic constraints on the further evolution of plasticity to site differences were apparent as strong genetic correlations between characters expressed in the two sites (Table 4). Genetic correlations across sites for plants grown at low density were strongly positive for all characters except branch and bud production. Correlations were significantly less than one for flowering date in the sun population and for length of the first internode in the woodland population. Correlations across sites for plants grown at natural density were strongly positive for all traits except height when populations were analyzed separately. The most common types of environments in the field are natural density in the sun site and low density in the woodland site (Table 3). In the sun population, correlations across these two environments were significantly positive for all traits except the length of the first internode, indicating constraints on the evolution of plasticity to environmental differences accompanying migration from the sun site to the most common conditions within the woodland site. However, this correlation was significantly less than one for flowering date, indicating some possibility of further evolution of plasticity in this trait. For the woodland population, these correlations were strongly and significantly positive for all traits except the length of the first internode, height, node number, and branch production. Therefore, constraints on the evolution of plasticity accompanying migration from the woodland site to the most common conditions within the sun site are also strong for some characters.

Environment-dependent phenotypic differences between populations were associated with environment-dependent fitness differences, such that the degree of home-site-advantage depended on both site and density (Fig. 1, Table 2). When populations were pooled, we observed a significant negative genetic correlation across sites for fitness at low density (Table 4), suggesting local adaptation and specialization to within-site environmental conditions at low density. This negative genetic correlation was due in part to variation between populations. Within populations, fitness at low density was not significantly correlated across the sun and woodland sites (although the correlations were negative), indicating that fitness at low density in the sun site did not strongly predict fitness in the woodland site relative to other individuals in the same population. In contrast, genetic correlations for fitness across sites were strongly positive at natural density, as were correlations between the most common environments in each site: natural density in the open site and low density in the woodland site (Tables 3 and 4). That is, genotypes that had high fitness at natural density in the sun site had high fitness at both densities in the woodland site, suggesting similarity in the selective environments under closed-canopy conditions and high density under open canopy conditions. Therefore, the selective environment that differed most from the others was low density under an open canopy whereas the other environments were similar to each other.

DISCUSSION

Inbred lines from the two *Impatiens capensis* populations differed in their ability to respond plastically to density and to environmental differences between sites. In general, genotypes from the open site were more plastic to density and

TABLE 4. Genetic correlations across sites at low and natural density. Genetic correlations are given for each population separately and for the sample pooled across populations. [a] Correlation is significantly different from 1 if positive or −1 if negative. NE, nonestimable. + $P < 0.1$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

	Low density			Natural density		
Trait	Sun pop	Wood pop	Pooled	Sun pop	Wood pop	Pooled
Internode 1	1.00***	0.66*[a]	0.81***	1.00*	0.76*	0.92**
Internode 2	0.97***	1.00***	0.99***	1.00***	1.00***	1.00***
Height	1.00***	0.83***	0.94***	1.00+	0.60	1.00**
No. of nodes	1.00**	1.00	0.98**	1.00***	1.00*	1.00***
Flowers	1.00***	0.97***	0.99***	1.00***	1.00***	1.00***
Branches	−0.28	0.31	0.24[a]	NE	1.00***	1.00***
Buds	−0.33	−1.00	−0.35[a]	1.00**	1.00**	1.00***
Leaf length	1.00*	1.00*	1.00**	1.00***	0.78**	0.91***
Flowering date	0.62*[a]	0.96***	0.89***[a]	0.97***	1.00***	1.00***
Fitness	−0.47	−0.16	−0.58*	0.79	1.00	1.00**

site manipulations in selectively important characters such as internode elongation and flowering time. This observation is consistent with the observation that plasticity to density was selectively favored in the open-canopy site but not in the woodland site (Donohue et al. 2000a). Taken together, these results suggest that the observed population differentiation represents evolutionary responses to divergent selection on plasticity. Specifically, they support Morgan and Smith's (1979) prediction that open-canopy conditions will select for stronger internode elongation responses to density than closed-canopy conditions (see also Dudley and Schmitt 1995). They also support the prediction that the sun population would be more plastic in flowering time than the woodland population, since plasticity in flowering time was adaptive in the open site, in which selection favored delayed reproduction of the long-lived plants at low density (see also Geber 1990; Schmitt 1995), but plasticity was maladaptive in the woodland site because of early mortality at both densities (see also Schemske 1984; Lacey 1986a,b; Fox 1990; Bennington and McGraw 1996). Moreover, divergent selection on plasticity to density appears to have caused correlated divergence in plasticity to site for some traits. This correlated divergence resulted in adaptive plasticity of flowering time to novel canopy environments.

Plasticity to density under open-canopy conditions resembled plasticity to site differences for most characters. This result suggests that the overhead canopy altered the light environment in a manner similar to that of conspecific neighbor proximity. Other factors, such as soil moisture or other edaphic factors, may also have been similar between the woodland site and the high density stands in the sun site (Donohue et al. 2000a). However, it is unlikely that differences in soil moisture elicited the observed plastic responses since, in this experiment, treatment-dependent water conditions were not apparent until after most of the phenotypes were measured. The similarity between plasticity to density and plasticity to site implies that plasticity that evolved in response to environmental variation within a site, namely variation in density, can influence phenotypic expression in a foreign site.

The evolved plasticity to density appears to give genotypes fitness benefits under altered canopy conditions as well; genotypes with the highest fitness at natural density under an open canopy also had the highest fitness under closed-canopy conditions at both densities. Appropriate responses to high density therefore can be appropriate responses to overhead canopy, and vice versa. This result is unexpected based on the hypothesis of Morgan and Smith (1979) that plastic shade avoidance responses to neighbor density are not appropriate responses to overhead canopy. Their hypothesis focused on phytochrome-mediated stem elongation responses. Selection on elongation, in fact, was not inconsistent with their predictions, since direct selection did not favor elongation at low density in the woodland site (Dudley and Schmitt 1996; Donohue et al. 2000a), but it did favor elongation at high density in the open-canopy site. The primary reason for the similarity in the selective environments between closed-canopy conditions and high density therefore appears to be due to selection not on elongation, but on the correlated character of flowering date. Early flowering was adaptive both in the woodland site and at high density in the sun site, while late flowering was adaptive only at low density in the sun site (Donohue et al. 2000a). Thus, the selective environment under closed-canopy conditions was similar to that within high density stands under an open canopy for flowering date. Such selection on flowering date strongly contributed to local adaptation in both sites, and it can even account for the relative maladaptation of the sun genotypes at high density in the sun site since woodland genotypes flowered earlier than sun genotypes. Consequently, correlated plasticity to density and overhead canopy can actually increase fitness in novel canopy environments.

The similarity in the selective environments of both closed-canopy density environments and high density under an open-canopy suggests that the divergence in plasticity was not caused primarily by the selective disadvantage of inappropriately elongating in response to overhead canopy. Rather, it seems to result largely from the similarity in selective environments between high and low density under closed-canopy conditions—a similarity that would not promote the evolution of plasticity. The selective environments did vary substantially in the sun site so as to favor plasticity in that site (Donohue et al. 2000a). Genetic constraints on the evolution of plasticity to density were also weaker in the sun site, and this difference could have contributed to the observed differentiation (Donohue et al. 2000b). Moreover, the evolution of shade-avoidance responses, and divergence in these responses, will depend not only on selection on elongation, but also on selection on correlated characters such as flowering time.

The cues that elicited similar plastic responses to these different ecological environments—light availability, R:FR, and possibly soil conditions—appear to be accurate predictors of the selective environments in both ecological contexts with respect to some traits. Plasticity to these particular environmental factors may influence ecological niche breadth by eliciting appropriate responses to different ecological environments. Plasticity is often proposed as a mechanism for extending niche breadth (e.g., Bradshaw 1965; Sultan 1987; Van Tienderen 1991; Sultan and Bazzaz 1993c; Oyama 1994b; Sultan et al. 1998). How commonly plasticity to particular cues may extend ecological niche breadth depends on how specifically environmental factors that elicit plasticity predict selective environments in different ecological contexts (Moran 1992; Getty 1996). Adaptive plasticity to more generally predictive cues would increase ecological niche breadth more than would plasticity to cues that accurately predict selection only under very specific ecological conditions. The relationship between factors that elicit plasticity and the specificity of the selective environment that such cues predict needs to be more fully explored in other systems to determine how plasticity influences ecological niche breadth.

Although plasticity to site differences reduced phenotypic and fitness differences between populations, population differentiation in plasticity caused phenotypic and fitness differences between populations to vary with the environment. For example, because genotypes from the open site were more plastic to site and density than woodland genotypes, the difference in flowering time between them was greatest in the open site at low density. Genotypes from the sun population

accelerated their flowering in the woodland site, and the difference between the populations in absolute fitness was thereby reduced in the woodland site. In contrast, genotypes from the woodland population did not delay flowering appreciably at low density in the sun site. Because delayed flowering was strongly favored in this treatment, differences in absolute fitness were extreme in this treatment. Environment-dependent phenotypic differences between populations therefore can contribute to environment-dependent fitness differences between foreign and native genotypes. How they contribute depends on patterns of natural selection in the different environments.

Interestingly, the difference between the populations in relative fitness, as opposed to absolute fitness, was greatest in the woodland site. This result suggests that, despite the increased adaptive plasticity displayed by the sun genotypes, immigrants from the sun population into the woodland site would be less likely to increase in frequency than immigrants from the woodland population into the sun site. Due to the similarity in selective environments between all treatments except the sun site, low density treatment, woodland genotypes are, in fact, rather well adapted to the high density conditions of the sun site. Only at low density in the sun site did they suffer a severe fitness disadvantage. Thus, the constitutively early-flowering specialists of the woodland population had slightly higher fitness than the plastic generalists of the sun population (sensu Van Tienderen 1991) even in the sun site, when they were grown at high density. (The nonsignificant difference between fitness of the sun and woodland populations in this treatment reported in Donohue et al. [2000a] can be attributed to their use of genotypic mean values for the genotypic selection analysis, as opposed to phenotypic values, as used here.) The comparatively rare, low-density environment in the sun site therefore appears to be important for maintaining the adaptive plasticity expressed by the genotypes from the sun population. Because of the strong genetic correlations across density (Donohue et al. 2000b), moreover, selection at low density appears to have constrained the phenotype expressed by sun genotypes at natural density to the extent that the woodland genotypes actually express the more favorable phenotype at natural density.

Although the plastic responses exhibited by the two populations resulted in more adaptive phenotypes in immigrants to a foreign site, limits to their plasticity often prevented them from attaining the selectively favored phenotype in the foreign site. Such constraints on plasticity are common (DeWitt et al 1998). Because of these limits to plasticity, both ecotypic differentiation and local adaptation (in most environments) were observed in the field. Furthermore, strong genetic correlations across environments were observed for many characters, as has been found in many studies (Via and Lande 1985; Via 1987; Platenkamp and Shaw 1992; Via and Conner 1995; Pigliucci et al 1995; Schmitt 1993; Sultan and Bazzaz 1993a,b). Such genetic constraints would limit the potential for further selection on plasticity to reduce phenotypic and fitness differences between foreign and native genotypes.

In summary, these populations appear to have responded evolutionarily to divergent selection on plasticity in response to density variation. Plasticity that has evolved in response to environmental variation within a site, moreover, can significantly influence phenotypic expression in foreign sites. Differentiation in plasticity can cause phenotypic and fitness differences between populations to vary with the environment. If differences among populations are more pronounced in certain environments, then selection against foreign genotypes may be stronger in some locations than in others and may thereby influence patterns of gene flow. Thus, divergence in phenotypic plasticity, and the resulting environment-dependent phenotypic differences between populations, can have important consequences for ecotypic differentiation, local adaptation, patterns of gene flow, and the evolution of ecological niche breadth. Genetic constraints and limits to plasticity can strongly mediate the ability of plasticity to influence these population processes.

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