Ecology, 83(7), 2002, pp. 1965–1980

SHADE-INDUCED PLASTICITY AND ITS ECOLOGICAL SIGNIFICANCE IN WILD POPULATIONS OF *ARABIDOPSIS THALIANA*

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Abstract. In laboratory studies of *Arabidopsis thaliana*, plants shaded by neighboring vegetation (or subject to treatments mimicking shade) flower at a younger developmental stage, and sometimes earlier in time. We examined whether this shade avoidance response varies among and within natural populations of *A. thaliana*, and whether it corresponds to variable selection regimes at shaded and unshaded field sites, by conducting a two-year reciprocal transplant study and a parallel greenhouse study that manipulated the presence and timing of shade. In the field, shading had limited or inconsistent impacts on survivorship across several phases of the growing season. The date of bolting was earlier at the shadier site compared to the less shady site, but in the greenhouse there was no significant shade-induced plasticity for this trait. In both studies, we detected directional selection gradients favoring earlier bolting in shade, but gradients favoring earlier bolting were as strong in nonshaded conditions. The number of rosette leaves at bolting (i.e., the developmental stage of flowering) was significantly reduced by shade in both studies. However, there was either no directional selection on this trait, or selection to flower with more rather than fewer leaves. Despite the contrast in habitats, there was limited differentiation between populations for survivorship, reproductive fitness, size-related or flowering time traits, and no differentiation for trait plasticities. Some traits were variable among families within populations. A trade-off between age and developmental stage may limit the response to selection for flowering time, possibly explaining limited local adaptation. The adaptive significance of shade-induced flowering time plasticity remains equivocal. Future studies of the plasticity of flowering time in *A. thaliana* should investigate the effects of shading regimes together with other environmental variables on size- and timing-related traits.

Key words: *age and size at reproduction; Agricultural Experiment Station, Tennessee;* Arabidopsis thaliana; *flowering time; life history evolution; light spectral quality; phenotypic plasticity; phenotypic selection; phytochrome; shade avoidance; Sharp Ridge Park, Tennessee.*

INTRODUCTION

Biologists interested in the evolution of life history traits have often argued that, all else being equal, fitness is enhanced if an organism can develop more rapidly and grow to a larger size before making the transition to reproductive maturity (King and Roughgarden 1982, Stearns 1992, Roff 1999). Because larger individuals tend to have greater fecundity, a trade-off exists between size or stage at maturity and the time required to reach the transition to maturity.

Since the environment is heterogeneous, all else is not equal. A complete understanding of life history evolution therefore requires examining how the environment induces plasticity in life history traits and investigating how such plastic traits relate to lifetime fitness across an appropriate range of environments (Stearns and Koella 1986, Stearns 1992). Phenotypic plasticity, i.e., the production of different phenotypes by the same genotype in response to environmental variation, is especially important for plants. Their passive seed dispersal and sessile habit may expose genetically similar individuals to substantial environmental variation. In particular, the presence of intra- or interspecific neighbors often reduces the availability of light, a critical resource, with important impacts on rates of development and growth, as well as on fitness distributions (Weiner and Thomas 1986, Geber 1989, Schmitt 1993).

Competition may lead to natural selection for novel morphologies that enhance resource capture or for shifts in the timing of growth or reproduction that obviate competition, and variation in the presence of competition may select for adaptive phenotypic plasticity of such traits (Sultan 1987, 1995, Via et al. 1995). The phenotypic plasticity expressed by a given genotype is considered adaptive (in the sense of being currently advantageous) if the phenotypes evoked by a relevant range of environments confer high relative fitness in those environments.

Several recent studies have demonstrated the adaptive significance of shade-induced plasticity. For example, plasticity in stem elongation may be selected

Manuscript received 14 May 1999; revised 16 March 2001; accepted 30 March 2001; final version received 13 October 2001.

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because taller stems increase light capture in shady conditions while shorter stems promote mechanical stability and minimize support costs in less shady environments (Givnish 1988, Casal and Smith 1989, Schmitt et al. 1995, Dudley and Schmitt 1996). Also, flowering earlier may be adaptive if the optimal tradeoff between the chronological timing and developmental stage of flowering differs between shaded and unshaded environments. For example, the potential for growth declines and the risk of mortality increases where encroaching competitors cast shade late in the growing season. In such habitats, selection should favor individuals that flower earlier in the face of increasingly scarce light resources. Under sparser canopies, the potential for continued growth and lower risk of mortality should instead select for delayed flowering. If genotypes have a high probability of encountering alternative environments, the shade-induced plasticity of flowering time traits may be altered by selection, and a more favorable norm of reaction may evolve (Stearns 1992, Mitchell-Olds 1996*a*, *b*, Via et al. 1995).

In addition to reducing light resources, competing neighbors alter the spectral quality of light, because chlorophyll absorbs red wavelengths but transmits and reflects far-red wavelengths. Accordingly, encroaching competition can be detected by a transduction of environmental signals mediated by phytochromes, a family of red- and far-red-sensitive apoproteins (Holmes and Smith 1977, Smith 1982). It has been argued that shade-induced, phytochrome-mediated plasticity syndromes are especially advantageous because they can operate in an anticipatory fashion (Smith 1982, Ballare et al. 1990, Schmitt and Wulff 1993, Schmitt 1997, Callahan et al. 1999). There is substantial evidence that responsiveness to the red:far-red ratio (R:FR) varies among species (Morgan and Smith 1979) and within species (Solangaarachichi and Harper 1987, Bain and Attridge 1988), but few studies have examined how selective factors operating within populations might alter or maintain a species' shade avoidance responses (Dudley and Schmitt 1996, Weinig 2000).

We have studied the shade-induced plasticity of flowering time in natural populations and natural habitats of the model species for plant genetics, *Arabidopsis thaliana*. The mechanistic and molecular genetic basis of its shade avoidance behaviors has been thoroughly investigated in the lab (Halliday et al. 1994, Reed et al. 1994, Yanovsky et al. 1995, Pigliucci and Schmitt 1999). Also, the species shows clear interpopulation variation for flowering time and flowering time plasticity (Jones 1971, Karlsson et al. 1993, Alonso-Blanco et al. 1998, Pigliucci and Schlichting 1998), which may be ecologically important for rosette-forming annuals like *A. thaliana* that do not use stem elongation to avoid shade cast by competing neighbors.

Variation in the shade-mediated phenotypic plasticity of flowering time and natural selection on flowering time have seldom been carefully studied among and within *A. thaliana* populations growing in natural habitats. To explore whether there is a correspondence between the selection regimes imposed by shaded and unshaded conditions and the shade-induced plasticity of flowering-time traits, we conducted a two-year field study at two contrasting sites. We chose one site where vegetation remained sparse throughout the spring and another where there was little vegetation during the winter, but vigorous growth throughout the spring and very dense vegetation by late spring. Using reciprocal transplants, we investigated local adaptation and variation within natural populations. Furthermore, we used families of plants from these two populations in a greenhouse experiment involving light regimes that were unshaded, shaded, or heterogeneous within the growing season. This parallel experiment aimed to minimize confounding differences between the two study sites such as differences in soil moisture or fertility, microclimate, and belowground competition.

In this paper, we first document spatial and temporal heterogeneity in photosynthetic photon flux density (PPFD) and spectral quality (R:FR). Second, we report on our examination of survivorship, flowering time, plant size, and reproductive output in both habitats to assess the impact of vegetation shade and related site factors. Third, we examine whether there is differentiation in the timing- or size-related traits between the two native populations or among families within populations. Finally, in both shaded and unshaded conditions, we conducted multivariate analyses of natural selection on flowering time and size traits (Lande and Arnold 1983, Endler 1986), and we present and compare phenotypic and genotypic analyses for both the field and greenhouse studies. The phenotypic analyses are powerful because of the large sample sizes employed. Genotypic analyses are relevant because selection gradients estimated in this manner are less likely to be biased by environmentally induced correlations between selected traits and fitness itself (Rausher 1992, Mauricio and Mojonnier 1997).

We address the following questions: (1) To what extent do contrasting light regimes alter survivorship, flowering time, overall plant size, and fecundity? (2) Is there differentiation between populations for survivorship, flowering time traits, size, and fecundity? (3) How is relative fitness affected by flowering time and plant size in shaded and unshaded conditions? (4) Does habitat-induced phenotypic plasticity of flowering time traits match directional selection gradients observed in those habitats?

Materials and Methods

Arabidopsis thaliana *in the southeastern United States*

Examination of 62 herbarium specimens from throughout Tennessee, USA, indicate that *Arabidopsis thaliana* is strictly spring flowering. This observation

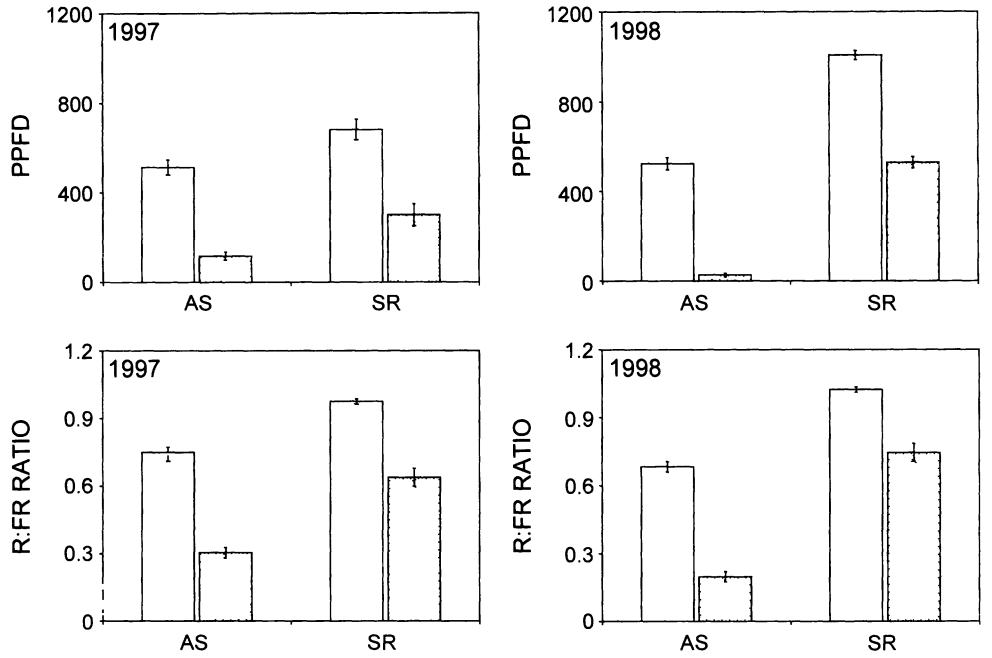

FIG. 1. Mean early-season (open) and late-season (shaded) photosynthetic photon flux density (PPFD) and red to far-red (R:FR) ratios at the two field study sites in Knox County, Tennessee, USA: the Agricultural Experiment Station (AS) and Sharp Ridge Park (SR) sites. For sample sizes, see *Materials and methods: Environmental and seed sampling*. Means ± 1 SE are shown.

is consistent with previous studies indicating that the species is a winter annual in Kentucky (Baskin and Baskin 1983), even though summer or fall-flowering populations have been observed in other regions (Napp-Zinn 1985; J. Schmitt and K. Donohue, *personal communication*). We also directly observed the winter annual habit in eight populations in Knox and Blount Counties, Tennessee, where we conducted initial surveys of vegetation density, light availability, and light spectral quality for this study.

Environmental and seed sampling

For the two-year, two-site study, we chose two sites with sharply contrasting light regimes. The Agricultural Experiment Station site (35°56′N, 83°57′W) and the Sharp Ridge Park site (36°00′N, 83°56′W) are both in Knox County, and are abbreviated hereafter as AS and SR, respectively. The vegetation at the AS site was predominantly fescue grass (*Festuca* spp.) with other early-spring-flowering weed species, such as *Stellaria media*, *Lamium amplexicaule*, and *Veronica arvensis*. The less shaded SR site lacked the fescue grass cover, but was patchily distributed with the same early-flowering weeds, along with scattered clumps of *Cardamine hirsuta* and *Plantago lanceolata*.

During both years of the study, we characterized the light regimes at the two sites, measuring the red to far-red ratio (R:FR) and photosynthetic photon flux density (PPFD) with a LI-COR 1800 Spectroradiometer (LI-COR, Lincoln, Nebraska, USA). We sampled at a stratified random array of points within study plots at each site (1997, $N \approx 80$; 1998, $N \approx 260$). Early-season measurements were taken during the hours of 1000–1500 under cloudless conditions during the first two weeks of March. Late-season measurements were taken under similar conditions during the first two weeks of April (Fig. 1). For both early- and late-season measurements, we took one measurement at each point in the array.

In May 1996, we collected seeds at the two sites. We gathered seeds from 60 maternal plants in the very large AS population (>500 plants) and from 40 families in the smaller SR population (~150 plants). Bulk collections of these seeds, and of five other Tennessee populations, are available from the Arabidopsis Information Resource (TAIR), which is an online resource hosted by at the Carnegie Institution of Washington, Stanford, California, USA.

First-year field study

We maintained seed families in separate envelopes and stored them dry at room temperature until 16 October 1996. We then randomly sampled 25 families from each of the two populations, and imbibed seeds in the dark at 4°C for about one week on moist filter paper. During 29 October–2 November 1996, we planted seeds from each of the 50 families into four random cells in each of 10 200-cell germination trays filled with fine, moist vermiculite, keeping trays covered until sowing was complete. We then transferred the trays to artificial light (two 40-W fluorescent tubes combined

TABLE 1. Differences in noncumulative survivorship, within each of the three stages of the growing season, examined with G tests (df = 1).

Year	Site	Survival (%)	Between-site G test		Population of origin	Survival (%)	N	Between-population-of-origin G test	
			G	P				G	P
a) Survivorship immediately after transplanting†									
1997	AS	63.9	172	<0.0001	AS	62.2	436	0.34	0.56
					SR	64.1	401	...	...
	SR	34.1	...	...	AS	36.0	370	1.63	0.20
					SR	31.5	356	...	...
1998	AS	94.0	10.2	0.0014	AS	88.3	411	4.11	0.04
					SR	92.5	400	...	...
	SR	85.2	...	...	AS	83.3	419	2.51	0.11
					SR	87.2	399	...	...
b) Prebolting survivorship (overwintering)‡									
1997	AS	72.3	0.005	0.9433	AS	73.0	271	0.14	0.71
					SR	71.6	257	...	...
	SR	72.6	...	...	AS	72.9	133	0.07	0.79
					SR	71.4	112	...	...
1998	AS	93.3	0.023	0.8799	AS	91.7	363	2.90	0.09
					SR	94.9	370	...	...
	SR	93.1	...	...	AS	91.1	349	4.40	0.04
					SR	95.1	348	...	...
b) Postbolting survivorship§									
1997	AS	83.5	4.24	0.0394	AS	81.3	198	2.28	0.13
					SR	87.0	184	...	...
	SR	90	...	...	AS	90.7	97	1.95	0.16
					SR	83.8	80	...	...
1998	AS	48.7	276	<0.0001	AS	48.6	333	0.003	0.99
					SR	48.7	351	...	...
	SR	90.8	...	...	AS	91.2	318	0.14	0.70
					SR	90.3	331	...	...

Note: Site abbreviations are AS, Agricultural Experiment Station; SR, Sharp Ridge Park.

† G test for difference between years at AS: $G = 172$, $P < 0.0001$. G-test for difference between years at SR: $G = 446$, $P < 0.0001$.

‡ G-test for difference between years at AS: $G = 105$, $P < 0.0001$. G-test for difference between years at SR: $G = 62$, $P < 0.0001$.

§ G-test for difference between years at AS: $G = 132$, $P < 0.001$. G-test for difference between years at SR: $G = 0.093$, $P = 0.7604$.

with two 25-W incandescent bulbs, placed 10 cm above the seedlings). Seedlings were germinated under an 11 h light, 13 h dark (11/13 L/D) photoperiod to approximate field conditions. We added 1 mL of standard Hoagland's solution to each seedling on 10 November and transferred the seedlings from growth racks at room temperature (20–22°C) to a 4°C cold room on 16 November. Before transplanting to the field, we thinned to a single seedling per cell. At this stage, juvenile plants had initiated two to four true leaves. We randomly assigned five trays to each of the two field sites and then transplanted the seedlings into extant vegetation, spaced ~10 cm apart. (This precluded intraspecific competition, but not competition with interspecific neighbors.) The identity of each individual plant was well marked. Transplanting at the AS site was done on 19–20 November; transplanting at the SR site was completed on 22–23 November.

After transplanting seedlings, we assessed mortality in mid-December (Table 1a) and then monitored growing rosettes periodically. We conducted a census to determine prebolting mortality on 27–28 February 1997. Thereafter, we monitored every other day for bolting, the first manifestation of the reproductive phase in *A. thaliana*. When we recorded the number of days to bolting, we also counted the number of rosette leaves and measured rosette diameters to the nearest 0.1 cm. We were unable to count and measure some bolting plants because mud obscured some rosettes. We recorded plants that never bolted as mortalities in the analysis of prebolting (overwintering) mortality (Table 1b). We monitored survivorship of all bolting inflorescences, recording a postbolting mortality (Table 1c) if a plant bolted, but we were subsequently unable to locate the inflorescence. When flowering and fruiting ceased, we harvested inflorescences and measured height and number of matured fruits. Previous studies indicate that seed number per plant is strongly correlated with number of fruits (Westerman and Lawrence 1970, Mauricio 1998).

Second-year field study

For the second year of the study, we propagated seed families from the maternal families collected in May

TABLE 2. ANOVA results (Type III MS and *P* values) from the greenhouse study for (a) bolting date, leaf number, and rosette diameter, and (b) inflorescence height and fruit production.

a) ANOVA for bolting date, leaf number, and rosette diameter

Effect	df	Bolting date		Leaves at bolting		Rosette diameter	
		MS	*P*	MS	*P*	MS	*P*
Shading treatment†	1	214.90	0.1704	**532.22**	**<0.0001**	**1242.78**	**<0.0001**
Population of origin†	1	**7902.35**	**<0.0001**	**402.63**	**0.0002**	17.65	0.3087
Shading treatment × Population†	1	278.27	0.1190	63.02	<0.1331	34.94	0.1527
Maternal family (Population)	44	**938.02**	**<0.0001**	**123.51**	**<0.0001**	**33.00**	**0.0014**
Block × Maternal family (Population)	187	113.44	0.6355	27.69	<0.0903	16.95	0.6535
Block	5	**373.05**	**0.0087**	9.67	0.8396	**65.42**	**0.0031**
Error	...	118.79	...	23.39	...	17.86	
(df)		(355)		(353)		(342)	

b) ANOVA for inflorescence height and fruit production

Effect	df	Inflorescence height		Fruit production	
		MS	*P*	MS	*P*
Early shading treatment†	1	1.29	0.6727	**220.51**	**0.0055**
Late shading treatment†	1	**85.20**	**0.0007**	**259.96**	**0.0026**
Early treatment × Late treatment†	1	18.47	0.1108	54.40	0.1647
Population of origin†	1	48.41	0.0103	125.44	0.0355
Early treatment × Population†	1	22.02	0.0819	55.90	0.1590
Late treatment × Population†	1	0.37	0.8198	8.13	0.5903
Early treatment × Late treatment × Population†	1	11.05	0.2168	38.98	0.2392
Maternal family(Population)	44	**26.84**	**<0.0001**	**73.01**	**<0.0001**
Block × Maternal family(Population)	181	7.19	0.7326	27.95	0.4507
Block	5	16.48	0.0644	54.43	0.0818
Error	...	7.82	...	27.54	...
(df)		(314)		(314)	

Note: Boldfaced type indicates significance ($P < 0.05$) after a sequential adjustment for separate univariate tests were conducted on five nonindependent traits.

† These terms were tested with the Block × Maternal Family(Population) term in the denominator.

1996. During July–December 1996, we grew plants for one generation in 5.5 × 5 × 5 cm pot inserts in potting soil (Peters Professional Potting Soil, Scott, Marysville, Ohio, USA) under artificial light (banks of 40-W fluorescent tubes) in a 16/8 L/D photoperiod at 20–22°C. Until plants began flowering, we sprayed rosettes approximately biweekly with a dilute fertilizer solution (11-11-11 N-P-K). We collected seeds from individual plants in envelopes and stored them dry at room temperature.

On 28 October 1997, we began imbibing seeds on moist filter paper at 4°C in the dark for approximately one week. We used a design similar to the one employed the previous year, randomly choosing 25 seed families from each of the two populations and similar protocols and schedules for sowing (28–30 October), pregerminating (starting 31 October), fertilizing (November 6), cold-hardening (starting November 21), transplanting (November 24–26 at the SR site; November 28 at the AS site), winter monitoring, and spring data collecting (beginning February 28).

Greenhouse study

This study was conducted during January–May 1997. We randomly selected 48 families, 24 from each population, from the seed families propagated under artificial light (see *Materials and methods: Second-year field study*). On 10 January 1997, we placed seeds on moist filter paper and imbibed them in the dark at 4°C for 15 d. Imbibed seeds were sown into standard growing mix (Fafard no. 2, Inkerman, New Brunswick, Canada) into every other cell of 24 96-cell (5 cm deep) greenhouse flat inserts. Flats were placed outdoors, adjacent to the University of Tennessee Botany Department Greenhouses on 24 January 1997. For certain families in both population, seed germination was poor in all or most of the four experimental treatments; in addition, some mortality occurred due to herbivory by slugs at the rosette stage. Consequently, many individual plants and sometimes an entire family (of the original 48) were dropped from analyses. Final sample sizes are indicated in Tables 2 and 3: Genotypic estimates.

The blocked factorial design involved six replicates. There were four experimental light treatments: (1) unshaded both early and late in the life cycle; (2) unshaded early, but shaded late; (3) shaded early, but unshaded late; or (4) shaded early and shaded late. To create shade treatments, we alternated pots containing *A. thaliana* individuals with pots containing three to four perennial ryegrass (*Lolium perenne*) plants. By

TABLE 3. Total selection and directional selection gradients within the four treatments used in the greenhouse study (with P values presented parenthetically); phenotypic estimates were based on individual plants, and genotypic estimates were based on family means.

Trait	Unshaded early			
	Unshaded late (Treatment 1)		Shaded late (Treatment 2)	
	Total selection	Direct selection	Total selection	Direct selection
Phenotypic estimates				
No. rosette leaves	0.12 (0.1378)	0.07 (0.5242)	0.05 (0.5463)	**0.26 (0.0103)**
Date of bolting	**−0.33 (<0.0001)**	**−0.37 (<0.0001)**	**−0.44 (<0.0001)**	**−0.61 (<0.0001)**
Rosette diameter	**0.51 (<0.0001)**	**0.48 (<0.0001)**	**0.59 (<0.0001)**	**0.45 (<0.0001)**
Sample size	...	139	...	144
R^2	...	0.456	...	0.571
Cross-validation				
R^2_{est}	...	0.465	...	0.561
R^2_{pred}	...	0.291	...	0.430
Genotypic estimates				
No. rosette leaves	0.6 (0.7050)	0.10 (0.6580)	0.08 (0.607)	0.31 (0.0976)
Date of bolting	**−0.43 (0.0051)**	**−0.62 (0.0011)**	**−0.54 (0.000)**	**−0.68 (<0.0001)**
Rosette diameter	**0.55 (0.0002)**	**0.61 (0.000)**	**0.62 (<0.0001)**	**0.38 (<0.0117)**
Sample size	...	40	...	42
R^2	...	0.613	...	0.631

Notes: Boldfaced type indicates significance at the $P < 0.05$ level after sequential Bonferroni correction. Sample sizes are for complete observations that were used for model estimation.

imposing grass canopy treatments in separate pots, we prevented belowground competition while creating environments that provided light cues normally associated with the absence or presence of competitors. The design also allowed us to control the timing of shade. An individual plant's date of bolting served as the division between the early and late treatments. Pots in treatments 2 (shaded late only) and 3 (shaded early only) were switched to shaded or unshaded flats, respectively, within two days of bolting. A border of ryegrass surrounded the shaded flats to minimize edge effects. We periodically checked R:FR in the shaded and unshaded flats using a LI-COR 1800 Spectroradiometer. The ratio in shaded flats ranged 0.55–0.65; in the unshaded flats it ranged 0.95–1.05. We watered flats during dry spells, sprinkled with slug pellets, and sprayed approximately biweekly to control for aphids. Flats otherwise experienced the same natural temperature, photoperiod, and rainfall regime.

After one week, we thinned germinated seedlings to a single plant per cell and began monitoring plants daily. Bolting occurred during 3 April–31 May. At bolting, we recorded the number of days between bolting and sowing (24 January 1997). We also counted the number of rosette leaves and measured rosette diameter to the nearest 0.1 cm. When plants ceased flowering and fruits were shedding seeds, we harvested infructescences and recorded main stem height and number of fruits.

Statistical analyses

In the field studies, a series of contingency table tests determined whether there were significant differences between sites and between years for survivorship at three stages: after transplant, before bolting, and after bolting. The α levels of these tests were Bonferroni corrected to a nominal level of $P < 0.05$ to account for nonindependence. Variation in plant size and phenology in both the field studies and the greenhouse study were analyzed using a split-plot ANOVA with blocks. For significance testing, we again used a sequential Bonferroni correction, because we were examining five traits on each plant. Distributions of residuals were approximately normal, with the exception of number of fruits in the field study, which was natural log-transformed to improve normality and homoscedasticity.

In the field studies, the ANOVA model examined the effects of block, year, site, and the year × site interaction (whole plots) as well as the effects of population of origin, interactions of population with site and year, and maternal family (subplots). In the greenhouse study, the ANOVA model that analyzed three early traits (rosette leaf number at bolting, date of bolting, and rosette diameter) included blocks and only the early-shading treatment (whole plots), as well as population of origin, the interaction of population and the early treatment, and maternal family (subplots). The ANOVA model that analyzed the two late traits (inflorescence height and number of fruits) included blocks, both early- and late-shading treatments, and the interaction of early and late shading (whole plots), as well as the effects of population of origin, interactions of population with the early and late treatments, and maternal family (subplots). Because we examined five traits on each individual plant, also in this case the α level of $P < 0.05$ for significance testing was corrected using a sequential Bonferroni procedure.

TABLE 3. Extended.

Shaded early			
Unshaded late (Treatment 3)		Shaded late (Treatment 4)	
Total selection	Direct selection	Total selection	Direct selection
0.08 (0.4074)	0.15 (0.1520)	−0.11 (0.2277)	**0.24 (0.0050)**
−0.41 (<0.0001)	**−0.45 (<0.0001)**	**−0.62 (<0.0001)**	**−0.75 (<0.0001)**
0.29 (0.0050)	0.17 (0.1203)	**0.38 (<0.0001)**	**0.27 (<0.0004)**
...	116	...	127
...	0.261	...	0.562
...	0.272	...	0.478
...	0.141	...	0.525
0.07 (0.6427)	0.01 (0.9625)	−0.39 (0.0138)	0.10 (0.6094)
−0.22 (0.1694)	−0.13 (0.4683)	**−0.68 (<0.0001)**	**−0.80 (<0.0001)**
0.34 (0.0299)	0.29 (0.1328)	0.18 (0.2642)	**0.28 (0.0329)**
...	42	...	40
...	0.129	...	0.552

When grown in common garden plots in the field, the two populations of origin showed very similar patterns of survivorship (Table 1) and were not significantly differentiated for most of the traits measured, with the exception of leaf number at bolting (Table 4). Therefore, to maintain large sample sizes for our selection analyses, we pooled data from the two populations in phenotypic and genotypic selection analyses.

We conducted selection analyses using two different models. In both, relative fitness was estimated as the total number of fruits (untransformed) divided by the mean number of fruits within a site and year, or within a treatment for the greenhouse study. One model included four independent variables: bolting date, number of rosette leaves at bolting, rosette diameter at bolting, and final inflorescence height. In the first model, the most influential independent variable was inflorescence height, a trait measured later than the other three traits and very strongly correlated with the number of fruits. This result is consistent with Myerscough and Marshall's (1973) observation of an allometric relationship between main stem height and reproductive output across a broad range of environments. We therefore conducted and present only the second selection analysis, a three-trait model that omitted inflorescence height.

Separate models were used to estimate linear and quadratic coefficients; quadratic models did not include cross-product terms (Brodie et al. 1995). We conducted phenotypic selection analyses on all individual plants with complete data for all variables. Models were fit using SAS PROC GLM (Version 7.0 for Windows), which generates F tests based on Type III sums of squares for testing significance of slopes. For each factor in the model, we also used SAS to estimate directional selection gradients (standardized partial-regression coefficients). We used a cross-validation procedure to examine the predictive ability of the phenotypic models: we randomly split the data set and used half of the data to reestimate regression parameters (i.e., unstandardized regression coefficients and an estimation coefficient of determination, multiple-R^2_{est}). We used the estimated models to predict relative fitnesses for the other half of the data set, and calculated a prediction coefficient of determination (multiple-R^2_{pred}) to compare to R^2_{est} (Mitchell-Olds and Shaw 1987, Montgomery and Peck 1992:447).

Genotypic selection analyses (Rausher 1992) were conducted using family means (within each environment) as a proxy for breeding values. These used relative fitness based on a given family's mean fruit number (within an environment), divided by the overall mean. Cross-validation was not conducted for the genotypic analyses due to much smaller sample sizes. Estimating selection gradients in this manner controls for the possibility that the microenvironment experienced by an individual plant may induce a correlation between the independent variables and the estimate of relative fitness (Rausher 1992, Mauricio and Mojonnier 1997).

To test for heterogeneity of directional selection gradients, we combined the data sets from two years and two sites in the field study, and from four different shading treatments in the greenhouse study, respectively. We then fit separate ANCOVA models for each of the three continuous, independent variables (i.e., number of rosette leaves, bolting date, and rosette diameter at bolting). Models for the field study added interactions between a particular variable and the main effect of year, the main effect of site, and the site ×

TABLE 4. ANOVA results for the five traits measured in the two-year, two-site field experiment.

Effect	df	Leaves at bolting		Bolting Date	
		MS	*P*	MS	*P*
Site†	1	**645.2**	**<0.0001**	**8364.2**	**<0.0001**
Year†	1	**5739.9**	**<0.0001**	**4703.3**	**<0.0001**
Site × Year†	1	8.1	0.6072	**250.4**	**0.0090**
Population of origin	1	**467.1**	**0.0001**	62.6	0.1530
Site × Population of origin	1	82.9	0.1074	3.1	0.7505
Year × Population of origin	1	**234.3**	**0.0068**	36.1	0.2777
Site × Year × Population of origin	1	76.3	0.1224	27.1	0.3472
Block(Site × Year)	15	**445.3**	**<0.0001**	**214.7**	**0.0001**
Population of origin × Block(Site × Year)	15	22.2	0.7908	**64.7**	**0.0077**
Maternal family (Population of origin)	73	**50.7**	**0.0017**	**156.2**	**<0.0001**
Block × Maternal family (Population of origin)	682	30.6	0.7294	**36.5**	**0.0051**
Error		31.9		30.6	
(Error df)		(986)		(1078)	

Notes: Type III mean squares (MS) and corresponding *P* values are given. Boldface type indicates significance after adjustment for separate univariate tests conducted on nonindependent traits.

† Factors tested using Block × Maternal family(Population of origin) as the whole-plot error term.

year interaction. Models for the greenhouse study added interactions between a particular variable and experimental conditions (unshaded early and late, unshaded early and shaded late, shaded early and unshaded late, or shaded early and late).

RESULTS

Field study

Environmental variation.—We confirmed that the shadier Agricultural Experiment Station (AS) site had both lower photosynthetic photon flux density (PPFD) and a lower red to far-red ratio (R:FR) than did the Sharp Ridge Park (SR) site. The difference between early- and late-season PPFD and R:FR was greater at the AS site, especially during the study's second year (Fig. 1).

Survivorship.—During the first year of the field study, seedlings survivorship immediately after transplanting was significantly higher at the AS site than at the SR site. In the second year, seedling survivorship was significantly higher at both sites; the between-site discrepancy in seedling survivorship was smaller, yet still significant (Table 1a). In both seasons, over-winter survivorship was similar at the two sites, but survivorship was overall higher in the second year (Table 1b). At the SR site, there was similar postbolting survivorship in both years of the study. Compared to the SR site, postbolting survivorship at the AS site was slightly lower in spring 1997 and much lower in spring 1998 (Table 1c). Table 1 includes survivorship data for plants either native or nonnative to the site. At both sites, differences in survivorship between native and nonnative plants were small and statistically nonsignificant.

Mean flowering time, plant size, and reproductive output.—Plants bolted with fewer rosette leaves at the shadier AS site than at the SR site, and in the first year compared to the second (Fig. 2a); there was no significant interaction between site and year (Table 4). In both years, bolting tended to occur earlier at the shadier AS site. In the second year, when bolting occurred later at both sites, between-site differences were also significantly larger (Fig. 2b; Table 4, significant effects of site, year, and site × year interaction). Rosette diameters also varied across sites and years; especially small rosettes were observed at the AS site during the first year (Fig. 2c, Table 4). Inflorescences were ~1.4-fold taller at the SR site than at the AS site during the first year, while the reverse was true in the second year (Fig. 2d; Table 4, significant site × year interaction). Finally, mean fruit production paralleled mean rosette diameter rather closely; it was markedly lower at the AS site than at the SR site in the first, but not in the second year (Fig. 2e; Table 4, site × year interaction).

In the second year, when all plants produced more leaves, plants native to the SR site produced more leaves than plants native to the AS site (Fig. 2a). The significance of this difference is supported by the significant year × population-of-origin interaction and the significant effect of population of origin (Table 4). For all other traits, the two populations were not significantly differentiated. Lack of differentiation for the shade-induced plasticity of these traits is suggested by the lack of a significant population × site term, although this term approached significance for size- and fitness-related traits (Table 4). Variation among maternal families was significant for number of rosette leaves at bolting, bolting date, and rosette diameter, but not for inflorescence height or fruit number (Table 4).

Phenotypic selection on flowering time and plant size.—Although seasonal light regimes differed consistently between sites in both years (Fig. 1), selection regimes were qualitatively similar between the two sites and in the two years of the study (Table 5). At both sites in both years, total selection favored larger plants, i.e., those with larger rosette diameters and more rosette leaves at bolting. There was also total selection

TABLE 4. Extended.

Rosette diameter		Main inflorescence height		Total fruit production (ln)	
MS	*P*	MS	*P*	MS	*P*
26.8748	**<0.0001**	53.43	0.1531	**32.11**	**<0.0001**
45.0591	**<0.0001**	**1960.17**	**<0.0001**	**63.35**	**<0.0001**
28.2102	**<0.0001**	**1713.91**	**<0.0001**	**46.27**	**<0.0001**
1.7794	0.0811	0.57	0.8813	0.003	0.9544
2.0352	0.0622	110.98	0.0382	4.54	0.0211
0.1581	0.6028	11.19	0.5098	0.62	0.3933
0.0848	0.7032	2.85	0.7396	0.37	0.5122
8.0790	**<0.0001**	**310.41**	**<0.0001**	**10.03**	**<0.0001**
0.5838	0.4524	42.55	0.0561	1.11	0.1907
0.9931	**0.0003**	25.74	0.4808	0.94	0.2686
0.5789	0.5458	26.11	0.4287	0.77	0.8766
0.5838		25.7		0.85	
(988)		(594)		(648)	

for earlier bolting, and it was somewhat stronger at the less shaded SR site. At that site, plants that bolted early had larger rosettes and tended to produce taller inflorescences (Appendix A).

Directional selection gradients for larger rosette diameters were always large and highly significant, but gradients for bolting with more rosette leaves were weaker, and their magnitude and significance were not consistent across sites or years. Gradients for bolting at an earlier date were also weaker than gradients for rosette diameter, and there was not a consistent tendency for these gradients to be stronger at a given site or within a given year (Table 5).

The cross-validation procedure supported the model, with the exception of a poorer fit to data at the AS site in the second year (Table 5). When quadratic terms were added to the model, they were only significant for rosette diameter. Also, quadratic coefficients were positive, but not strictly interpretable as disruptive selection, since visual inspection revealed no local minima within the ranges of the data (Mitchell-Olds and Shaw 1987).

The results of the genotypic analyses essentially parallel those of the phenotypic model (Table 5). Directional selection gradient favoring larger rosettes were strong, especially at the SR site during the second year. In both years, directional selection gradients on date of bolting differed in magnitude between sites. This gradient was significant only at the shadier AS site during the first year; it was marginally significant at the less shady SR site during the second year. Directional selection gradients on number of rosette leaves at bolting were always nonsignificant (Table 5).

Table 6 summarizes the ANCOVA analyses of heterogeneity of directional selection gradients from the field study. The magnitude of directional selection gradients on rosette diameter and number of leaves at bolting differed significantly between combinations of years and sites. This was not true for the directional selection gradients on date of bolting.

Greenhouse study

Mean flowering time, plant size, and fitness.—When we measured bolting date, number of rosette leaves and rosette diameter, plants had been exposed to two contrasting early treatments only, rather than to the full factorial array of early and late treatments. Plants that were shaded bolted with significantly fewer rosette leaves and larger rosette diameters, but bolting date was not significantly affected (Fig. 3a–c, Table 2). With inflorescence height and fruit production, we were able to examine the main effect of late-shading treatments, as well as the main effect of early-shading treatments and the interaction of early- and late-shading treatments (Fig. 3d, e). Shade imposed after bolting induced significantly taller inflorescences, but shade prior to bolting did not affect inflorescence height (Fig. 3d, Table 2). Both late and early shading reduced fruit production, but in an additive rather than an interactive manner (Fig. 3e, Table 2).

The ANOVAs indicate a highly significant effect of maternal family on all five traits. Population of origin was a significant main effect only for mean bolting date and mean number of rosette leaves; slight differences in inflorescence height and fruit production between the two populations approached significance (Table 2). In both the shaded and unshaded treatments, the population native to the shadier AS site flowered earlier and with fewer leaves than the population native to the SR site (Fig. 3). However, there was no significant interactions between population of origin and either the early treatment or the late treatment (i.e., the two populations had similar plastic responses to shading regimes; Table 2).

Selection on flowering and size traits.—In all four treatment combinations used in the greenhouse study, total selection was qualitatively similar. Total selection always favored larger diameters, but this selection was weakest in treatment 3 (shading early, but not late), a novel environment that does not correspond to con-

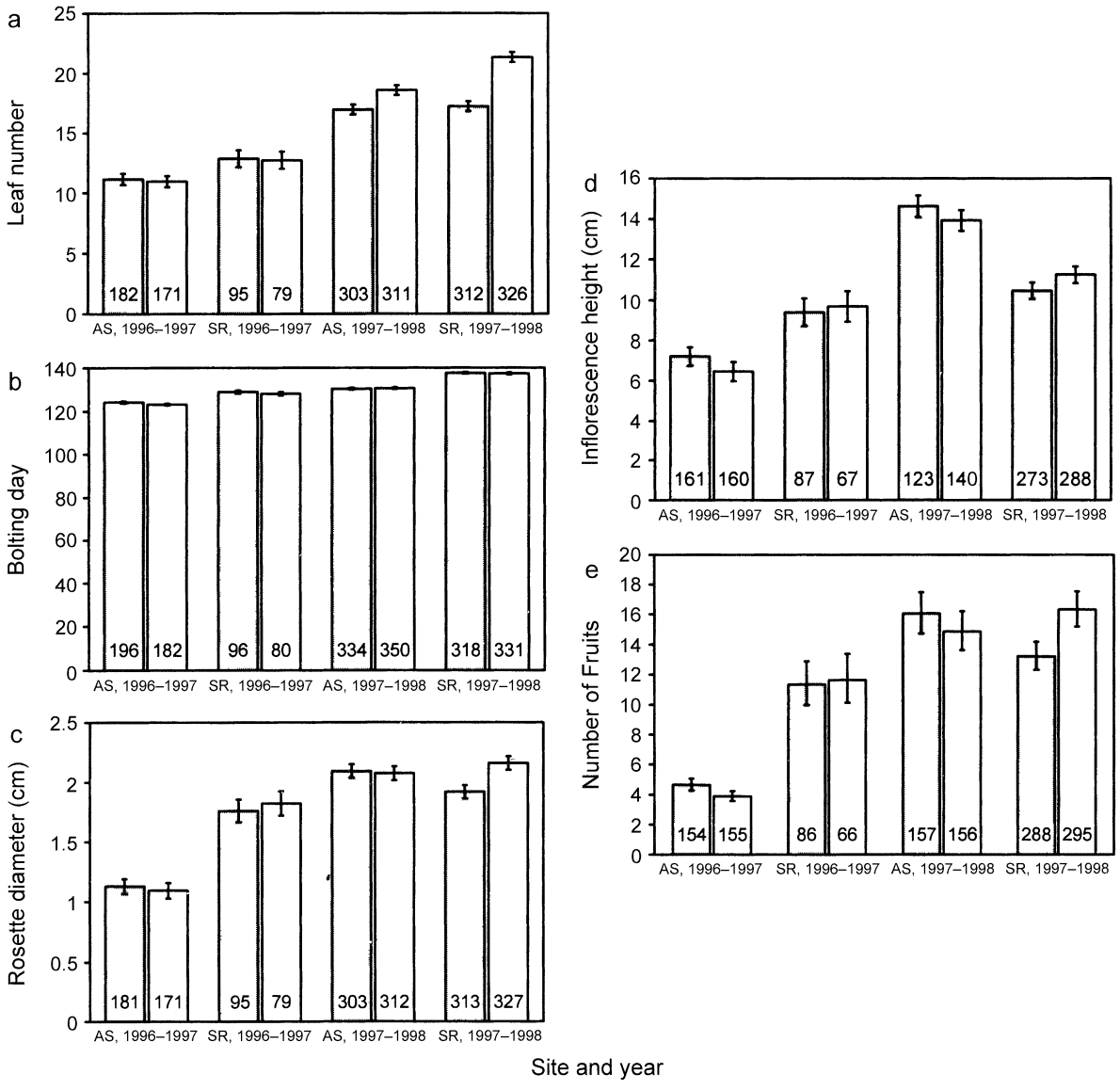

FIG. 2. (a) Mean rosette leaf number at bolting, (b) bolting date, (c) rosette diameter, (d) inflorescence height, and (e) fruit production of *Arabidopsis thaliana* at two field sites in Tennessee during two consecutive growing seasons. Population of origin is as follows: open bar, Agricultural Experiment Station (AS); gray bar, Sharp Ridge Park (SR) (least-squares means ± 1 SE are shown). *N* value appears within the bar for the associated treatment.

ditions observed in the field. (Note that for treatment 3, the model fit poorly and was poorly supported by the cross-validation procedure; Table 3). Although there was total selection for an earlier date of bolting, there was no total selection for bolting with more rosette leaves. Directional selection gradients for earlier bolting were always significant. This was also the case for larger rosette diameters, except in the novel treatment 3. Finally, there were significant directional gradients for flowering with more rosette leaves, but only in treatments where rosettes were shaded after bolting (treatments 2 and 4).

A genotypic analysis found selection gradients similar to the phenotypic ones (Table 3: Genotypic estimates). There were significant directional selection gradients favoring larger rosette diameters and an earlier date of bolting, except in the novel treatment 3. There were no significant selection gradients on number of rosette leaves at bolting.

For the greenhouse study, we again used ANCOVA models to examine heterogeneity of phenotypic directional selection gradients. We found that across the four treatments the direct selection gradients were significantly heterogeneous only for bolting date ($F_{3,509}$ =

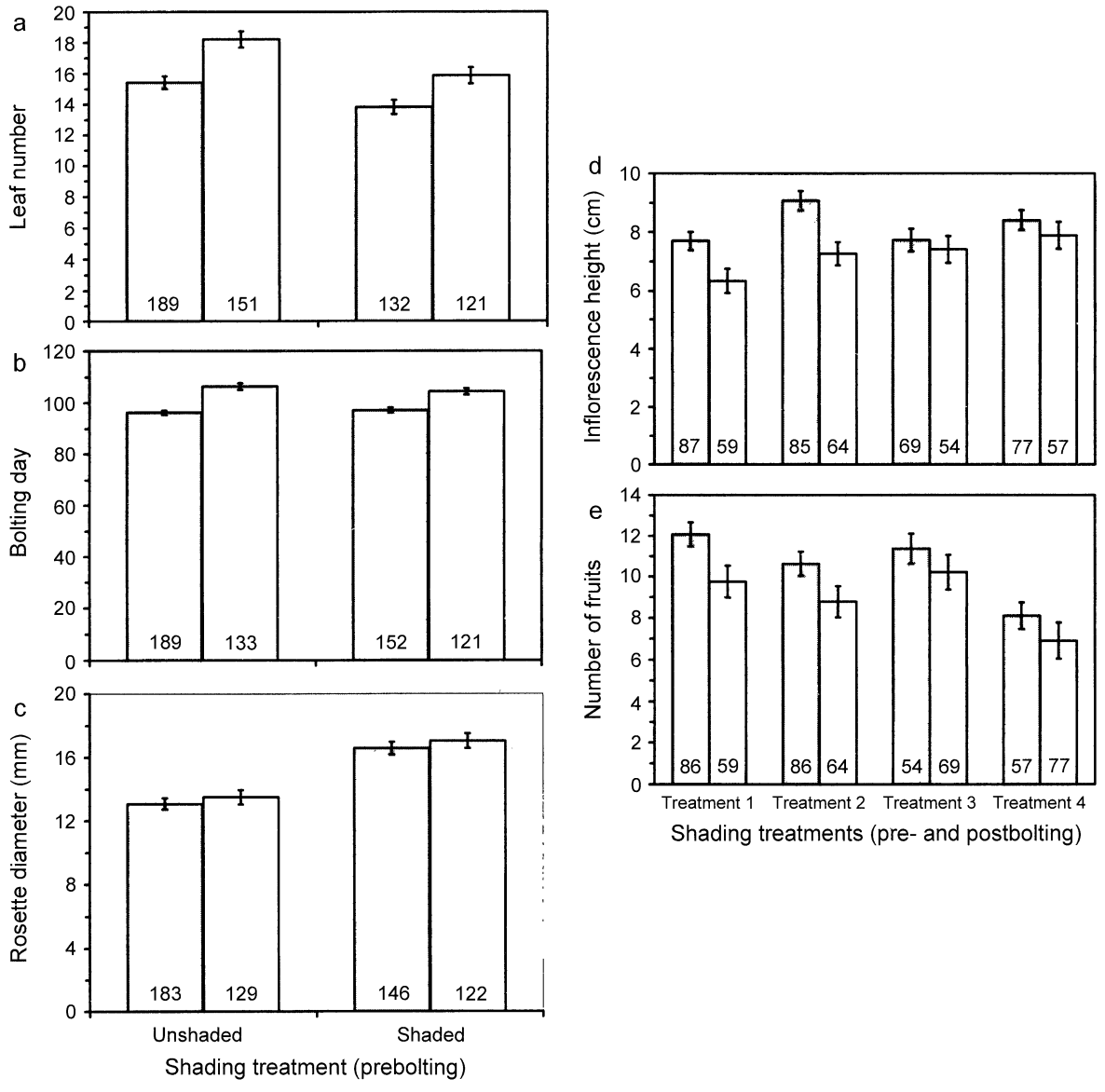

FIG. 3. (a) Mean rosette leaf number at bolting, (b) bolting date, (c) rosette diameter, (d) inflorescence height, and (e) fruit production of *A. thaliana* in the greenhouse experiment. For panels (d) and (e), results are shown separately for Treatment 1 (unshaded late, unshaded early), Treatment 2 (shaded late, unshaded early), Treatment 3 (unshaded late, shaded early), and Treatment 4 (shaded late, shaded early). Population of origin is as follows: open bar, Agricultural Experiment Station (AS); gray bar, Sharp Ridge Park (SR) (least-squares means ± 1 SE are shown). *N* value appears within the bar for the associated treatment.

4.43, $P < 0.004$). This result reflects our detection of strong directional selection gradients for earlier bolting in all but one treatment.

DISCUSSION

Numerous studies of *A. thaliana* have focused on how decreased photosynthetic photon flux density (PPFD) and a reduced red-to-far-red ratio (R:FR) can affect the germination and morphology of seedlings and the flowering time of adult plants (Halliday et al. 1994, Reed et al. 1994, Quail et al. 1995, Yanovsky et al. 1995, Pigliucci and Schmitt 1999). Our field study is among the first to quantify the survivorship, flowering time behavior, and reproductive output of natural populations of *A. thaliana* across contrasting shaded and unshaded natural habitats in which spatial and temporal variation in PPFD and light spectral quality were also documented. We found differentiation both between and within populations for flowering time traits and fitness, but evidence for local adaptation was not especially strong. Although shade did induce plants to bolt earlier, this plasticity was not clearly adaptive.

TABLE 5. Total selection and direct linear selection gradients from the field study (with P values presented parenthetically): (a) phenotypic estimates (based on individual plants) and (b) genotypic estimates (based on family means).

Trait	AS (Agricultural Experiment Station)			
	1996–1997		1997–1998	
	Total selection	Direct selection	Total selection	Direct selection
Phenotypic estimates				
No. rosette leaves	**0.59 (<0.0001)**	**0.30 (<0.0001)**	**0.36 (<0.0001)**	0.16 (0.0178)
Date of bolting	**−0.14 (0.0120)**	**−0.13 (0.0006)**	**−0.25 (<0.0001)**	**−0.18 (0.0012)**
Rosette diameter	**0.69 (<0.0001)**	**0.52 (<0.0001)**	**0.41 (<0.0001)**	**0.30 (<0.0001)**
Sample size	...	294	...	256
R^2	...	0.585	...	0.320
Cross-validation				
R^2_{est}	...	0.513	...	0.218
R^2_{pred}	...	0.610	...	0.330
Genotypic estimates				
No. rosette leaves	**0.45 (0.0010)**	0.21 (0.1414)	0.07 (0.630)	−0.26 (0.1413)
Date of bolting	−0.10 (0.4822)	**−0.26 (0.0284)**	−0.22 (0.132)	−0.03 (0.8244)
Rosette diameter	**0.59 (<0.0001)**	**0.51 (0.0007)**	**0.38 (0.007)**	**0.52 (0.0076)**
Sample size	...	49	...	49
R^2	...	0.423	...	0.186

Notes: Boldfaced type indicates significance after adjusting for multiple tests. Sample sizes are for complete observations used for model estimation.

Nonshaded conditions may select for accelerated bolting just as strongly as shaded conditions. Also, selection does not tend to favor bolting at an earlier developmental stage. Rather, delaying the developmental timing of bolting is sometimes favored in both shaded and unshaded environments.

Shade and survivorship

The risk of postbolting mortality was higher at the shaded Agricultural Experiment Station (AS) site than at the Sharp Ridge Park (SR) site, at least during the second year of the study, when PPFD reached extremely low levels late in the season. This provides good circumstantial evidence that *A. thaliana* plants face an increased risk of mortality if they encounter vigorous aboveground crowding during the longer, warmer, wetter days of spring. Prebolting (i.e., over-winter) mortality was low, especially in the second year, and rates were similar at the two sites. This is circumstantial evidence that over-winter survivorship is not affected by differences in shading regimes or other competitive factors between the AS and SR sites (Fig. 1). Finally, lower mortality of seedlings at the more shaded site contrasts with previous reports of increased seedling and prereproductive mortality with increasing density of conspecific or interspecific neighbors (Myerscough and Marshall 1973, Yanovsky et al. 1995, Ballare and Scopel 1997). Our results for seedling survivorship may differ for several reasons. We were working with natural populations, rather than with photoreceptor mutants (Yanovsky et al. 1995). Additionally, there may be differentiation among natural populations in seedling survivorship, due to genetic differences or to differences among maternal environments that alter seed and seedling behavior (Koornneef and Karssen 1994). Finally, our results may reflect the relative ease of successfully transplanting seedlings onto a flatter site, rather than a difference due to the local competitive and light regime.

Shade-induced phenotypic plasticity

The most consistent impact of shade was its tendency to induce bolting at a significantly earlier developmental stage (i.e., with fewer rosette leaves). Although this plasticity was consistently significant, its magnitude varied from year to year in the field. Shade also tended to shift bolting to an earlier point in time, although this effect also varied in magnitude between years, and was not as consistent as shade's tendency to induce bolting earlier in development. In fact, the shaded treatment in the greenhouse did not induce an earlier bolting date. The disparity between these two flowering time traits is somewhat puzzling, because they are often almost perfectly correlated in studies of laboratory lines, and in such studies it is common to measure the number of rosette leaves rather than the date of bolting (Koornneef et al. 1991).

The correlation between flowering date and leaf number may be positive and highly significant, but <1.0 in more variable laboratory populations (Mitchell-Olds 1996*a*) or in late-flowering ecotypes (Pigliucci and Schlichting 1998). We therefore advocate using both traits to fully characterize flowering time, because it seems likely that shade can affect a plant's flowering time in two distinct fashions. It can shift bolting to an earlier developmental stage and also slow overall plant growth, including the rate of new leaf production. The two trends can essentially cancel, resulting in no net

TABLE 5. Extended.

SR (Sharp Ridge Park)			
1996–1997		1997–1998	
Total selection	Direct selection	Total selection	Direct selection
0.45 (<0.0001)	0.10 (0.1480)	**0.62 (<0.0001)**	**0.21 (<0.0001)**
−0.33 (<0.0001)	**−0.17 (0.0046)**	**−0.37 (<0.0001)**	**−0.10 (0.0003)**
0.63 (<0.0001)	**0.54 (<0.0001)**	**0.72 (<0.0001)**	**0.56 (<0.0001)**
...	151	...	555
...	0.593	...	0.644
...	0.569	...	0.672
...	0.550	...	0.610
0.41 (0.0047)	0.04 (0.8288)	**0.58 (<0.0001)**	−0.04 (0.7023)
0.30 (0.0422)	−0.20 (0.1165)	**−0.43 (0.0020)**	−0.17 (0.0504)
0.52 (<0.0001)	**0.52 (0.0044)**	**0.83 (<0.0001)**	**0.81 (<0.0001)**
...	46	...	49
...	0.375	...	0.718

change in the date of bolting. This was observed in our greenhouse study and also by Pigliucci and Schmitt (1999), who were studying photomorphogenic mutants and their plasticity to neutral or vegetation shade. In contrast, our field study found variation between sites and years in both rosette leaf number at bolting and bolting date. Perhaps we detected differences because field conditions encompassed a broader range in the intensity, timing, or other details of shaded vs. open regimes.

Finally, shaded conditions in the greenhouse increased some size-related traits (Fig. 3c, d), but the corresponding differences were not consistently observed between the more shaded and less shaded field sites (Fig. 2c, d). In the field, the phenotypic plasticity of these traits may reflect inevitable responses to the high density of surrounding vegetation, which lowers the R:FR, but also may result in competition for above- and belowground resources (Sultan and Bazzaz 1993). Size-related traits may have been affected not only by light quality, but also by local variability in other abiotic and biotic factors. This possibility is further supported by the significant effect of year in the field study (Table 4) and by highly significant block effects in both the field and greenhouse studies (Table 4, Table 2).

Are natural populations locally adapted?

The significant effect of site for most traits (except inflorescence height) suggests that there is significant shade-induced plasticity in both populations. Despite this plasticity, the generally nonsignificant effect of population of origin indicates little differentiation between two populations. Furthermore, nonsignificant or only marginally significant site × population-of-origin interaction terms indicate that shade-induced plasticity does not differ between populations. Examining the results in detail reveals, however, that the shade-native AS population shows less plasticity to variation in shading. During the second year only, the AS population bolted with fewer leaves and produced fewer fruits than the SR population (Fig. 2) when the two populations were grown at the less shady SR site. This suggests that this shade-native population may be less capable of delaying bolting to a later developmental stage in order to take advantage of nonshaded conditions. Thus, its flowering time plasticity may be maladaptive or suboptimal.

The greenhouse study provides stronger evidence for differentiation between populations. As in the field study, the shade-native AS population bolted earlier.

TABLE 6. Results of statistical tests for heterogeneity of linear selection gradients from the two-year, two-site field study.

Continuous variable	Site × Continuous variable		Year × Continuous variable		Site × Year × Continuous variable	
	F	*P*	*F*	*P*	*F*	*P*
No. rosette leaves	0.215	0.6429	**18.23**	**<0.0001**	**18.5**	**<0.0001**
Day of bolting	0.190	0.6627	5.07	0.0245	1.05	0.3055
Rosette diameter	**41.24**	**<0.0001**	2.28	0.1315	**4.65**	**<0.0001**

Notes: Separate ANCOVA models were fit for each of the four variables. All models used the same combined data set ($N = 1256$).

This occurred not only in the shaded treatments, which resembled the native habitat of the AS population, but also in the unshaded treatments. The AS population also had slightly taller inflorescences and greater fitness in all treatments. Despite these differences, populations native to the AS and SR sites showed similar plasticity to shade for all traits.

In both populations and in both studies, we detected significant variation among families for the three traits measured at the time of bolting. Moreover, our genotypic selection analyses suggest that natural selection, if it were consistent within a site from year to year, could favor families with earlier bolting dates (Table 4, Table 5). It is unclear whether such a selection regime is acting or has acted within either of these populations. We detected significant variation among families for two traits that estimate fitness (inflorescence height and fruit number), but only in the greenhouse study, not in the field study. In the field, response to natural selection may be quite weak because a large proportion of the variance for fitness may be due to environmental rather than genetic variation.

For several reasons, we might predict rather limited differentiation between the two populations (currently or in the future) for flowering time traits or their shade-induced plasticity. First, directional selection gradients for number of rosette leaves at bolting may differ between years as much as or more than they do between the two contrasting sites. Year-to-year variation also indicates that factors other than shading may mediate heterogeneous selection on this trait. Second, directional selection gradients favoring earlier bolting dates were not generally stronger at the more shaded AS site. This result suggests that selection for bolting at an earlier date may not be mediated by vegetation shade, but instead by temperature, rainfall, soil moisture, or other factors. Indeed, the importance of small-scale variation in such factors is underscored by the significant block effects found in our field study (Table 4) and even among the spatial blocks in our greenhouse study (Table 2), as well as previous studies (Bell and Lechowicz 1991, Stratton and Bennington 1996). Third, the greenhouse study found that gradients for number of rosette leaves at bolting were weak in unshaded, shaded, and heterogenous treatments. Finally, in some habitats competitors may encroach so rapidly that it is not possible to escape competition, possibly rendering shade avoidance disadvantageous. For example, the weedy annual *Abutilon theophrasti* experiences asymmetric competition or differences in the timing of competition that negate the selective advantage of shade avoidance (Weinig 2000).

It is also possible that estimates of phenotypic selection gradients are either inaccurate or biased. Their accuracy could also be compromised by mortality that resulted in unequal sample sizes between populations, or among maternal families. Also, we did detect some between-population genetic structure for trait means (e.g., rosette leaf number), indicating that selection analyses should be interpreted cautiously. They may be less accurate than if estimates had been obtained using only the plants native to each site. Data were pooled, however, to maximize both sample sizes and the range of natural trait variation, both important for an analysis examining multiple traits. This approach is appropriate to the general aim of gaining some of the first insights into the ecology of shade avoidance in the natural habitats of *A. thaliana*. Additionally, bias in phenotypic selection analyses may result because an individual plant's microsite can induce correlations between traits and relative fitness (Rausher 1992), a problem addressed by the genotypic selection analyses, which were qualitatively similar to phenotypic analyses (i.e., gradients changed in magnitude or significance, but not in sign).

Is shade-induced plasticity adaptive?

As in many laboratory studies, we observed bolting with fewer rosette leaves and at an earlier date under shaded conditions. If this plasticity is adaptive, selection regimes in shaded conditions should favor plants that bolt earlier and with fewer leaves. Selection regimes under shaded conditions did favor plants that bolt chronologically earlier, but a similar selection regime was also observed in less shaded or unshaded conditions. Also, we observed selection to flower with more rather than fewer leaves, or no selection on this trait. In the densely shaded conditions we studied, it was not advantageous to bolt at a developmentally earlier stage.

Since distinct flowering time traits may be subject to conflicting selective pressures, it appears that the plasticity of these traits may be only partly adaptive, and perhaps subject to constraint. In many laboratory studies of *A. thaliana*, the strong positive correlation between leaf number and date of bolting means that few plants flower both early and with many rosette leaves. We also observed a strong correlation between these two flowering time traits in our greenhouse study (Appendix B). Furthermore, it appears that the positive correlation between these two traits is not easily altered by selection, even when mutation rates are increased artificially (Mitchell-Olds 1996*a*, Camara and Pigliucci 1999). Such results suggest pleiotropy of genes controlling the two traits, or perhaps a functional or physiological connection between them.

While it may be possible to detect pleiotropy or other constraints in the lab, they may not be relevant in field environments, where we found either no correlation or a negative correlation between these two traits (Appendix A). This may be due to local environmental heterogeneity, a possibility supported at the SR site where correlations between bolting date and leaf number calculated with block means were weakly negative (1997, $r = -0.14$; 1998, $r = -0.13$). At the AS site, however, correlations calculated with block means

were strong and positive (1997, $r = 0.81$; 1998, $r = 0.96$), indicating that local environmental heterogeneity, if important, probably imposes heterogeneous selection at much finer spatial scales (Stratton and Bennington 1996). Large discrepancies between either phenotypic or genotypic correlations estimated in benign laboratory vs. harsher or more variable field environments may not be the norm, but they have been detected in some quantitative genetic studies (Roff 1996, 1999).

Finally, in addition to considering pleiotropy, other constraints, and environmental dependence of correlations and covariances, it may be important to bear in mind that plasticity may be induced by multiple factors in the external environment (Bell and Lechowicz 1994, Schlichting and Pigliucci 1998). For example, spring-flowering annuals like *A. thaliana* are likely to encounter imperfectly concordant environmental factors, such as a predictable photoperiod regime, but interannual variation in shading or temperature regimes. Future ecological studies of *A. thaliana* should continue to examine the genetic architecture of multiple life history traits, but they should also be conducted in field sites or in more controlled experiments where multiple ecological factors vary, and not necessarily in a concordant fashion. Without an appreciation for such complexities, it will be difficult to gain a full understanding of the plasticity of flowering time and its ecological significance.

Acknowledgments

For assistance in the field and greenhouse, we thank N. Byrd, M. Camara, K. Hayden, J. James, A. Maleszyk, B. Marlow, K. McFarland, R. Smejterowicz, B. Stawicki, and K. Tran. The University of Tennessee Herbarium, the University of Tennessee Agricultural Experiment Station Plant Science Unit, and the Knoxville/Knox County Parks Department helped with selection and maintenance of field sites. We thank M. Camara, H. Pollard, C. Wells, M. Cruzan, J. Agren, T. Mitchell-Olds, and two anonymous reviewers for careful reading and comments that improved the manuscript. This research was funded by National Science Foundation grant DEB 95-27551 to M. Pigliucci.

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APPENDIX A

Pearson product-moment correlation coefficients for the populations in the Agricultural Experiment Station and Sharp Ridge Park study sites are detailed in ESA's Electronic Data Archive: *Ecological Archives* E083-034-A1.

APPENDIX B

Pearson product-moment correlation coefficients for the four treatments (treatment 1, always unshaded; treatment 2, unshaded early/shaded late; treatment 3, shaded early/unshaded late; treatment 4, shaded early and late) are detailed in ESA's Electronic Data Archive: *Ecological Archives* E083-034-A2.