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NICHE CONSTRUCTION THROUGH GERMINATION CUEING: LIFE-HISTORY RESPONSES TO TIMING OF GERMINATION IN *ARABIDOPSIS THALIANA*

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Abstract.—Germination responses to seasonal conditions determine the environment experienced by postgermination life stages, and this ability has potential consequences for the evolution of plant life histories. Using recombinant inbred lines of *Arabidopsis thaliana*, we tested whether life-history characters exhibited plasticity to germination timing, whether germination timing influenced the strength and mode of natural selection on life-history traits, and whether germination timing influenced the expression of genetic variation for life-history traits. Adult life-history traits exhibited strong plasticity to season of germination, and season of germination significantly altered the strength, mode, and even direction of selection on life-history traits under some conditions. None of the average plastic responses to season of germination or season of dispersal were adaptive, although some genotypes within our sample did exhibit adaptive responses. Thus, recombination between inbred lineages created some novel adaptive genotypes with improved responses to the seasonal timing of germination under some, but not all, conditions. Genetically based variation in germination time tended to augment genetic variances of adult life-history traits, but it did not increase the heritabilities because it also increased environmentally induced variance. Under some conditions, plasticity of life-history traits in response to genetically variable germination timing actually obscured genetic variation for those traits. Therefore, the evolution of germination responses can influence the evolution of life histories in a general manner by altering natural selection on life-history traits and the genetic variation of these traits.

Key words.—Dormancy, maternal effects, natural selection, phenotypic plasticity, seasonal cues.

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The timing of seed germination is highly responsive to environmental conditions. For a seed to germinate, certain environmental conditions must be present to break dormancy, and additional environmental conditions must exist subsequently to permit germination (Simpson 1990; Bewley 1997; Baskin and Baskin 1998). The timing of germination can therefore be a precise mechanism of habitat selection or niche construction that determines the environment experienced by the plant (Donohue 2003, 2005). If the environmental conditions that elicit germination accurately predict the environment experienced by adults, germination behavior has the potential to influence the environment experienced by the plant throughout its life. It can thereby influence the phenotypic expression of plastic postgermination characters and the mode and strength of natural selection on those characters (Donohue 2002).

Niche construction occurs when organisms alter the environment they experience (Odling-Smee et al. 1996), either through direct habitat modification, habitat choice, or resource garnering and depletion (Bazzaz 1991; de Kroon and Hutchins 1995; Huber et al. 1999). The environmental modification may be beneficial or not. In plants, perhaps the most effective way to alter their environment is through phenotypic plasticity. The ability of plants to sense the environment also provides them with some ability to respond to that environment in ways that can alter their exposure to it (Donohue 2003). Plastic environmental cueing of germination can be a precise mechanism of habitat selection in plants that can determine both biotic and abiotic conditions that germinants experience (McCullough and Shropshire 1970; Hayes and Klein 1974; Evans and Cabin 1995; Gutterman 2000; reviewed in Baskin and Baskin 1998).

Germination responses to cues that predict season, in particular, have the potential to influence the environment experienced by young germinants and also by adult plants. For example, in winter annuals germination responses to warm after-ripening followed by a brief cold period would indicate autumn conditions in a temperate climate (Baskin and Baskin 1972, 1974, 1983, 1990; Baskin et al. 1992; Galloway 2001, 2002). The seedling or rosette would then be exposed to winter conditions after seed germination. Alternatively, in spring germinants exposure to a prolonged cold period as a seed would indicate the passage of winter. The germinant and rosette would then not be exposed to prolonged cold, but spring and summer conditions would follow immediately after germination (Baskin and Baskin 1998; Galloway 2002; Kalisz and Wardle 1994). Therefore, germination responses to seasonal cues are likely to be especially important for determining subsequent life-history expression and selection on postgermination life-history characters because cues perceived by seeds can predict conditions experienced by adult plants.

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Niche construction has some interesting consequences for life-history evolution. In particular, if life-history characters respond plastically to the selected environment, and if that ability to select the environment exhibits genetic variation, plastic life-history characters can evolve even if they themselves do not exhibit any genetic variation within a particular environment (Moore et al. 1997, 1998; Wolf et al. 1998, 1999; reviewed in Donohue 2003). That is, the genetic variation for niche construction (e.g., germination cuing and the environment that results from that) is essentially transferred to the life-history character (e.g., flowering time) whose expression depends on that environment (e.g., the postgermination season). In addition, habitat selection can enable specialization of subsequently expressed characters to the selected habitat (Levins 1968; Holt 1987; Rosenzweig 1987; Brown 1990; Evans and Cabin 1995; reviewed in Donohue 2003), because exposure to other selective environments is diminished. Therefore, plasticity in characters responsible for niche construction, such as germination, can increase the adaptation of subsequent characters to a particular postgermination environment.

To determine empirically the importance of niche construction to life-history evolution in plants, we need to know first whether life-history characters respond plastically to the constructed environment. We also need to know whether the ''constructed'' environment influences natural selection on life-history characters and whether the expressed life-history characters are specialized to their particular environment. Finally, the contribution of genetically based variation in habitat-determining traits to genetic variation in life-history characters needs to be determined. In this study, we consider germination timing to be a habitat-determining character. We tested whether postgermination life-history characters exhibit phenotypic plasticity in response to the seasonal environment experienced after germination. We also measured natural selection on adult life-history characters in plants that germinated in different seasons to determine which life-history traits that were expressed in different postgermination seasonal environments were adaptive in that particular environment. Finally, we determined whether genetically based variation in germination timing augmented or diminished differences among genotypes in life-history expression.

We used diverse recombinant inbred lines of *Arabidopsis thaliana* in a field manipulation of geographic location and season of seed dispersal to test how the importance of habitat determination through germination varies with geography and seed dispersal season. *Arabidopsis thaliana*, a highly selfing (Abbott and Gomes 1989) annual mustard, matures and disperses seeds in both spring and autumn in New England (see also Thompson 1994), and seasonal environmental factors influence germination timing. In companion papers, we documented highly plastic and genetically variable responses of germination timing to geographic location and season of seed dispersal (Donohue et al. 2005a), and we showed that germination timing explained a large proportion of the variation in fitness among genotypes in some conditions but not others (Donohue et al. 2005b). Here we examine indirect effects of germination timing on fitness operating through the phenotypic expression of postgermination characters and through natural selection on those postgermination characters.

Artificially outcrossed segregants, such as those used here, provide a powerful tool to investigate the adaptive consequences of genetically possible phenotypes that may have already been selected out of natural populations (Jordan 1991; Schemske and Bradshaw 1999). The use of recombinant inbred lines enabled us to determine which life-history responses were adaptive in each season of germination and whether any novel adaptive genotypes existed after hybridization between natural lineages.

In this study, we first tested whether the expression of adult life-history characters depended on the season of seed germination. We then determined whether natural selection on life histories depended on the season of germination, and also whether the observed plasticity of life-history traits in response to season of germination was in the adaptive direction for any genotypes in this sample of recombinant lines. Last, we tested whether variation in germination timing altered the degree of genetic variation expressed for postgermination life-history characters.

Materials and Methods

The Genetic Material

We used recombinant inbred lines derived from two accessions of *A. thaliana*: one from Calver, England (Cal), and the other from Tacoma, Washington (Tac), with the Tac line as the maternal parent. Seeds of Cal, were acquired through the Arabidopsis Biological Resource Center at Ohio State University (stock CS1062), and Tac seeds were collected by T. Mitchell-Olds. Please see Donohue et al. (2005a) for details on the lines and their maintenance.

Experimental Treatments

Experimental design.—Seeds were matured under two photoperiods that represent the photoperiods during late spring and autumn when plants are maturing seeds in the field. The seeds were then deposited (dispersed) into the field during late spring (June) and autumn (November) in two sites: Rhode Island (RI) and Kentucky (KY). In KY, natural populations flower and disperse seeds only during the spring, so we dispersed seeds in the spring in KY. In RI, plants flower and disperse seeds both during the spring and the autumn, so we dispersed seeds in RI during the late spring and autumn. We imposed different germination cohorts and monitored plants that germinated during each season in each site and season of dispersal. From this design, we quantified the effect of germination season on life histories in the two geographic locations by comparing seeds dispersed during June in KY and RI, and we quantified the effect of germination season in both seasons of seed dispersal by comparing the seeds dispersed in RI during June and November. See Donohue et al. (2005b, fig. 1) for the experimental design.

Maternal treatments.—Plants were grown in two batches to provide seeds to be dispersed into the field during June and during November. A total of 110 recombinant inbred lines were available for the first planting for June, and 10 additional lines were available for the second planting in

November. In each planting, six replicates of each recombinant genotype were grown in a short-day treatment (10 h of full-spectrum light followed by 14 h of darkness) and a long-day treatment (14 h of full-spectrum light followed by 10 h of darkness) in Conviron E7/2 (Controlled Environment Ltd., Pembira, ND) growth chambers at 22°C. See Donohue et al. (2005a) for details on the growing conditions. Seeds were collected from each plant as they matured, and they were pooled across replicates. Ten seeds from a given genotype and photoperiod combination were put into separate microcentrifuge tubes. Seed collection and processing took three to four weeks, during which seeds were kept in centrifuge tubes at 23°C.

Field treatments.—In both KY and RI, experimental gardens were prepared in old-field sites. Peat pots of 2.25-in diameter (Jiffy Poly-Pak, Jiffy, Products; www.jiffyproducts.com) were filled with sterile soil medium (Metromix 360; Scotts Sierra, Marysville, OH) and planted into the blocks with approximately 5 cm between pots. The vegetation canopy was minimal at this early successional stage in both sites, and the same soil was used in both sites, so the primary difference between sites was climatic. Three blocks were established in KY, and three replicates of each genotype and photoperiod combination were randomly assigned to each germination-cohort treatment (see below) and randomly positioned within each block. In RI, nine blocks were established for each season of dispersal. June-dispersal blocks alternated with November-dispersal blocks on the site. One replicate of each genotype and photoperiod combination was randomly assigned to each germination-cohort treatment within each block, with the position of each being randomly assigned. To compare KY to RI, blocks in RI were combined to give three blocks of the same size as the KY blocks. Each tube of 10 seeds was poured into its respective pot, which was then covered with a Mason jar screen lid. Lids prevented mechanical disturbance but permitted ambient environmental conditions (for more details see Donohue et al. 2005a). For the June dispersal season, seeds were dispersed in KY from 1 to 4 June 2001, and they were dispersed in RI from 20 to 23 June 2001. For the November dispersal season, seeds were dispersed in RI from 3 to 6 November 2001.

Germination cohort treatment.—For seeds dispersed in June, three germination cohorts were established by plucking all germinants from pots except those that germinated during the correct randomly assigned season. One focal germinant in each pot was randomly chosen from those remaining so that competition between germinants did not occur. The germination cohorts were: summer (June through July), autumn (August through January), and spring (after February). Two germination cohorts existed for seeds deposited in November: winter (November to January) and spring (after February). To compare across dispersal seasons in RI, only seeds that germinated during November and afterward in both dispersal treatments were compared. These seasonal germination cohorts correspond to peaks of germination in the field (Donohue et al. 2005a).

When dispersed in June, some genotypes germinated only during summer or during a subset of seasons (see Donohue et al. 2005a, table 4). Therefore, germination cohorts frequently comprised different sets of genotypes. When that was the case, germination cohorts were also compared using only the subset of genotypes that germinated in multiple seasons, and results are presented in the text when they differed from the analysis of the complete sample.

Phenotypic measurements.—Each focal individual was followed throughout its life. Its date of germination, date of death, and the total number of fruits produced throughout its life was recorded by censusing every week during the growing season. Total fruit production was used as an estimate of fitness. The following life-history characters were recorded. As a measure of the timing of reproduction, the interval between germination and bolting date, or bolting age, was recorded. It indicates a developmental switch from vegetative growth to reproductive activity (Dorn and Mitchell-Olds 1991; Mitchell-Olds 1996). The interval between bolting and the first date on which a flower was observed, or flowering interval, was also recorded. Flowering interval is a measure of the rate of inflorescence development. The size at reproduction was measured as the rosette diameter, or the largest diameter on the day of bolting. Allocation to vegetative growth after reproductive initiation was measured as the number of basal branches that were produced throughout the life of the plant (number of basal branches). It is also an indicator of overall size of the plant. Results did not differ if we analyzed total branch number, so only results using basal branches are provided.

Statistical Analyses

The effects of photoperiod, site, and season of seed dispersal on germination were presented in a companion paper (Donohue et al. 2005a). To determine the effects of season of germination, photoperiod, site, and season of seed dispersal on life history-characters, we used analysis of variance (ANOVA; Proc GLM in the SAS; SAS Institute 1990). Individual phenotypes were analyzed with block as a random factor, nested in site or dispersal season, and with genotype as a random factor. In addition, genotypic means were calculated by pooling over blocks. Analysis of individual phenotypes and genotypic mean values produced very similar results, so only results of the analyses of genotypic means are provided. Photoperiod did not influence most traits, but it interacted significantly with season of dispersal to influence basal branch production of plants in RI ($F = 7.11$, df = 1, $P = 0.008$). Because photoperiod did not significantly influence the other traits, and because the effect of photoperiod was not significant for any treatment analyzed separately, all subsequent analyses calculated genotypic means by pooling over block and photoperiod. To analyze the effect of geographic location (site), seeds dispersed during June in KY and RI seeds were analyzed, with germination cohort, photoperiod, and site as fixed factors. To analyze the effect of dispersal season, seeds dispersed in RI during June and November were analyzed in a separate model, with germination cohort, photoperiod, and dispersal season as fixed factors. Data were natural log-transformed to normality, and the residuals of most analyses were normally distributed. When they were not, we performed Kruskal-Wallis tests.

For seeds dispersed during June, some genotypes germinated only during the summer (specialists) and others ger-

TABLE 1. Results of analysis of variance to test for effects of germination season (cohort) and site on life history, using seeds dispersed during June. Genotypic means were used based on individual phenotypes pooled across block and photoperiod. df (season) = 2, df (site) = 1, df (interaction) = 2; $N = 352$. F-ratios are given for the full model and for the analyses within each site based on Type III sums of squares. Effects of germination season were highly significant ($P < 0.001$) in both sites when based on Kruskal-Wallis tests. Tests for effects of site, within each germination cohort, are based on Kruskal-Wallis χ^2. See Appendix 1 for the number of genotypes in each cohort and treatment.

Source	Bolting age	Flowering interval	Rosette diameter	No. basal branches	Total fruits
Full model					
Germination season	142.30***	57.86***	92.91***	26.19***	156.44***
Site	107.41***	13.67***	0.43	3.57	30.80***
Germination season × site	218.22***	45.57***	3.05*	6.91**	107.21***
Effect of germination season in each site					
Germination season in KY	72.73***	15.49***	17.53***	10.05***	294.41***
Germination season in RI	1442.85***	110.90***	232.01***	58.33***	48.96***
Effect of site in each germination season					
Site in summer	50.66***	34.88***	0.08	0.40	83.18***
Site in autumn	17.10***	0.67	5.86	20.74***	20.33***
Site in spring	2.29	4.33*	2.00	4.68*	0.27

* $P < 0.05$, *** $P < 0.001$.

minated over multiple seasons (generalists). We compared life histories of summer germinants of specialist versus generalist genotypes using ANOVA.

To test for environment-dependent natural selection on life history, a phenotypic (Lande and Arnold 1983; Arnold and Wade 1984) and a genotypic selection analysis (Mitchell-Olds and Shaw 1987; Rausher 1992) were performed. Genotypic selection analysis is preferable to phenotypic selection analysis because it controls for environmentally induced correlations between phenotypes and fitness. Results did not differ much between the two selection analyses, so only results of the genotypic selection analysis are presented. Relative fitness within each germination cohort in each site and dispersal season was calculated as the total lifetime fruit production divided by the mean fruit production within each combination of site, dispersal season, and germination season. The life-history characters were standardized within each treatment combination to have a mean of zero and standard deviation of one. Linear and quadratic selection gradients were calculated through multiple regression analysis. To test for significant differences in selection across treatments, analysis of covariance (ANCOVA) was performed—which included life-history traits (and their quadratics), treatments, and interactions between traits and treatments—to test for differences in the slopes of the regression of life-history characters on fitness across treatments. Specifically, differences across germination cohorts were tested within each site and dispersal season. Differences between site (using seeds dispersed in June only) and differences between dispersal seasons (using seeds dispersed in RI only) were tested separately in each germination cohort. Interactions between the life-history trait and any treatment would indicate that selection differed across treatments.

We tested whether genetically based variation in germination timing contributed to genetic variation in postgermination life-history characters. First, we calculated the residual values of life-history traits from an ANCOVA with each life-history trait as the dependent variable and germination date as the predictor in each treatment. We then tested for significant differences among genotypes for the residual values of life-history traits and compared the differences among genotypes to those based on raw values of life-history traits. If differences among genotypes were smaller when based on residuals (after factoring out the influence of germination timing), then genetically based variation in germination timing can be considered to augment genetic variation in life-history characters. To quantify this potential difference, total phenotypic variance in life-history traits was estimated on the raw characters and also on the residuals of the characters. The percentage of the phenotypic variance that was explained by variance among genotypes, or the genetic variance component, was also calculated for raw phenotypes and for residuals using Proc Varcomp in SAS (SAS Institute 1990). The environmental variance was calculated as the total phenotypic variance minus the genetic variance. The ratio of the genetic to the total phenotype variance is the heritability of the trait. Significance of heritabilities was determined from the significance of the main effect of genotype in an ANOVA conducted separately within each treatment but pooled across germination cohorts. We did not estimate genetic parameters separately within each germination cohort because different genotypes were represented in the different cohorts, severely confounding the interpretation of environment-dependent genetic estimates.

RESULTS

Plasticity of Life-History Characters in Response to Season of Germination and Dispersal

Effects of germination season on fitness and life history.—Germination season significantly altered total lifetime fruit production, but its effect depended on geographic location (site) and dispersal season (Tables 1, 2; Figs. 1, 2). In both KY and RI, seeds that germinated in autumn had the highest fitness, but the effect of germination season was stronger in KY (Fig. 1). Plants in RI had higher fitness than those in KY if they germinated in summer, but plants in KY had higher fitness than those in RI if they germinated in autumn. Winter

TABLE 2. Results of analysis of variance to test for effects of germination season (cohort) and season of dispersal on life history, using seeds dispersed in Rhode Island. Genotypic means were used based on individual phenotypes pooled across block and photoperiod. df (season) = 2, df (season) = 1, df (interaction) = 2; $N = 328$. F-ratios are given for the full model and for the analyses within each dispersal season based on Type III sums of squares. Effects of germination season were highly significant ($P < 0.001$) in both dispersal seasons when based on Kruskal-Wallis tests. Tests for effects of dispersal season within each germination cohort are based on Kruskal-Wallis χ^2. See Appendix 2 for the number of genotypes in each cohort and treatment.

Source	Bolting age	Flowering interval	Rosette diameter	No. basal branches	Total fruits
Full model					
Germination season	6164.67***	99.42***	174.09***	0.33	106.86***
Dispersal season	80.20***	78.35***	103.02***	10.56***	25.34***
Germination season × dispersal season	14.26***	92.30***	0.45	1.34	31.06***
Effect of germination season in each dispersal season					
Germination season in June	38.30***	19.80***	16.39***	0.04	19.95***
Germination season in November	182.25***	3.52	157.95***	0.98	97.44***
Effect of dispersal season in each germination season					
Winter	2.44	23.38***	10.45**	0.23	3.59
Spring	73.54***	1.71	72.79***	0.00	16.70***

** $P < 0.01$, *** $P < 0.001$.

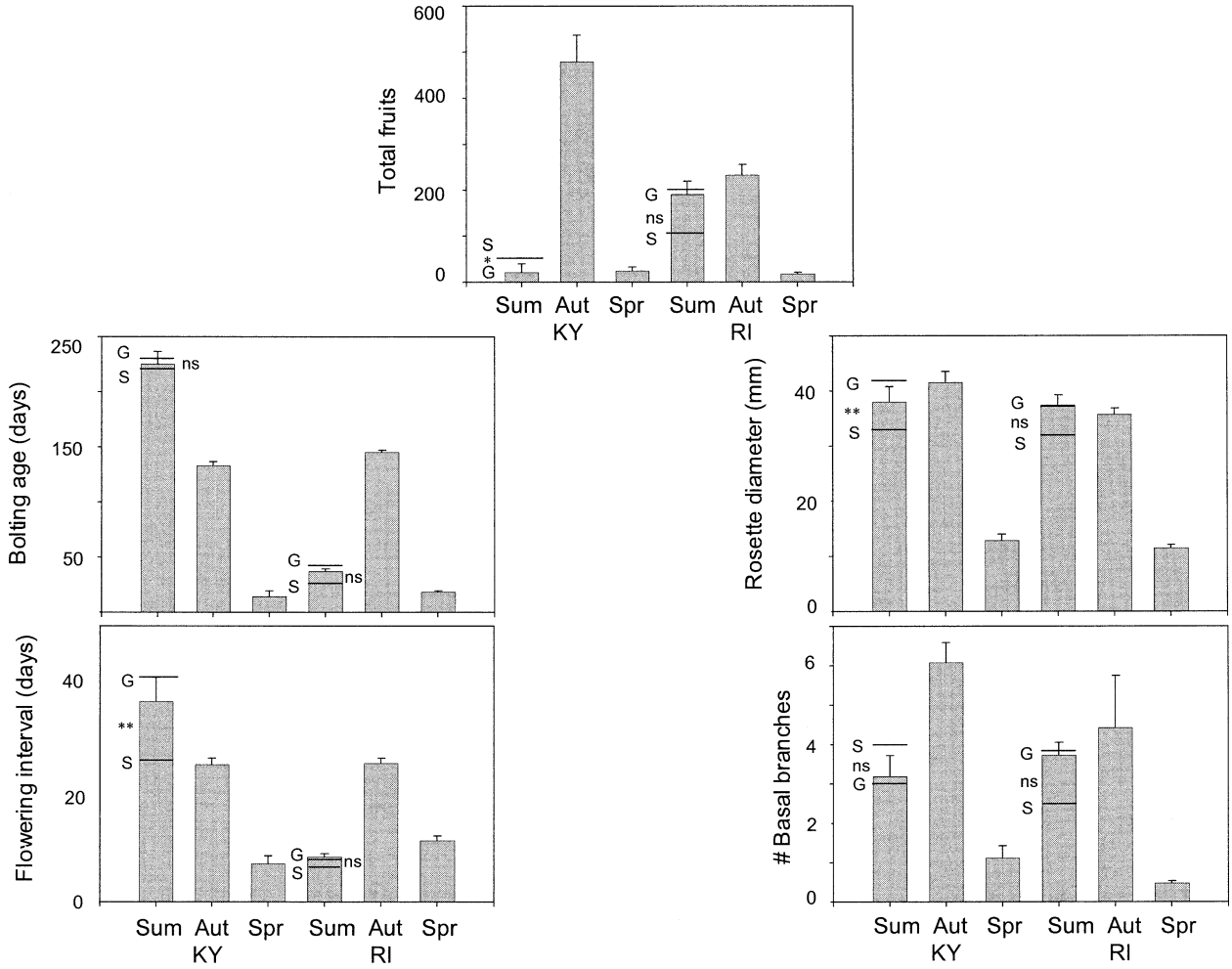

FIG. 1. Total lifetime fruit production and life-history traits of plants from seeds dispersed during June in Kentucky (KY) and Rhode Island (RI). Germination seasons are summer, autumn, and spring. S indicates the mean value of specialist genotypes, which germinated only in the summer; and G indicates the mean value of the summer germinants of generalist genotypes, which germinated in multiple seasons. Means and untransformed standard errors are indicated. Asterisks indicate significant differences between summer-specialist S genotypes and generalist G genotypes. The number of specialist and generalist genotypes (S, G) were as follows. KY: total fruits = 27, 32; bolting age = 21, 32; flowering interval = 11, 25; rosette diameter = 24, 32; basal branches = 4, 19. RI: total fruits = 6, 54; bolting age = 4, 40; flowering interval = 2, 35; rosette diameter = 3, 50; basal branches = 6, 53. ns, not significant; **$P < 0.01$.

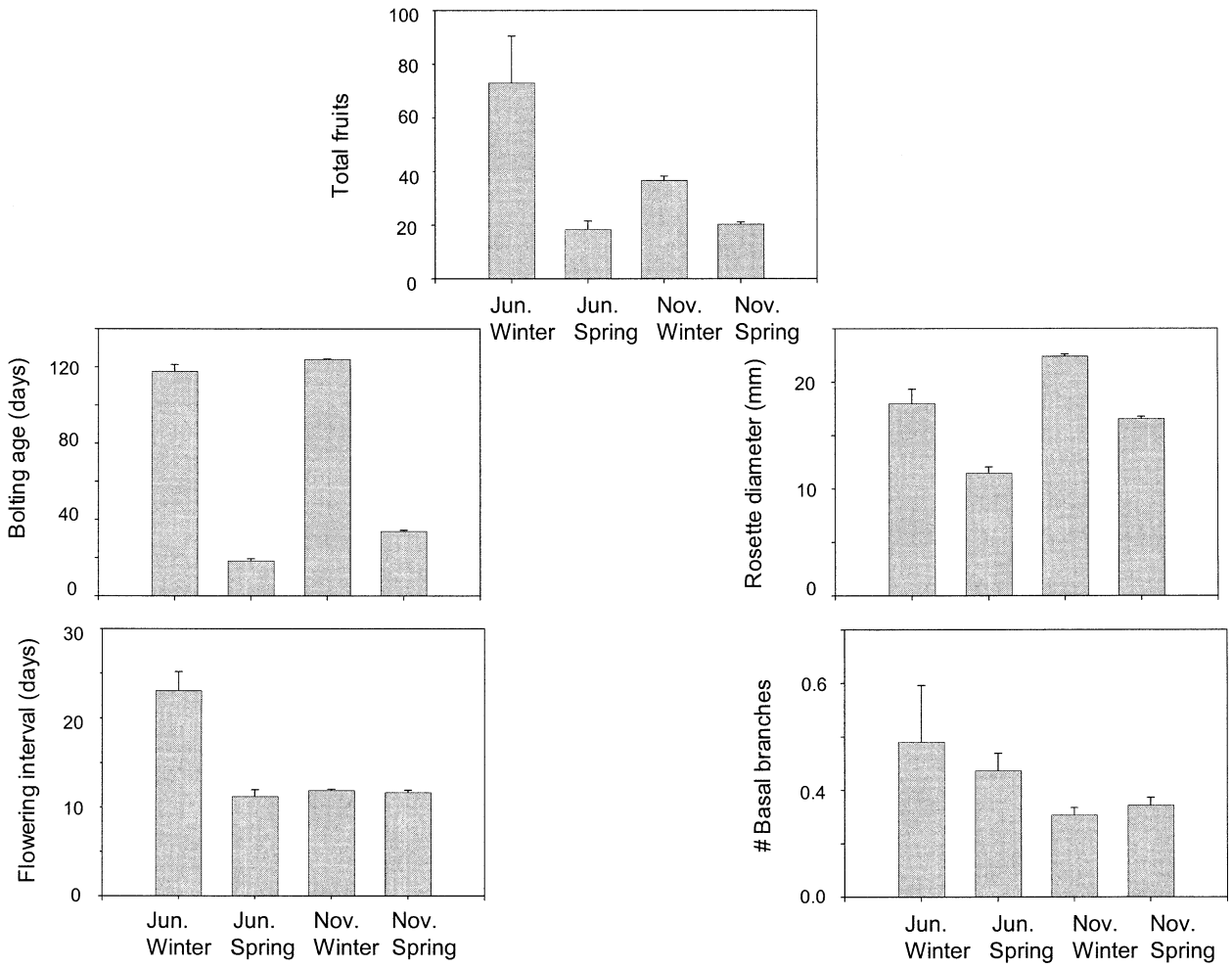

FIG. 2. Total lifetime fruit production and life-history traits of plants from seeds that were dispersed in Rhode Island during June and November. Germination seasons are winter and spring. Means and untransformed standard errors are indicated.

germinants had higher fitness than spring germinants in both seed dispersal seasons, with winter germinants dispersed in June having the highest fitness (Fig. 2).

Germination season also influenced life-history expression (Table 1, Fig. 1). In KY, spring germinants were fastest to reach reproductive maturity (shortest bolting age), but they were the smallest (small rosettes and few branches). Summer germinants were slowest to reach reproductive maturity, but their slow development did not result in their being the largest. Rather, autumn germinants developed at an intermediate rate and were the largest, most fit plants in KY. In RI, autumn germinants were the most slowly developing cohort, germinating and sometimes bolting in the autumn but flowering in the spring, while summer and spring germinants were comparable, since many of them reproduced in the same season in which they germinated. The slowly developing autumn germinants were larger than spring germinants (had larger rosettes and more branches). Summer germinants were also larger than spring germinants, most likely due to the longer growing season between germination and bolting.

Differences in plants grown in KY and RI depended on the season in which they germinated (Table 1, Fig. 1). For example, differences in bolting age between KY and RI were far more pronounced in summer germinants than in autumn or spring germinants. Therefore, the season of germination can influence the degree to which adult life-history characters vary geographically.

Effects of dispersal season on life history.—Dispersal and germination season interacted to influence life-history expression (Table 2, Fig. 2). For example, spring germinants from seeds dispersed in November bolted later ($F = 45.26$, df = 1, $P < 0.001$) and at a larger size ($F = 33.89$, df = 1, $P < 0.001$) than those dispersed in June, even when only the 67 genotypes that germinated in both dispersal cohorts were analyzed, and even when differences in germination timing within a season were controlled for. Winter germinants from seeds dispersed in November also bolted at a larger size than those from seeds dispersed in June (Table 2, Fig. 2), and this difference was significant even when only the 16 shared genotypes that germinated from both dispersal cohorts was analyzed, correcting for differences in germination timing within the season ($F = 8.92$, df = 1, $P < 0.01$). Thus dispersal season influenced life history through mechanisms other than influencing germination timing; the pregermination conditions experienced by seeds apparently influenced development and growth rates of germinants.

TABLE 3. Analysis of covariance to test for differences in selection across treatments. The left two columns are results of tests for differences in selection among germination cohorts within each site. The middle three columns show results of tests for differences in selection across site within each germination cohort. The last two columns are results of tests for differences in selection across dispersal season within each germination cohort. *F*-ratios are based on Type III sums of squares and indicate the *F*-ratio for the interaction between the trait (or its quadratic) and the treatment. *F*-ratios to test for differences in quadratic selection are given in parentheses. *N* is the number of genotypes in the analysis. NE, not estimable due to small sample size.

	Across germination cohort		Across site Kentucky vs. Rhode Island			Across dispersal season (June vs. November)	
Trait	Kentucky	Rhode Island	Summer	Autumn	Spring	Winter	Spring
Bolting age	5.38**	8.31***	7.22**	1.60	2.19	2.79†	0.63
(quadratic)	(390.31***)	(2.36†)	(243.94***)	(1.93)	(NE)	(1.07)	(1.52)
Flowering interval	0.01	2.60†	0.00	0.65	0.20	0.04	14.09***
(quadratic)	(5.78*)	(1.70)	(3.16†)	(0.13)	(NE)	(1.67)	(5.10*)
Rosette diameter	6.47**	1.64	8.79**	2.86†	0.00	15.15***	6.43*
(quadratic)	(303.74***)	(0.42)	(188.15***)	(6.27*)	(NE)	(6.16*)	(6.52*)
No. basal branches	0.20	5.15**	0.26	0.06	0.05	0.24	0.78
(quadratic)	(0.45)	(1.65)	(0.43)	(12.38***)	(NE)	(4.04*)	(0.04)
N	96	180	60	150	66	138	183

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

Natural Selection on Life-History Traits

Effect of germination season on natural selection.—The strength and mode of natural selection on life-history traits depended on the season of germination in both KY and RI (Table 3, Fig. 3; see Appendix 1 for selection coefficients). In KY the strength of selection varied across germination cohorts, whereas in RI the direction of selection also changed. In KY (Fig. 3, upper panels), most summer germinants did not produce fruits, but a very small number produced many fruits. This led to statistically significant, but likely not biologically significant, stabilizing selection that favored intermediate bolting and flowering rates, and disruptive selection that disfavored intermediate sized rosettes only in summer germinants. In RI (Fig. 3, lower panels), summer germinants were selected to bolt quickly, in contrast to spring germinants, which were selected to delay bolting. Selection to allocate resources to branch production was detected only in autumn germinants, in contrast to spring germinants, which were selected only to be larger at the time of reproduction rather than to increase in size after reproduction as well.

Specialist versus generalist germinants.—In KY, the specialist genotypes that germinated only in summer had higher fitness than generalist genotypes (those that germinated in multiple seasons) when they germinated in summer (Fig. 1). They also had smaller rosettes and shorter flowering intervals. Flowering interval was not under selection in summer germinants in KY, but when variation in rosette diameter and its quadratic were controlled for, the fitness difference between generalist and the specialist summer germinants was no longer significant ($F = 0.07$, df = 1, $P > 0.05$). Therefore, the smaller size at bolting of specialist summer germinants was closer to the putative optimum than that of generalist germinants in KY, and it fully accounted for the fitness advantage of the specialists. It should be noted, however, that the observed fitness difference between specialist and generalist genotypes in KY depends on a very small number of genotypes that produced any fruits at all. No significant differences in fitness or life history were detected between specialist and generalist germinants in RI, in part because of the small number of specialist genotypes (Fig. 1).

Effect of dispersal season on selection.—Natural selection on some life-history traits, and the effect of germination season on selection, depended on the season of seed dispersal (Table 3, Fig. 4; see Appendix 2 for selection coefficients). Seeds dispersed in June (Fig. 4, upper panels) were under stronger selection for reproduction at a larger size, and seeds dispersed in November (Fig. 4, lower panels) were under stronger selection for postreproductive vegetative growth through branch production. The only significant difference between selection on winter versus spring germinants was that disruptive selection on rosette size was significantly stronger in winter germinants than in spring germinants, but this was true only if seeds were dispersed in November ($F = 5.04$, df = 1, $P < 0.05$; other nonsignificant results are not shown).

Adaptive Significance of Plasticity of Life-History Traits

Plasticity to season of germination.—The observed plasticity of life history in response to season of germination was not adaptive in KY because the direction of natural selection on each trait was consistent across all germination cohorts (Table 3, Appendix 1). While some phenotypes were adaptive, such as many basal branches in autumn germinants, the plastic responses themselves (i.e., the phenotypes expressed in the other season) were not.

In seeds that were dispersed in RI during June, most of the observed plastic responses to germination season were not adaptive. For example, the adaptive response of bolting age would be to exhibit early bolting in summer germinants and late bolting in spring germinants (Appendix 1). Bolting was in fact accelerated in spring germinants, so the observed response was not adaptive. However, 66 of 106 genotypes exhibited appropriate bolting responses. Of those with appropriate bolting responses, six maintained large rosettes (which was adaptive; specifically, they were among the 20% of the genotypes with the largest rosettes) in summer and autumn, six maintained large rosettes in summer and spring, five maintained large rosettes in autumn and spring, but only two maintained large rosettes across all germination seasons. Five genotypes with appropriate bolting also produced many

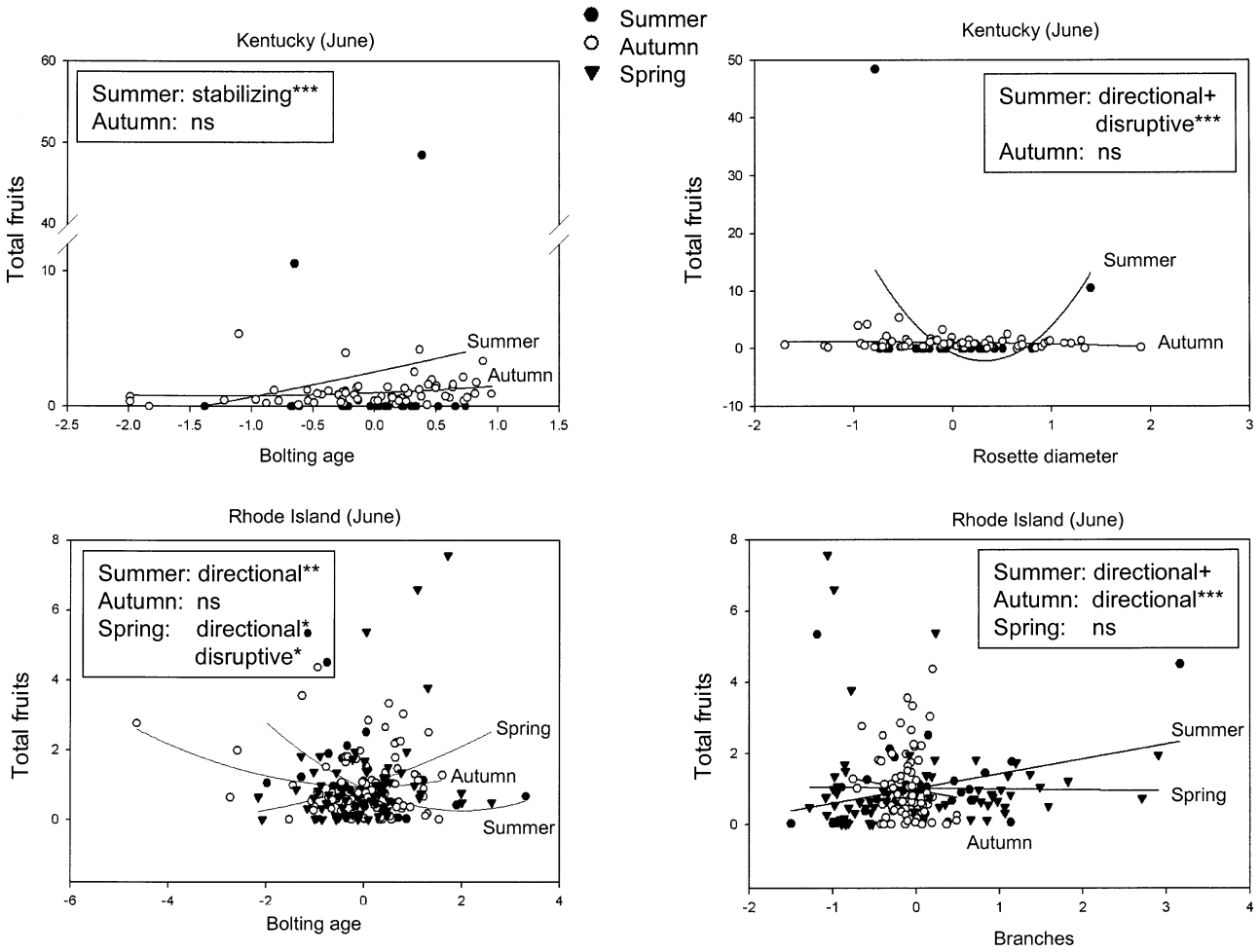

FIG. 3. Scatter plots of relative fitness versus residuals of life-history traits for plants grown in Kentucky (upper) and Rhode Island (lower) from seeds dispersed in June. Residuals were obtained from a regression of the life-history trait against all other life-history traits used in the selection analyses to indicate the strength of the association between the trait and fitness after controlling for correlations among traits. The relationship reflects the approximate strength of direct selection (β) on the trait, although effects of the quadratics of correlated traits are not factored out. The significant stabilizing and disruptive selection in Kentucky was due to the outliers. Too few spring germinants were available for analysis in Kentucky, so they are not shown. ns, not significant; $+P < 0.1$; $*P < 0.05$; $**P < 0.01$; $***P < 0.001$. (See Appendix 1 for selection gradients.)

branches (adaptive) if they germinated in either summer or autumn. Only three genotypes that had adaptive bolting responses also exhibited both favorable rosette diameter and branch production, and only one of those exhibited the favorable flowering interval as well. Only four genotypes were able to exhibit adaptive responses in multiple traits in both sites.

In seeds dispersed in RI during November, the direction of natural selection did not change with germination season for any trait (Table 3, Appendix 2), so the observed plasticity in these traits was not adaptive. The adaptive response would be to maintain large rosettes and many branches across all germination seasons. Six genotypes maintained large rosettes and six maintained high branch production across all germination seasons. None were able to do both.

Plasticity to dispersal season.—The direction of selection on all measured life-history traits did not differ significantly across dispersal season, so plasticity to season of dispersal would not be adaptive. The larger rosettes of plants from seeds dispersed in November were more adaptive than those of seeds dispersed in June, and maintaining large rosettes and many branches regardless of dispersal season would be the adaptive response. One genotype was able to maintain branch production in both dispersal seasons, but no genotypes maintained the largest rosettes in both dispersal seasons.

The Contribution of Germination Timing to Genetic Variation in Life-History Taits

Genetic variation was detected for many life-history traits when pooled across all germination cohorts (Table 4). In KY, significant genetic variation was detected for all traits except flowering interval. In seeds dispersed during June in RI, significant genetic variation was detected only for basal branch production. In seeds dispersed during November in RI, significant genetic variation was detected in all traits except bolting age.

When variation in life-history traits due to variation in

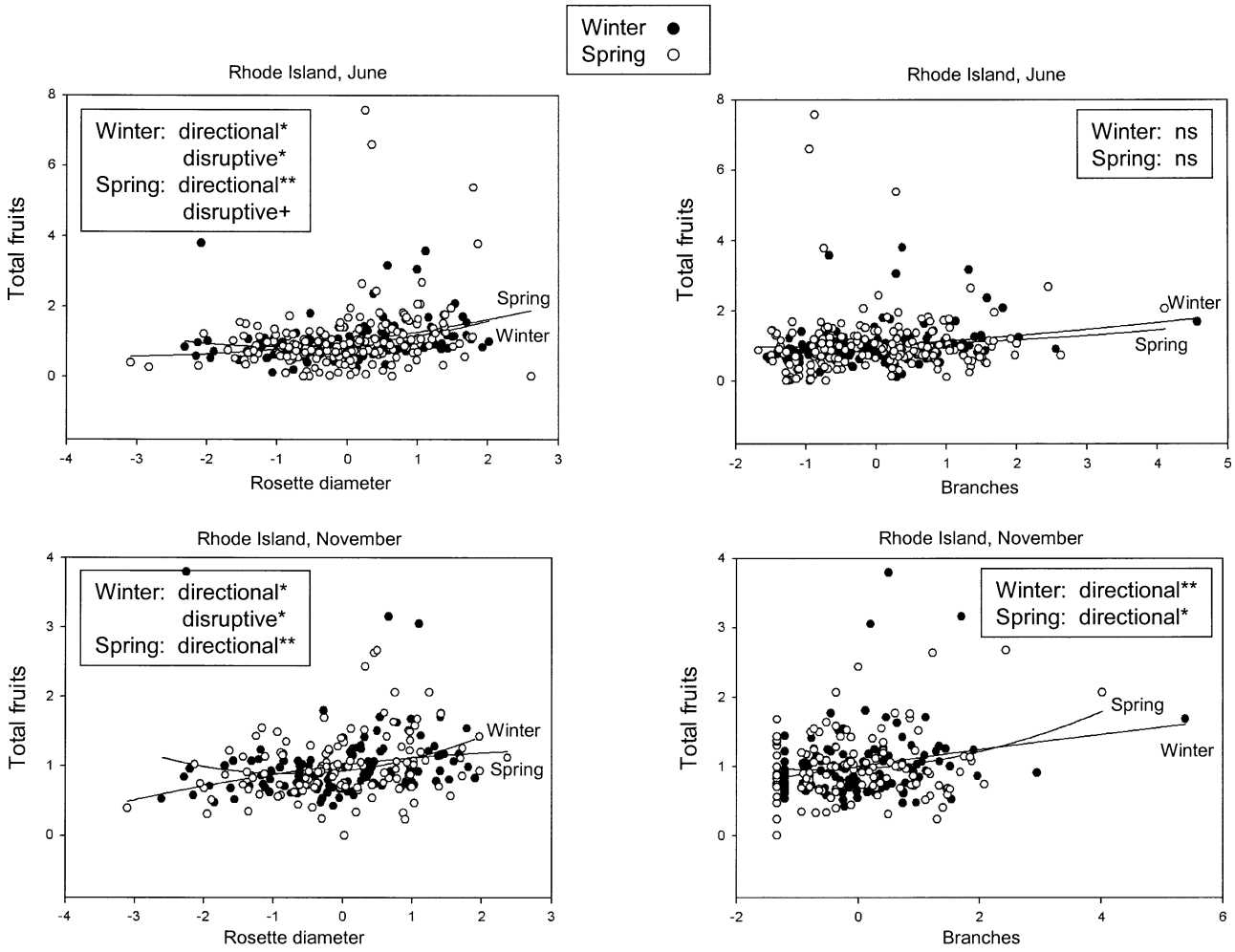

FIG. 4. Scatter plots of relative fitness versus residuals of life-history traits for plants from seeds dispersed in Rhode Island during June (upper) and November (lower). See legend of Figure 4 for explanation of residuals. ns, not significant; $+P < 0.1$; $*P < 0.05$; $**P < 0.01$; $***P < 0.001$. (See Appendix 2 for selection gradients.)

germination timing was factored out, genetic variances and heritability estimates changed (Table 4). In KY, heritabilities of bolting age and rosette diameter increased in magnitude, while the heritability of basal branch production decreased slightly. The increased heritability of bolting age based on residual values was due to both an increase in genetic variation and a decrease in environmentally induced variation. Plasticity of bolting age in response to germination timing caused genotypes to resemble each other more in bolting age, thereby decreasing genetic variation for bolting age. In addition, environmentally induced variation in germination timing magnified environmentally induced variation in bolting age. For rosette diameter, genetic variance did not change much when variation due to germination was factored out, but environmental variance declined. In contrast, for branch production, genetic variance declined slightly and environmental variance increased slightly, leading to lower heritabilities when based on residual values. In RI June-dispersed seeds, genetic variances for all characters decreased when variation due to germination timing was factored out. However, environmental variance was also reduced in most cases, so heritability estimates for all characters decreased only very slightly. For November-dispersed seeds, genetic variance of residuals was usually lower or remained very similar to that based on raw values, and environmental variances of residuals were also usually smaller or very similar.

The results indicate that for the majority of cases, in which genetic variance decreased when germination timing was controlled for, genetic variation in germination timing augmented genetic variation in life-history traits. In those cases in which heritability estimates increased when germination timing was controlled for, plasticity to germination timing apparently caused genotypes to resemble each other more closely, and environmentally induced variation in germination augmented environmentally induced variation in the life-history trait. Therefore, plasticity in response to genetically variable germination timing can either augment or diminish genetic variation and heritability of life-history traits.

DISCUSSION

Life-history traits responded plastically to season of germination in both geographic locations and in both seasons of dispersal. The season of germination also influenced the

TABLE 4. Genetic variation for life-history traits and the contribution of germination timing to genetic differences in life-history traits. Significance levels are based on analyses of variance that test for significant genotype effects using raw values or residuals after factoring out effects of germination timing. V_g, the genetic component of variance; V_e, environmental variance, calculated as the total phenotypic variance (V_p) minus the genetic variance; h^2, broad sense heritability, defined as V_g/V_p. Values were calculated separately for each site and season of seed dispersal.

	Raw values			Residuals		
Trait	V_g	V_e	h^2	V_g	V_e	h^2
Kentucky, June						
Bolting age	712.380	972.17	0.42***	744.22	266.83	0.74***
Flowering interval	−0.33	0.95	0.00	−0.34	0.96	0.00
Rosette diameter	0.20	0.15	0.56***	0.21	0.08	0.72***
Basal branches	0.69	0.19	0.78**	0.66	0.25	0.73
Rhode Island, June						
Bolting age	4073.35	43.33	0.99	1995.16	39.67	0.98***
Flowering interval	0.24	0.3	0.44	0.17	0.28	0.38
Rosette diameter	0.50	0.06	0.89	0.20	0.06	0.77
Basal branches	0.78	0.08	0.91*	0.61	0.09	0.87
Rhode Island, November						
Bolting age	1908.53	291.03	0.87	182.42	31.06	0.85
Flowering interval	0.29	0	1.00***	0.30	0	1.00**
Rosette diameter	0.21	0	1.00***	0.17	0.01	0.94***
Basal branches	0.16	0	1.00***	0.22	0	1.00**

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

strength and mode of natural selection on life-history characters. In no case was the plastic response of life-history traits to season of germination adaptive in these recombinant lines, despite adaptive phenotypes being expressed by some germination cohorts. However, a modest number of genotypes did express adaptive responses to germination season in some traits, although very few were able to express adaptive responses in more than one treatment. Moreover, genetic variation in germination timing sometimes altered genetic variation in life-history traits because these life-history traits exhibited plasticity to season of germination.

Plasticity of Life History in Response to Germination

The observed plasticity of life-history traits in response to the season of germination indicates that germination timing alters the environment experienced by postgermination life stages in a lasting manner. Therefore, not only does germination timing influence the survival of young seedlings (Biere 1991; Gross and Smith 1991; Simons and Johnston 2000; Donohue et al. 2005b), but it has persistent effects on adult life stages as well. The season of germination has been known to alter basic life-history expression in other systems, determining whether plants will be annuals as opposed to biennials (Kalisz 1989; Kalisz and Wardle 1994; Galloway 2001, 2002) or summer annuals as opposed to winter annuals (Ratcliffe 1976; Effmertova 1967; Evans and Ratcliffe 1972; Nordborg and Bergelson 1999). Such plasticity has the potential to determine basic demographic properties such as generation time, a fundamental parameter that can strongly influence population growth rates of weedy species like *A. thaliana*.

The season of seed dispersal also influenced the expression of life history, and it did so independently of germination timing. Plants from seeds that experienced summer conditions were more slowly developing, but they were able to compensate through postreproductive branch production.

The particular nature of the plastic response of life history to germination season depended on season of seed dispersal and geographic location. The difference in responses by plants growing in the two geographic locations was due primarily to whether summer germinants were able to reproduce in the autumn, as they often did in RI, or whether they remained nonreproductive until spring, as they did in KY. Similar environmental cues early in life therefore appear to predict different conditions later in life, depending on geographic location. Such geographic variation in the relationship between seasonal environments experienced early and late in life implies that responses of later life-history traits to postgermination environments would be subjected to geographically variable natural selection.

Life-history differences between plants grown in KY and RI depended on the season in which they germinated. This result indicates that ecotypic differences in adult life histories observed in situ can depend on ecotypic differences in germination timing. Differences in germination timing can thereby influence geographic patterns of life-history variation.

Natural Selection on Life History and Its Plasticity

The season of germination influenced natural selection on life-history traits, but its influence depended on geographic location and season of dispersal. In KY the season of germination influenced the strength of natural selection; in RI, even the direction of selection on life-history traits changed depending on the season of germination. The evolution of germination timing therefore is expected to impose changes in selection on postgermination life histories. The degree to which the evolution of germination timing can alter selection on life histories also is expected to depend on geographic location.

The geographic location that enabled germination season to alter natural selection most conspicuously, RI, is also the

geographic location that is more likely to exhibit a broad range of seasons of germination. While small amounts of spring germination have been observed in typically winter-annual populations of *A. thaliana* (Baskin and Baskin 1983), populations in New England have been observed to have high frequencies of spring germination, possibly because of their ability to set seeds in the autumn (Griffith et al. 2004). We predict therefore that adaptive plastic responses to germination season would evolve most easily in such populations.

We found no evidence that the lines used in this experiment exhibited adaptive plasticity of life-history characters in response to season of germination or season of dispersal. We expect to find nonadaptive or even maladaptive phenotypes in recombinants since coadapted gene complexes have been disrupted through recombination. The distinct lack of adaptive responses suggests that adaptive plasticity of life histories in response to germination and dispersal season could involve a coordinated phenotype controlled by separate, unlinked genes. Some genotypes did exhibit adaptive phenotypes in more than one germination cohort and a very small number of them exhibited adaptive phenotypes under several conditions. Therefore, the possibility exists for adaptive plasticity to evolve in response to germination timing, especially after the creation of transgressive segregants following hybridization between distinct lineages (Ellstrand and Schierenbeck 2000; Lexer et al. 2003; Rieseberg et al. 2003a,b). However, no genotype exhibited adaptive phenotypes in all conditions, so variation in germination timing in different geographic locations may contribute to the maintenance of genetic variation for plant life histories. Further studies of intact natural genotypes must test whether populations that harbor variation in the season of germination have actually evolved adaptive responses of adult life histories in response to germination and dispersal season.

The Influence of Germination Timing on Genetic Variation of Life Histories

Genetically based variation in germination timing augmented genetic variation for most adult characters. That is, germination timing is genetically variable in this sample (Donohue et al. 2005a), and plasticity in life histories in response to this genetically based variation supplies an additional source of genetically based differences in adult characters. This augmentation accords with theoretical work that emphasizes the role of indirect genetic effects in enabling responses to selection in characters that may not in themselves harbor genetic variation (Moore et al. 1997, 1998; Wolf et al. 1998, 1999). In contrast to these predictions, we also found that plastic responses to genetically based variation in germination timing sometimes obscured differences among genotypes in some adult life-history characters (in KY). That is, plasticity to postgermination seasonal environments caused distinct genotypes to behave similarly under certain seasonal conditions. The ability of phenotypic plasticity to mask genetic variation has been appreciated for decades (Via and Lande 1985), although this dynamic in indirect genetic effects and habitat selection has not been previously documented.

Germination timing also had a strong effect on environmentally induced variation. Because evolutionary responses to selection depend on the ratio of genetic to total phenotypic variance, or heritability, germination timing had only a very weak effect on the heritability of most life-history traits. The influence of environment-determining traits on environmentally induced variance needs to be considered in addition to their influence on genetic variance to evaluate their contribution to the evolutionary dynamics of other traits.

Conclusions: Consequences of Plasticity of Life History in Response to Germination Timing

Plasticity of life-history characters to season of germination is important for two reasons. First, it indicates that germination season influences the environment experienced by plants throughout their life. It can determine the opportunity for natural selection on life-history traits and influence the strength and mode of natural selection that adult life stages are exposed to. Therefore, germination timing acts as a form of habitat determination and influences the environment to which later life stages must adapt. Second, plasticity of life history to germination timing can influence the degree of genetic variation and the heritability that is expressed for adult life-history characters.

We found strong plasticity of adult life histories in response to germination season, and we found that germination season significantly altered the strength, direction, and mode of natural selection to which life histories are exposed. Moreover, genetic variation in germination timing both augmented and diminished expressed genetic variation for adult life-history traits. Germination responses can therefore be considered to be important habitat-determining traits in plants. As such, the evolution of germination can influence adult life-history evolution by determining the selective environment that postgermination traits are exposed to, influencing the degree of genetic variation and environmentally induced variation that is expressed for post-germination traits, and determining the adaptive value of the coordinated expression of traits in different life stages and that are likely to be controlled by different genes.

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APPENDIX 1.

Results of genotypic selection analysis of seeds dispersed during June in Kentucky (upper) and Rhode Island (lower). Selection gradients are given for each site and germination season. β indicates the strength of directional selection on the trait while controlling for correlations among characters. γ indicates the strength of quadratic selection on the character while controlling for correlations among characters. NE, not estimable due to small sample size.

	Summer		Autumn		Spring	
Trait	β	γ	β	γ	β	γ
Kentucky						
Bolting age	9.59 (5.89)	−41.37 (4.36)***	0.17 (0.14)	−0.06 (0.07)	NE	NE
Flowering interval	1.01 (3.89)	−2.15 (1.94)	0.20 (0.14)	0.11 (0.07)	NE	NE
Rosette diameter	−12.32 (6.94)†	43.35 (5.21)***	−0.06 (0.15)	−0.21 (0.11)†	NE	NE
No. basal branches	1.30 (2.25)	0.83 (1.60)	0.44 (0.15)**	0.30 (0.10)**	NE	NE
R^2	0.25	0.91	0.26	0.38		
N	23	23	68	68		
Rhode Island						
Bolting age	−0.54 (0.17)**	0.06 (0.12)	−0.04 (0.10)	0.07 (0.06)	0.38 (0.16)*	0.34 (0.13)*
Flowering interval	0.06 (0.15)	−0.04 (0.07)	0.07 (0.09)	0.08 (0.04)†	0.42 (0.16)*	0.16 (0.12)
Rosette diameter	0.63 (0.17)***	0.10 (0.10)	0.23 (0.10)*	0.07 (0.05)	0.38 (0.17)*	0.15 (0.08)†
No. basal branches	0.34 (0.18)†	0.17 (0.10)	0.40 (0.10)***	−0.04 (0.04)	−0.12 (0.15)	−0.03 (0.17)
R^2	0.51	0.59	0.40	0.48	0.37	0.51
N	37	37	82	82	61	61

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

APPENDIX 2.

Genotypic selection analysis of seeds dispersed in Rhode Island during June (upper) and November (lower). Selection gradients are given for each season of dispersal and germination season. β indicates the strength of directional selection on the trait while controlling for correlations among characters. γ indicates the strength of quadratic selection on the character while controlling for correlations among characters. *N* indicates the number of genotypes in the analysis.

	Winter		Spring	
Trait	β	γ	β	γ
June				
Bolting age	0.17 (0.21)	0.15 (0.19)	0.11 (0.20)	0.11 (0.12)
Flowering interval	−0.05 (0.24)	−0.22 (0.17)	0.49 (0.18)**	0.18 (0.12)
Rosette diameter	0.64 (0.21)*	0.54 (0.20)*	0.48 (0.17)**	0.18 (0.09)†
No. basal branches	0.21 (0.22)	0.17 (0.26)	−0.01 (0.16)	−0.02 (0.16)
R^2	0.53	0.87	0.35	0.46
N	16	16	61	61
November				
Bolting age	−0.06 (0.04)	−0.03 (0.03)	−0.01 (0.04)	−0.01 (0.03)
Flowering interval	−0.01 (0.04)	−0.01 (0.03)	−0.04 (0.04)	−0.04 (0.03)
Rosette diameter	0.09 (0.04)*	0.09 (0.03)*	0.13 (0.04)**	−0.01 (0.03)
No. basal branches	0.13 (0.04)**	−0.01 (0.02)	0.10 (0.04)*	0.04 (0.03)
R^2	0.12	0.18	0.12	0.17
N	122	122	122	122

† $P < 0.1$, * $P < 0.05$, ** $P < 0.01$.