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GERMINATION TIMING INFLUENCES NATURAL SELECTION ON LIFE-HISTORY CHARACTERS IN *ARABIDOPSIS THALIANA*

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Abstract. An experimental manipulation of germination timing was conducted to test whether germination timing influences the phenotypic expression of postgermination life-history characteristics and whether it alters natural selection on those characters. Seeds collected from five natural populations of *Arabidopsis thaliana* in Kentucky were forced to germinate in early November, early December, and early March. Life-history characters such as timing of reproduction, size at reproduction, and size at senescence were measured, and fruit production and mortality were monitored. Germination timing significantly altered subsequent life-history characters and reproduction. November germinants were larger than December germinants when they began reproducing, and they commenced reproduction sooner. All spring germinants died before reproducing. Germination timing also influenced natural selection on life-history characters. December germinants were more strongly selected to be large at the time of reproduction than November germinants, indicating stronger selection for a faster growth rate in December germinants. Stabilizing selection on timing of reproduction was detected in December germinants but not in November germinants. Therefore, variation in germination timing influenced fitness, modified the phenotypic expression of important life-history characters, and altered the strength and mode of natural selection on them.

Key words: Arabidopsis thaliana; *dormancy; germination; habitat selection; life-history characters; maternal characters; phenology; phenotypic plasticity; seasonal dormancy; variable selection.*

INTRODUCTION

Timing of germination is highly responsive to environmental conditions. Consequently, if environments change, due to habitat alteration or global warming for instance, germination behavior is likely to change as a direct and immediate response. For example, in species that typically germinate in the early autumn, protracted summer drought conditions could cause germination to be delayed until later in the autumn. Such a delay may not only have direct fitness consequences, but it may also affect other aspects of the plants' life history. Germination timing may also be altered by evolutionary responses to selection on germination. Characterizing how germination timing influences fitness, phenotypic expression, and natural selection on life-history characters therefore provides information on the manner in which plants might be affected by environmentally induced or evolutionary changes in their germination behavior.

Plants vary greatly in their life histories, including the size and timing of reproduction, the number of reproductive bouts, and the number of generations completed in a single growing season. Germination timing is closely associated with subsequent life-history characters in plants, such as annual vs. biennial strategies and summer vs. winter annual habits (Chouard 1960, Venable 1984, Kalisz 1986, Kalisz and Wardle 1994, Nordborg and Bergelson 1999). Variation in the timing of germination and flowering may even lead to variation in the number of generations per year. Thus determining the effects of variation in germination timing is useful for explaining the persistence of variation in other life-history characters.

Many plants, including many important weeds, typically display a ''winter annual'' life history, in which seeds germinate in the autumn, small plants overwinter as rosettes, and in the springtime they grow, flower, set seeds, and die as the summer approaches. Other plants display a ''summer annual'' life history, in which seeds germinate in the early spring, and they flower, set seeds, and die all during the same season. This basic life-history sequence varies among known *A. thaliana* ecotypes worldwide (Ratcliffe 1965, Effmertova 1967, Evans and Ratcliffe 1972, Nordborg and Bergelson 1999), as well as among natural populations in North America (L. Dorn and K. Donohue, *personal observations*). Populations in Kentucky have been characterized as winter annuals that germinate in autumn (Baskin and Baskin 1983), whereas New England (Rhode Island) populations have been seen to germinate either in autumn or in spring (L. Dorn and K. Donohue, *personal observation*). The mechanism of ''summer annual'' vs. ''winter annual'' life histories in *A. thaliana* is likely to be a function of both seed dormancy and vernalization requirements for flowering

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(Napp-Zinn 1976, Nordborg and Bergelson 1999). Therefore, investigating the relationship between the season of germination and subsequent life-history characters is relevant for understanding the basic ecology of summer and winter annual strategies.

Seasonal seed dormancy can delay germination until conditions are appropriate for growth and consequently may strongly influence fitness. In temperate climates, delaying germination until spring can prevent overwinter mortality. Autumn germination, however, may provide a selective advantage when the risk of winter mortality is low by enabling the plant to flower earlier in the spring or at a larger size. Consequently, selection for spring vs. autumn germination depends on the relative risks of winter mortality and the selective advantage of being developmentally advanced in the spring (Masuda and Washitani 1992). Germination timing within a season, moreover, may influence fitness by altering the size of overwintering and the size attained at the beginning of the spring growing season. Measuring the fitness consequences of the seasonal timing of germination can elucidate the selective mechanisms for the prevalence of these particular life-history strategies.

Germination timing not only can influence fitness directly, but it can influence the selective environment experienced by the plant after germination. If seeds germinate only under particular conditions, then the seeds effectively choose the selective environment that determines the evolution of postgermination traits. In this manner, genetically based associations can evolve between germination and postgermination characters (Evans and Cabin 1995). Germination timing therefore is likely to influence the evolution of life-history characters in plant populations by influencing the expression of life history variation and by determining the selective environment that acts on that life-history variation.

Using five populations of *Arabidopsis thaliana* collected from central Kentucky, I conducted an experimental manipulation of germination timing to assess its effects on life-history characters and fitness. Specifically, I asked the following questions. Does germination timing influence the phenotypic expression of life-history characters? Does germination timing influence mortality and reproduction? Does germination timing alter natural selection on postgermination characters?

Methods

Experimental design

Seeds were collected from five natural populations of *Arabidopsis thaliana* around Lexington, Kentucky, USA. Two populations, ''Hort'' and ''Ag,'' were collected from agricultural field edges at the University of Kentucky's Horticultural Research Station and Agricultural Research Farm, respectively. One population, ''TC,'' was collected from a fallow field which had not been cultivated for approximately two years. One population, ''RR,'' was collected from an older fallow field with substantial secondary succession and a vegetation canopy of herbaceous annuals and perennials. The last population, ''Garden,'' was collected from plants growing between bricks in a garden. Seeds were collected in May 1999 and were stored dry at room temperature until they were planted.

Seeds were planted into 96-well plug trays filled with Strong-Lite bedding plant mix (Strong-Lite Horticultural Products, Seneca, Illinois, USA). All seeds were forced to germinate synchronously by stratifying seeds on soil for three days at 5°C. After cold stratification, seeds were placed in a growth chamber at 25°C with full-spectrum light on a 12-h photoperiod. After germination, seeds were transferred to an unregulated greenhouse for three days for acclimation and then transferred to a field plot on the University of Kentucky's Lexington campus. Seeds from the five populations were randomly distributed within three plug trays (blocks) per treatment, with 18 replicates per population per block in each treatment. This gave a total of 810 seedlings. All plug trays were kept at a moisture level comparable to that of the surrounding vegetation throughout the season through occasional supplemental watering since the plugs tended to dry out slightly more quickly than the surrounding vegetation.

As a phenotypic manipulation of germination timing, three germination treatments were imposed: early autumn, late autumn, and spring. ''Early autumn'' germinants were forced to germinate (placed at 5°C) in early November and were transferred into the field on 14 November. Natural germination occurs primarily in mid-October in Kentucky (Baskin and Baskin 1983), so the early autumn germination treatment was approximately two and a half weeks behind the peak germination time of the natural populations in the field. However, germination in the field continues at least through the third week of November (K. Donohue, *personal observation*), so these experimental seedlings germinated well within the natural range of germination timing. This treatment reflects the most natural germination timing in this experiment. ''Late autumn'' germinants were forced to germinate during late November and were transferred into the field on 4 December. This treatment delayed germination beyond the germination timing naturally observed in the field and thereby functioned to extend the range of variation to include phenotypes that may have already been selectively eliminated from the population (Wade and Kalisz 1990). This treatment simultaneously manipulated both the timing of germination and the size of overwintering, since later germinants are necessarily smaller at the onset of winter conditions. Experimentally extending the range of variation in these two traits permitted more powerful tests of whether germination timing and size of overwintering influences mortality over the win-

ter or growth and reproduction in the spring. Spring germinating seeds were cold treated in late February and were transferred into the field on 8 March. This treatment represents the germination timing observed by individuals in some New England populations with summer annual strategies.

The diameter of each seedling was recorded on the day that it was transferred to the field. The number of leaves was counted and the rosette diameter was measured on the early autumn germinants approximately three weeks later, when the late autumn germinants were placed into the field. The number of rosette leaves was recorded for all autumn germinants five weeks later as an estimate of overwintering size. The number of rosette leaves and rosette diameter were recorded on all plants when the spring germinants were transferred into the field. The following characters were measured on all plants that expressed them: bolting date (the date on which the apical meristem begins to develop into the inflorescence, which signifies a switch from vegetative to reproductive allocation of the meristem), number of rosette leaves at bolting, rosette diameter at bolting, flowering date, and date of death. The following characters were determined at senescence: number of basal branches (branches originating from the rosette), inflorescence branches (branches on the main inflorescence stem), secondary and higher level branches, and total number of fruits, as an estimate of total lifetime fitness.

The spring germinants did not bolt by the time that most of the plants had died. One block of the spring germinants was kept alive by watering in order to determine the phenotype they would express if the dry summer conditions were postponed. These plants were monitored until they died, and the same characters as mentioned above were recorded.

Statistical analysis

The SAS (SAS Institute 1990) statistical package was used for all analyses. Characters were transformed to normality when necessary. A multivariate analysis of covariance was used to determine whether the measured characters differed among populations and germination treatments. Separate analyses of covariance then determined which of the traits were significantly influenced by these factors. Block was nested within treatment, giving a split-plot design. Population and germination treatment were considered fixed effects, and block was considered a random effect. Main effects of germination treatment were tested over the block and error mean squares, and main effects of population were tested over block–population interactions and error mean squares. Despite efforts to transfer seedlings into the field at the same size at the different planting times, there was significant variation in initial size among the planting treatments. Therefore, initial seedling size was used as a covariate in these analyses. A population main effect indicates that the populations differed due to either genetic differences between populations or to maternal effects of field-collected seeds. A population by treatment interaction indicates that populations differ in their response to germination treatment. All results reported are based on Type III sums of squares. Analysis of fruit number included all plants, including those with no fruits. The spring germinants did not naturally express any of the phenotypes because they never bolted. Therefore, only the early and late autumn germinants were compared in the analyses presented in the tables.

A Kaplan-Meier survival analysis using Proc Lifetest (SAS 1990) was used to compare mortality schedules across germination treatments. The data were uncensored.

A phenotypic selection analysis (Lande and Arnold 1983) was performed to determine the magnitude of natural selection on the measured characters. Rosette diameter, bolting date, the time interval between flowering and bolting, and total number of branches were used in the analysis. Two characters were excluded from the selection analysis a priori: The number of rosette leaves was not used because, like rosette diameter, it is a measure of size at the time of reproduction. Flowering time also was not used because of colinearity with bolting date and flowering interval. Characters were standardized to have a mean of zero and standard deviation of one within each treatment. Relative fitness was calculated within each treatment by dividing the total number of fruits produced by an individual by the mean number of fruits of all individuals within a given treatment. If the individual expressed the phenotypes but did not set fruit, it had a fitness of zero. Population was included as a fixed effect in the analysis in order to control for differences among populations in unmeasured characters that influence fitness (Donohue et al. 2000). Direct selection was estimated by analysis of covariance that included all traits, with relative fitness as the dependent variable. Total selection (direct selection plus indirect selection through correlations with other characters) was estimated in a model that included only one trait at a time. In additional analyses, selection coefficients were compared between early and late autumn germination treatments using analysis of covariance in which germination treatment was included as a fixed factor; significant differences between treatments in selection coefficients were apparent as significant interactions between the character and germination treatment (Donohue et al. 2000). These estimates of the strength of direct and total selection are not completely comparable to those of Lande and Arnold (1983). They differ from them due to the inclusion of population as a main effect in the analyses and the use of regression coefficients from analysis of covariance, rather than covariance, for an estimate of total selection. This method, however, has the advantage of controlling for effects of unmeasured characters that differ between populations that may si-

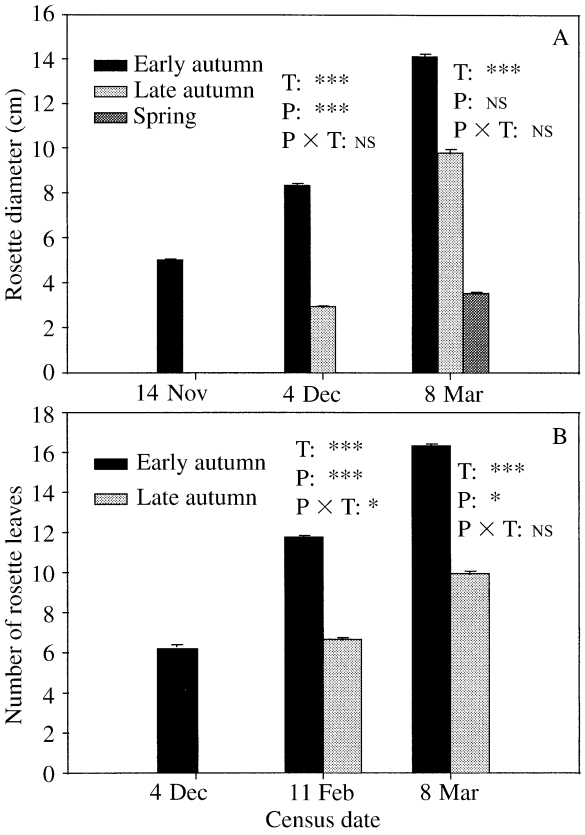

FIG. 1. Size throughout the winter and early spring. Rosette diameter at the time at which the seedlings were placed into the field is shown in the upper figure. The number of rosette leaves throughout the winter and spring for each of the treatments is shown in the lower figure. For all treatments, the seedlings had no rosette leaves when they were placed into the field; hence the bar is not visible for those treatments with no rosette leaves. T refers to a main effect of germination treatment; P refers to a main effect of population; P × T refers to a population-by-treatment interaction.

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

multaneously influence both phenotype and fitness, and inclusion of fixed treatment factors in the additional analyses enables direct tests for significant differences in selection in different treatments. Selection analysis was performed separately on the spring germinants that were watered because the unwatered spring germinants did not express the phenotypes.

Pearson correlations were calculated for all traits within each germination treatment. The spring germinants that were analyzed were those that had been kept alive through supplemental watering.

RESULTS

Starting conditions and characterization of the treatments

Early autumn germinants remained larger than late autumn germinants throughout the winter (Fig. 1), although the difference between treatments decreased over time, as indicated by significant interactions between germination treatment and census (rosette diameter, $F = 582.41$, df = 2, 512, $P < 0.001$; number of rosette leaves, $F = 7.78$, df = 2, 512, $P < 0.01$). Both early and late autumn germinants were substantially larger than the spring germinants when spring germinants were put into the field. Populations differed in the number of rosette leaves throughout the winter. Populations also differed in the degree to which the number of rosette leaves was reduced in the late autumn germination treatment, as indicated by a significant population by germination treatment interaction. Populations ceased to respond differently to the autumn germination treatments by the time the spring germinants were put into the field, however. Growth trajectories throughout the winter and spring also differed among populations, as indicated by the significant interaction between population and census in a repeated measures analysis of leaf number. Such differences were expressed only in early autumn germinants ($F = 3.33$, df = 8, 504, $P = 0.001$; based on Wilk's λ) but not in late autumn germinants ($F = 1.10$, df = 8, 504, $P > 0.05$; based on Wilk's λ). Thus, experimentally determined timing of germination resulted in persistent size differences among the treatments throughout the winter and into spring, but early in the experiment the magnitude of the difference in size depended on the population the seedling was from.

Treatment effects on postgermination life-history characters and fitness

Experimentally determined germination timing significantly altered the expression of postgermination life-history characters (Table 1, Fig. 2). MANOVA detected significant effects of autumn germination treatment ($F = 73.27$, df = 1, 500, $P < 0.001$) and population ($F = 21.33$, df = 4, 500, $P < 0.001$) on life-history characters. Early autumn germinants had more rosette leaves than late autumn germinants at the time of reproduction, and the switch from vegetative to reproductive growth (bolting time) was earlier in early germinants. Floral development time, or the interval between bolting and flowering, was not significantly influenced by germination timing, so early autumn germinants also flowered significantly earlier than late autumn germinants. Within the treatments, the initial size at which the seedling was put into the field influenced the size attained at reproduction and the timing of reproduction in the spring.

Fitness was influenced by the timing of germination. No early autumn germinants exhibited any sign of frost damage. Some frost damage was apparent in the late autumn germinants; meristems appeared to be damaged in 1.9% of the plants. Overwinter mortality was low in both germination classes. Early autumn germinants had <1.0% overwinter mortality, and 2.7% of the late autumn germinants died during the

TABLE 1. Results of analysis of covariance to test for effects of germination timing and population on morphological and phenological characters. Only early and late autumn germination treatments are included.

Trait	Initial size	Block	Germination time	Population	Germination time × Population
No. rosette leaves	61.42***	3.27*	12.57**	12.02***	0.63
Rosette diameter	0.71	1.23	2.86	1.70	0.25
No. branches	0.25	5.33**	0.01	5.07**	1.23
Bolting date	28.42***	13.71**	99.38***	125.73***	0.92
Flowering interval	7.76**	17.44***	1.97	8.22***	6.80**
Flowering date	19.71***	26.77***	68.69***	194.84***	0.95
No. fruits	2.37	1.71	3.47†	0.51	0.82

Notes: The *F* ratios given are based on Type III sums of squares. Numerator df = 1 for initial size and germination time, df = 2 for block, df = 4 for population and germination time × population. Denominator df = 503 for all factors except germination time. Denominator df for germination time ranged from 5 to 25 due to missing values.

† $P = 0.08$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

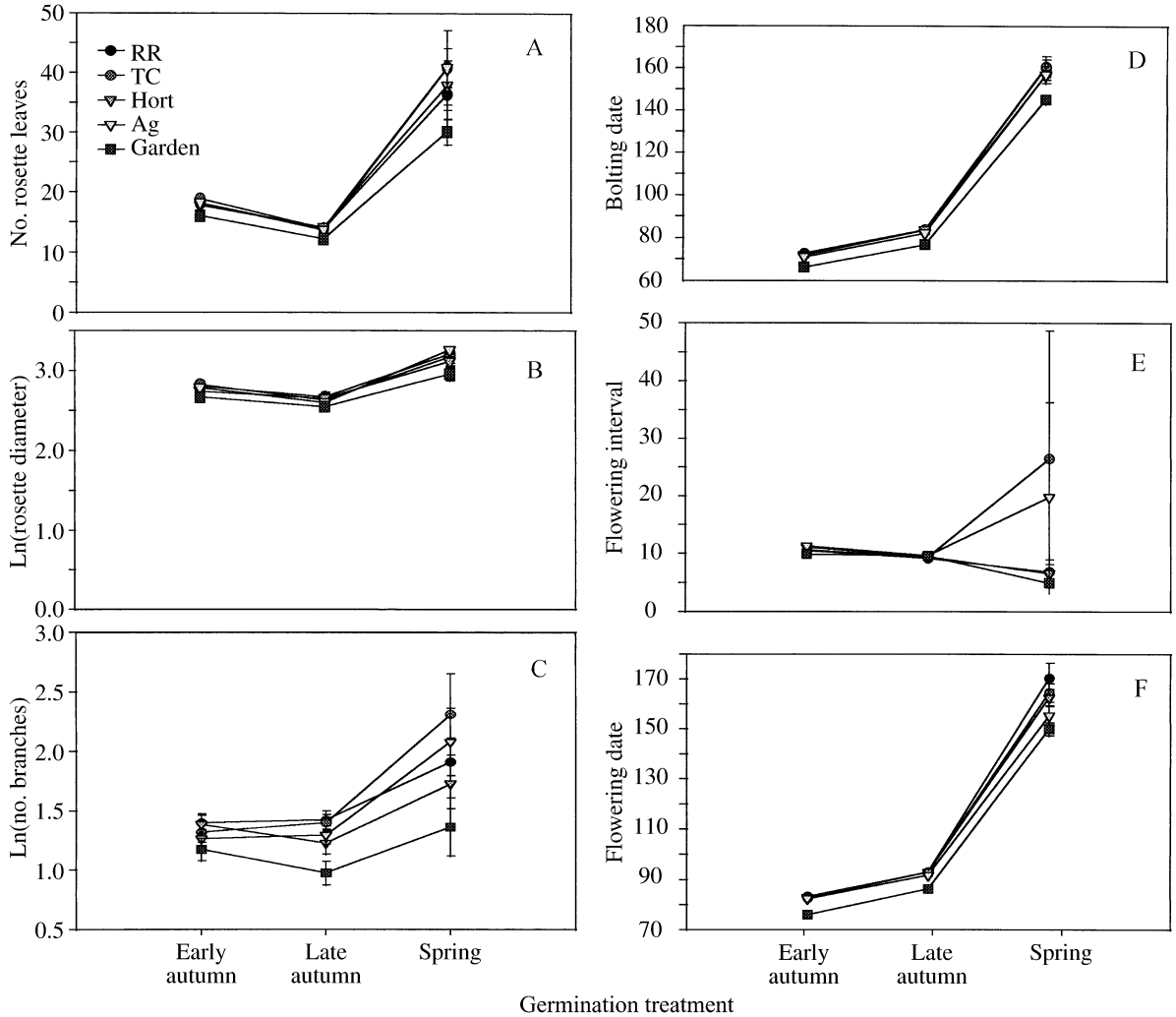

FIG. 2. Means (± 1 SE) of life-history traits of early autumn germinants, late autumn germinants, and spring germinants that had been kept alive through supplemental watering. Each line connects the mean value of a population in the three treatments. Populations are described in *Methods*: *Experimental design*. Characters related to the size at reproduction are shown in panels A–C. Characters related to the timing of reproduction are shown in panels D–F. Rosette diameter was measured in cm; bolting date and flowering date are expressed as day of year.

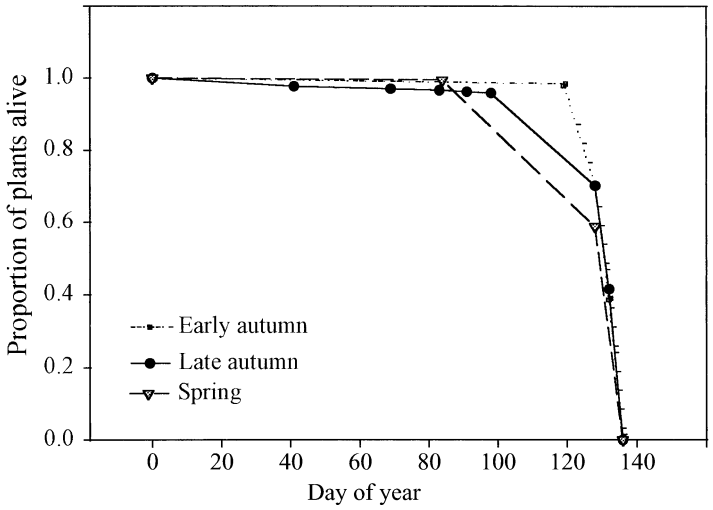

FIG. 3. Mortality schedules of plants that germinated in early autumn, late autumn, and spring. Only one plant died before 1 January (*not shown*).

winter. Mortality schedules did not differ significantly among autumn germination treatments (Fig. 3), and most mortality was episodic and corresponded with stressful drought conditions in the summer ($P \geq 0.05$ in Kaplan-Meier survival analysis comparing early and late autumn germinants). Ninety-six percent of both early and late autumn germinants survived to bolt. Early autumn germinants produced a mean of 12 more fruits (~240 more seeds) than late autumn germinants, but this difference was only nearly significant (Table 1, Fig. 4).

The spring germinants died slightly earlier than the autumn germinants (Fig. 3), as indicated by a significant effect of germination timing in a Kaplan-Meier survival analysis ($P = 0.01$ in an analysis that included all three treatments, excepting the spring germinants that had supplemental water). Every spring germinant died before switching from vegetative growth to reproduction, except for those that were kept alive by supplemental watering. The development of the watered plants, moreover, was noticeably altered (Fig. 2). Eventually, 56% of these plants bolted, but some lived for many weeks without bolting. Bolting time was significantly later than early and late autumn germinants ($P = 0.0001$ for both comparisons). Plants that did bolt had many more rosette leaves and had larger diameters by the time they bolted than did early and late autumn germinants ($P = 0.0001$ for both comparisons). They also had significantly more branches ($P = 0.0001$ for both comparisons). Fruit production of watered spring germinants was much less than that of either of the autumn germinants (Fig. 4; $P = 0.0001$ for both comparisons).

Populations differed significantly in all life-history characters except rosette diameter (Table 1, Fig. 2). In particular, plants from the population collected from the bricks in a garden bolted and flowered significantly earlier and at a smaller size than did plants from the

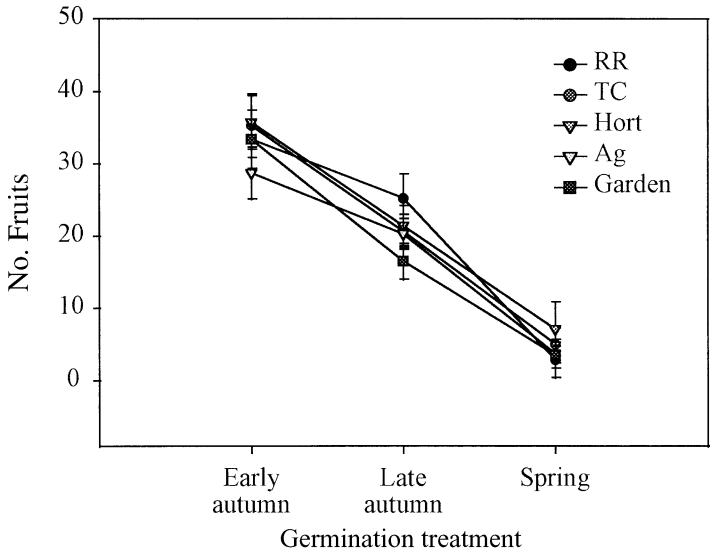

FIG. 4. Means and standard errors of fruit production by early autumn germinants, late autumn germinants, and spring germinants that had been kept alive through supplemental watering. All unwatered spring germinants had no fruit production. Each line connects the mean value of a population in the three treatments.

TABLE 2. Results of phenotypic selection analysis.

Character	Early autumn		Late autumn		Germination timing × total selection (F_S)‡	Germination timing × direct selection (F_β)§
	Total selection (S)	Direct selection (β)	Total selection (S)	Direct selection (β)		
Rosette diameter	0.010***	0.007***	0.023***	0.018***	32.22***	22.19***
No. branches	0.012***	0.010***	0.023***	0.013***	21.08***	1.77
Bolting date	0.004†	0.001	0.000	0.001	0.02	0.29
Flowering interval	0.003†	0.004**	0.004	−0.001	0.17	2.90+

Notes: Selection differentials measuring the magnitude of total selection (S) and selection gradients measuring the magnitude of direct selection (β) are given. F ratios are given for the tests for significant differences between germination timing treatments in the magnitude of total selection (F_S) and direct selection (F_β). Significant nonlinear selection coefficients for univariate (g) and multivariate (γ) selection analyses are as follows. Number of branches for early germinants ($g = 0.007$, $P < 0.001$; $\gamma = 0.005$, $P < 0.001$), number of branches for late germinants ($g = 0.008$, $P < 0.001$; $\gamma = 0.005$, $P < 0.001$), bolting time for late germinants ($g = -0.006$, $P < 0.001$). $N = 258$ for early autumn germinants. $N = 260$ for late autumn germinants.

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

‡ df = 1, 511; § df = 1, 502.

other populations. Populations did not differ in total fruit production. Populations responded differently to the autumn germination treatments. Plants from the garden population did not increase the interval between bolting and flowering as much as the other populations in the early autumn treatment. In the watered spring germinants, significant differences between populations were detected for rosette diameter ($F = 3.51$, df = 4, 40, $P < 0.05$), bolting time ($F = 3.85$, df = 4, 40, $P < 0.01$), and flowering time ($F = 4.37$, df = 4, 40, $P = 0.005$).

Natural selection on postgermination characters

The strength of natural selection on life-history characters was significantly influenced by the timing of germination (Table 2). Plants that germinated later in the autumn were more strongly selected to be larger (have a larger rosette diameter) upon switching from vegetative growth to reproduction. There was also stronger total selection (S) for larger overall size in these plants, as indicated by the significantly stronger total selection for more branches and larger rosettes. The stronger total selection for more branches in late autumn germinants could be due in part to the somewhat stronger positive correlation between rosette diameter and branch production in these plants (Table 3), since rosette diameter was also under strong positive direct selection. Significant disruptive selection was detected for branch production in both early and late autumn germinants, but the phenotype with the minimum fitness was not within the range of phenotypic variants in this study.

Significant stabilizing selection was detected for bolting time in late autumn germinants, and the optimal phenotype was within the range of phenotypic variants (Table 2). Therefore an intermediate bolting time was optimal in this sample. Early autumn germinants exhibited significant direct selection (β) and nearly significant total selection (S) for a shorter time interval between bolting and flowering. However, the difference in the strength of selection on this character between early and late autumn germinants was only nearly significant.

Some relationships among life-history characters differed between early and late autumn germinants (Table 3). Bolting date was significantly positively correlated with the size at which reproduction was initiated (number of rosette leaves and rosette diameter) in early autumn germinants but not in late autumn germinants, suggesting that late autumn germinants that delayed bolting did not necessarily attain a large size. In contrast, the number of leaves at bolting significantly in-

TABLE 3. Pearson correlations among characters for early autumn germinants (below diagonal) and late autumn germinants (above diagonal).

Character	No. leaves	Diameter	No. branches	Bolting date	Interval	Flowering date	No. fruit
No. leaves	1.00	**0.43*****	**0.34*****	−0.01	0.12	0.05	**0.31*****
Diameter	**0.20****	1.00	**0.49*****	−0.04	0.08	−0.01	**0.60*****
No. branches	−0.07	**0.31*****	1.00	0.09	**0.19****	**0.19****	**0.59*****
Bolting date	**0.25*****	**0.30*****	**0.19****	1.00	−**0.33*****	**0.95*****	0.05
Flowering interval	**0.21*****	−0.04	0.02	−**0.20*****	1.00	−0.01	0.09
Flowering date	**0.35*****	**0.26*****	**0.19****	**0.85*****	**0.34*****	1.00	0.05
No. fruit	0.05	**0.45*****	**0.56*****	0.08	0.11	0.14*	1.00

Notes: Boldface indicates significance after Bonferroni corrections for multiple comparisons. $N = 258$ for early autumn germinants. $N = 260$ for late autumn germinants.

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

TABLE 4. Results of phenotypic selection analysis of spring germinants that had been watered to prolong their growing season.

Character	Selection differential (S)	Selection gradient (β)	Nonlinear selection coefficients	
			Univariate (g)	Multivariate (γ)
Rosette diameter	−0.06	0.01	0.02	0.02
No. branches	0.23***	0.30***	0.09**	0.09*
Bolting date	−0.05	−0.04	−0.06	0.05
Flowering interval	−0.07	−0.19	−0.07	−0.40

Notes: Selection differentials (S) and selection gradients (β) are given. Nonlinear selection coefficients are given for univariate (g) and multivariate (γ) selection analyses; $N = 50$.
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

fluenced the total number of reproductive branches and fitness in late autumn germinants but not in early autumn germinants. It therefore appears that the variation in the size attained before reproduction by the late autumn germinants more strongly influenced their overall size and reproductive output, whereas reproduction at a consistently more advanced stage by early autumn germinants mitigated the association between size at reproduction and size at senescence.

A different pattern of selection was found for spring germinants that were kept alive beyond the natural growing season (Table 4). In these plants, only the total number of reproductive branches was associated with fitness. Significant disruptive selection was detected for branch production, but the phenotype with minimum fitness was outside of the range of phenotypic variants in this study. Size attained at reproduction and the timing of reproduction had no significant influence on fitness in this sample that did not experience summer drought conditions. Correlations among characters were quite weak in general (Table 5), indicating atypical variation in the characters and reflecting the apparently disrupted developmental pattern. These results indicate that natural selection on the measured characters was relaxed when the growing season was artificially prolonged or that life-history characters that were selectively important under more typical conditions contributed little to fitness variation when normal development was disrupted.

DISCUSSION

Life-history characters and fitness were shown to depend on the timing of germination. A difference in germination timing of three weeks in the autumn significantly altered basic life-history characters such as the timing of reproduction and size at reproduction. Germination timing also clearly had strong fitness consequences, with the early autumn germinants having the highest fitness. Even though late autumn germinants were significantly smaller than early autumn germinants throughout the winter, overwinter mortality differed very slightly between early and late autumn germinants. The size that seedlings attained for overwintering therefore did not determine the degree of overwinter mortality. Instead, the marginally nonsignificant difference in fitness between early and late autumn germinants was due to the difference in size attained before reproduction, which then influenced total fruit production.

Much of the influence of germination timing on life-history expression appears to be mediated by size. Although the differences in size between germination treatments decreased over time, size differences between treatments persisted throughout the life history of the plants in this experiment. Later germinants appear to have grown slightly faster than early germinants, reducing size differences in the spring, but this difference in growth rate was not sufficient to eliminate

TABLE 5. Pearson correlations among characters for spring germinants that had been watered to prolong their growing season.

Character	No. leaves	Diameter	No. branches	Bolting date	Flowering interval	Flowering date	No. fruits
No. leaves	1.00	0.27	0.23	0.05	0.06	−0.02	0.09
Diameter		1.00	−0.07	0.16	0.27	0.15	−0.18
No. branches			1.00	0.11	0.01	0.21	**0.71*****
Bolting				1.00	0.31*	0.97*	−0.10
Interval					1.00	0.21	−0.21
Flowering						1.00	−0.05
No. fruits							1.00

Notes: Boldface indicates significance after Bonferroni corrections for multiple comparisons; $N = 50$.
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

size differences due to variation in germination timing in the autumn.

Natural selection on life-history characters also depended on the timing of germination. Selection on the size attained before switching to reproduction was stronger in plants germinating in the late autumn than in those germinating earlier in the autumn, suggesting stronger selection for increased growth rate in later germinants. In addition, stabilizing selection on bolting time was detected in late autumn germinants but not in early autumn germinants. Bolting too early at a small size apparently caused a decrease in size-dependent reproduction, whereas bolting too late caused mortality before fruit maturation was completed. These patterns of selection and the failure of spring germinants to reproduce at all indicate that the advantage of a winter annual strategy and autumn germination in the temperate climate of Kentucky is due to the developmental head start that enables plants to reproduce both earlier and at a larger size.

The total failure of spring germinants to reproduce, and the altered development of those with an artificially protracted growing season, suggests that spring germination by these Kentucky populations is not likely to be a successful life-history strategy. However, these seeds experienced somewhat anomalous conditions from the beginning; all seeds were stratified (treated with cold as a seed) for only three days before being put into the field. Natural spring germinants in Kentucky would have been stratified much longer before germination. The duration of stratification and vernalization (cold treatment as a plant) has been shown to influence the probability and timing of reproduction in *Arabidopsis thaliana* (Nordborg and Bergelson 1999). Therefore, the coordination between stratification and vernalization was disrupted in these artificial spring germinants, and neither the seed nor the rosette received an adequate duration of cold for normal development. Consequently, the extremely poor performance of the spring germinants was probably due to a combination of an inappropriate delay in germination which caused a fatal delay in reproduction, and a disrupted developmental program caused by the unusual experimental conditions. This experiment therefore demonstrated a distinct constraint on plasticity in the development of important life-history characters that is very likely caused by absolute requirements for a minimal period of cold, either as a seed or a plant. In addition, it suggests an adaptive coordination of stratification and vernalization requirements which is mediated by germination timing. Germination timing influences the duration of stratification, and short stratification duration may be associated with natural selection that favors longer vernalization requirements to avoid flowering during the winter. Clearly, the unnatural experimental combination of short stratification and short vernalization duration was associated with lower fitness in this sample than the more natural combination of short stratification and long vernalization duration. This result suggests adaptive coordination of germination and vernalization requirements in nondisrupted plants, but determining the role of germination timing in mediating selection on vernalization requirements requires further study.

The populations differed significantly in their life-history characters and in the plasticity of one of these characters (the time interval between bolting and flowering) in response to germination treatment. Population differences expressed in the spring germination treatment with a protracted growing season suggests that genetic variation may exist for life-history expression in novel environments (such as environments with very short cold spells and long growing seasons). Because these seeds were collected directly from the field, these differences could be due to both genetic differences between populations and to maternal effects accompanying different field conditions. The combined influences cause variation in important life-history characters and their plasticity even at a small regional scale in *A. thaliana*.

The results of this experiment demonstrate a correlation between germination timing and postgermination life-history characters. They also show that postgermination life-history characters are under selection. The combined results suggest that selection on germination timing can operate indirectly through selection on postgermination characters. Thus selection on later life-history stages can potentially influence the evolution of germination timing in a general manner.

This experiment also demonstrated that the timing of germination significantly influenced the selective environment experienced at later life stages. Evans and Cabin (1995) have argued that germination timing can strongly influence the evolution of postgermination life-history characters (see also Tyler et al. 1978) and can thereby foster adaptive associations between germination and postgermination characters. Others have demonstrated theoretically that the timing of germination can influence the evolution of other characters (Venable and Lawlor 1980, Ritland 1983, Brown and Venable 1986, Venable and Brown 1988), and thereby cause associations between germination and nongermination characters, but there exist few empirical studies to test the selective mechanisms for such associations. Many such studies focus on the selective consequences of dormancy as a risk-reducing strategy in a variable environment (e.g., (Venable and Lawlor 1980, Brown and Venable 1986, Klinkhammer et al. 1987, Venable and Brown 1988), and they investigate the coevolution of other risk-reducing strategies such as seed dispersal. The studies by Evans and Cabin (1995) suggest a broader influence of germination timing on character evolution beyond its role as a risk-reducing trait; they demonstrate how germination in response to particular environmental conditions can determine the selective environment in which the ger-

minated seedlings and plants grow. In their system, *Lesquerella fendleri*, which experiences variable moisture conditions that strongly influence reproduction, both germination percentage and postgermination traits vary with moisture conditions. The conditions under which seeds germinate can potentially mediate the moisture-dependent selection on postgermination characters. Supporting their hypothesis that germination behavior can influence the evolution of postgermination characters, this study of *Arabidopsis* showed a direct relationship between the timing of germination and selection on postgermination life-history characters.

Because germination timing is often environmentally labile, this result also has implications for the evolution of life-history characters under conditions of environmental change. For example, if autumns become drier or warmer, a postponement of germination timing until later in the autumn could cause changes in natural selection on life-history characters similar to the differences in selection observed in this study.

It is interesting to note that such changes in selection on life-history characters can occur even if the environment experienced by adult plants is not altered. In this experiment, all plants experienced the same environmental conditions in the spring, yet the different treatments were at different developmental stages. Thus, germination timing can influence natural selection on postgermination characters through two mechanisms. First, it can alter the selective environment experienced by plants. In this experiment, germination timing determined whether or when seedlings would be exposed to winter conditions. However, this difference in environmental conditions had little effect on fitness in this study. Second, it can alter the developmental stage of the plant that is exposed to particular selective environments. It was this latter mechanism that caused the most difference in natural selection on life-history characters in this experiment. Thus stage-specific natural selection is likely to be influenced significantly by germination timing in plant populations.

The ability of plants to influence their own selective environment has important consequences for the evolution of life histories. Habitat selection in other organisms has been shown theoretically (e.g., Jones and Probert 1980, Templeton and Rothman 1982, Rausher 1984) and empirically (Jaenike and Holt 1991, Barker 1992) to influence adaptive evolution and contribute to the maintenance of genetic variation. Habitat selection in plants has received comparatively less attention because of their sessile habit, but plasticity such as germination cueing, seed dispersal, or morphological and physiological modifications in response to the local environment all have the potential to alter the environment experienced by plants. For example, shade-avoidance responses (Givnish 1982, Ballaré et al. 1990) and phototropism (Kendrick and Kronenberg 1994) cause plants to occupy altered light environments. Clonal foraging (e.g., MacDonald and Lieffers 1993, Alpert 1999) and selective root placement (e.g., Birch and Hutchings 1994) can result in altered nutrient or water conditions for plants. Habitat selection is probably more common in plants than has previously been appreciated. How such behaviors alter subsequent selection on other characters has not been well explored.

In plants, habitat selection is frequently affected by the maternal parent. Maternal parents can influence the selective environment experienced by their progeny by altering dispersal (Donohue 1999) or requirements for germination (reviewed in Gutterman 1992, and Baskin and Baskin 1998; see also Kugler 1951, Dobrovolna and Cetl 1966, Gutterman 1978, Goto 1982, Roach and Wulff 1987, Biere 1991*a*, *b*, Platenkamp and Shaw 1993, Léon-Kloosterziel et al. 1994). This critical consequence of maternal effects can strongly influence not only the evolution of postgermination life-history characters, as discussed above, but it can influence the adaptive value of the maternal effect itself. This is because the adaptive value of maternal effects depends on the degree of covariance between maternal and progeny selective environments and on how well maternal parents predict the selective environments of their progeny and prepare their progeny for them (Donohue and Schmitt 1998).

In summary, germination timing in this winter annual strongly influenced not only the expression of life-history characters but selection on those characters as well. By altering germination timing, plants can alter their selective environment and the developmental stage that is exposed to selection. This ability has important consequences for life-history evolution and for the evolution of plasticity and maternal effects on germination. These results also demonstrate that alterations in germination timing that may accompany environmental and evolutionary changes are expected to have important consequences for the expression and evolution of postgermination life-history characters.

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