THE EVOLUTIONARY ECOLOGY OF SEED GERMINATION OF *ARABIDOPSIS THALIANA*: VARIABLE NATURAL SELECTION ON GERMINATION TIMING

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Abstract.—Germination timing of *Arabidopsis thaliana* displays strong plasticity to geographic location and seasonal conditions experienced by seeds. We identified which plastic responses were adaptive using recombinant inbred lines in a field manipulation of geographic location (Kentucky, KY; Rhode Island, RI), maternal photoperiod (14-h and 10-h days), and season of dispersal (June and November). Transgressive segregation created novel genotypes that had either higher fitness or lower fitness in certain environments than either parent. Natural selection on germination timing and its variation explained 72% of the variance in fitness among genotypes in KY, 30% in June-dispersed seeds in RI, but only 4% in November-dispersed seeds in RI. Therefore, natural selection on germination timing is an extremely efficient sieve that can determine which genotypes can persist in some locations, and its efficiency is geographically variable and depends on other aspects of life history. We found no evidence for adaptive responses to maternal photoperiod during seed maturation. We did find adaptive plasticity to season of seed dispersal in RI. Seeds dispersed in June postponed germination, which was adaptive, while seeds dispersed in November accelerated germination, which was also adaptive. We also found maladaptive plasticity to geographic location for seeds dispersed in June, such that seeds dispersed in KY germinated much sooner than the optimum time. Consequently, bet hedging in germination timing was favorable in KY; genotypes with more variation in germination timing had higher fitness because greater variation was associated with postponed germination. Selection on germination timing varied across geographic location, indicating that germination timing can be a critical stage in the establishment of genotypes in new locations. The rate of evolution of germination timing may therefore strongly influence the rate at which species can expand their range.

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

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Timing of seed germination is critical to the lifetime performance of the resulting plant. The conditions that elicit germination are the same conditions with which the young seedlings must contend, and those conditions can determine or predict conditions experienced throughout the life of a plant. Germination timing strongly influences seedling survivorship (Biere 1991; Gross and Smith 1991), total lifetime fitness (Miller 1987; Kalisz 1989; Masuda and Washitani 1992), life-history expression (Chouard 1960; Venable 1985; Kalisz 1989; Kalisz and Wardle 1994; Nordborg and Bergelson 1999; Galloway 2001, 2002), and selection on post-germination characters (Evans and Cabin 1995; Weinig 2000; Donohue 2002). An appropriate germination response to environmental cues is thereby the first requirement for establishment of plants in a location; before plants can display adaptive phenotypes for any adult character, they must first survive through germination.

The timing of seed germination strongly depends on conditions experienced during seed maturation and after dispersal from the maternal plant. In particular, the photoperiod during seed maturation is a reliable indicator of season. Maternal photoperiod has been shown to influence germination and dormancy in several species (Gutterman 1992, 1993; Baskin and Baskin 1998; Munir et al. 2001). Such maternal environmental effects have been viewed as a form of potentially adaptive cross-generational phenotypic plasticity when the maternal environment predicts the offspring environment (Schmitt et al. 1992; Schmitt 1995; Bernardo 1996; Donohue and Schmitt 1998; Mousseau and Fox 1998). The environment experienced after dispersal also strongly influences germination behavior. Certain environmental conditions may be required to break dormancy, and other conditions are often required to permit germination after dormancy is broken (Bewley 1997; Foley 2001). Seeds of many species require days, weeks, or months at low temperatures to break dormancy (Bewley and Black 1994; Vleeshouwers et al. 1995), whereas others require warm temperatures for after-ripening to germinate when permissive conditions arrive (Baskin and Baskin 1972, 1983). Therefore, the evolution of germination timing can be viewed as the evolution of plasticity of germination to both the maternal and the progeny environments.

Investigating the adaptive value of germination responses to particular seasonal environmental factors can identify which factors are most important in eliciting adaptive germination behavior. In highly mobile species, such as many weeds, some seasonal factors may be more reliable than others as predictors of environmental conditions throughout the range of the species. For example, short days may predict

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the onset of winter cold in some locations, but they may indicate the beginning of a favorable rainy season in another location. Postdispersal temperature, however, may be a reliable predictor of postgermination temperature over broader geographic ranges. Under conditions of global environmental change, moreover, seasonal environmental factors may change in their predictive value. It is therefore important to characterize germination responses to different seasonal environmental factors to predict how germination responses can influence performance in different locations or novel climates.

In this study, we measured natural selection on germination timing to identify which plastic responses to different seasonal environmental factors and geographic locations are adaptive in the annual weed *Arabidopsis thaliana*. This species is a weedy mustard (Brassicaceae) that inhabits highly disturbed areas, and different ecotypes express different life histories that vary in the timing of germination and flowering (Ratcliffe 1976; Effmertova 1967; Evans and Ratcliffe 1972; Nordborg and Bergelson 1999). Winter annuals, the most typical life history, germinate in the autumn, overwinter as vegetative rosettes, and flower, mature, disperse seeds, and die in the following spring or early summer (Baskin and Baskin 1983). Spring annuals germinate, flower, mature, disperse seeds, and die in the spring or early summer. Plants in some populations flower and disperse seeds in the autumn (Thompson 1994; Griffith et al. 2004). Therefore, variation exists among natural populations in the seasonal conditions during which seeds mature and disperse and the season during which seeds germinate.

Arabidopsis thaliana is native to central Asia and was spread throughout Europe and North America through long-distance dispersal caused in large measure by human activities (Sharbel et al. 2000; Vander Zwan et al. 2000; Hoffmann 2002). It is therefore an ideal system for investigating the biology of range expansion of introduced species (e.g., Griffith et al. 2004). Like many weedy species, *A. thaliana* self-pollinates to a high degree (Abbott and Gomes 1989), creating highly homozygous lineages (Todokoro and Terauchi 1995; Berge et al. 1998; Bergelson et al. 1998) in natural populations that can maintain high levels of linkage disequilibrium (Nordborg et al. 2002). Because *A. thaliana* is efficiently dispersed, populations that are geographically close are not necessarily closely related genetically (Sharbel et al. 2000; Hoffmann 2002). Rare outcrossing events between such populations can create diverse recombinant genotypes. Transgressive segregation after hybridization between distinct lineages creates novel genotypes that can contribute to adaptive evolution and ecological diversification in hybrid species (Lexer et al. 2003; Rieseberg et al. 2003a,b), and hybridization is also hypothesized to contribute to invasiveness (Ellstrand and Schierenbeck 2000). Hybridization of colonizing lineages and the resulting creation of novel genotypes followed by lineage sorting may be an especially effective process for the rapid creation and subsequent preservation of adaptive gene complexes in selfing species (Weinig et al. 2003; Griffith et al. 2004). We simulated this sort of dynamic of novel genotypes in novel environments by experimentally creating a set of recombinant inbred lines to

measure natural selection on germination timing and other life-history traits in two geographic locations.

Recombinant inbred lines are powerful tools for studying natural selection because they frequently provide character combinations and phenotypic diversity beyond the range of adaptive variation that may have already been eliminated from populations by natural selection (Jordan 1991; Schemske and Bradshaw 1999). Therefore, they enable us to investigate the earliest stages of evolutionary dynamics in populations with limited hybridization. Recombinant inbred lines are especially useful for studies of phenotypic plasticity and environment-dependent natural selection because replicates of identical genotypes can be planted in different environments simultaneously. To identify which plastic responses of germination timing to seasonal environmental factors and geographic location are adaptive, we manipulated pre- and postdispersal seasonal environmental factors and deposited replicates of recombinant lines in two geographic locations. From this design, we were able to observe plasticity of germination timing in response to seasonal environmental factors and to measure the direction and strength of natural selection on germination timing under different conditions.

In a companion paper, we documented the direction of plasticity of germination in response to maternal photoperiod, season of seed dispersal, and geographic location (Donohue et al. 2005). Here, we measure natural selection on germination timing and determine what manner of plasticity was adaptive. We ask the following questions. Are maternal photoperiod effects adaptive? Specifically, do seeds matured under short days perform better when they are dispersed under short days, and do seeds matured under long days perform better if they are dispersed under long days? Does natural selection on germination timing depend on geographic location and season of dispersal? Is plasticity of germination timing in response to geographic location adaptive, such that the germination timing expressed in each location is adaptive in that location? Is plasticity in response to season of dispersal adaptive? Do seeds dispersed in June alter their germination timing in a manner that results in higher fitness for June-dispersed seeds, and likewise for seeds dispersed in November?

MATERIALS AND METHODS

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 Tac as the maternal parent. Seeds from Calver were acquired through the Arabidopsis Biological Resource Center at Ohio State University (stock CS1062). Seeds from Tacoma were collected by T. Mitchell-Olds. One hundred twenty recombinant lineages were maintained for six to eight generations by single seed descent, resulting in 98.4–99.6% homozygosity. See Donohue et al. (2005) for more details on the lines and their maintenance. One hundred ten recombinant inbred lines were available for the first planting for June, and an additional 10 lines were available for the second planting in November. Unfortunately, the Cal parental line did not produce enough seeds for adequate representation in all of the treatments, and
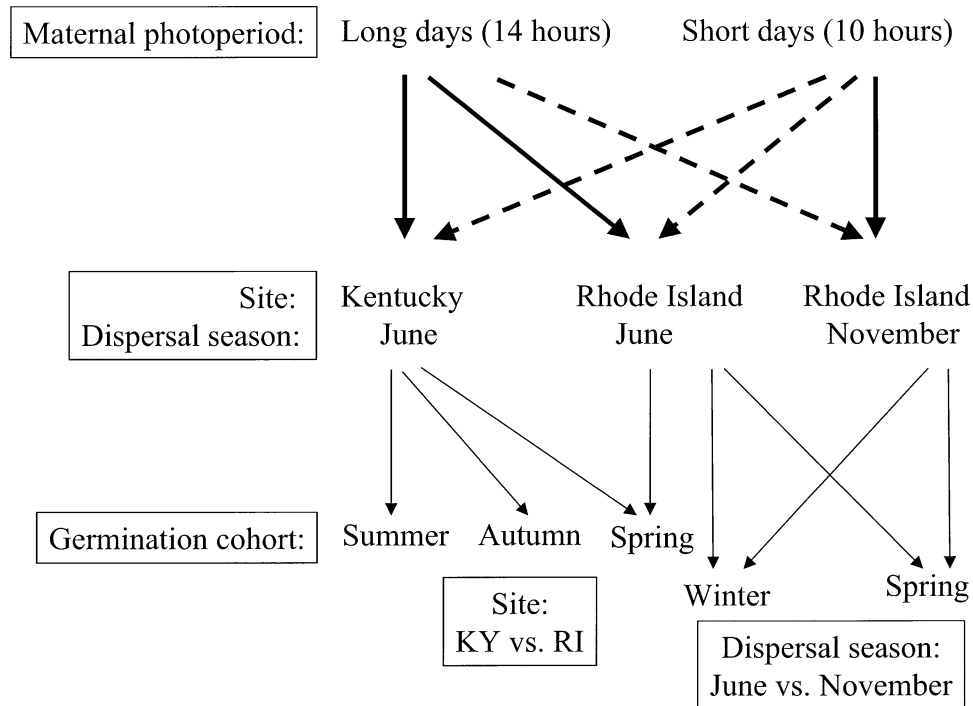
Fig. 1. Experimental design (see text for details). Solid lines indicate treatments in which the maternal photoperiod matches the season of dispersal. Dashed lines indicate treatments in which maternal photoperiod and dispersal season are mismatched.

less than 100% germination in the field caused some treatments to have no representative of the Cal parental line.

Experimental Treatments

We manipulated the photoperiod under which seeds were matured (''maternal photoperiod'' of short days and long days) and the season during which seeds were dispersed (''dispersal season'' during June and November) to decouple the photoperiod of seed maturation from the season that it predicts (Fig. 1). We dispersed these seeds in Kentucky (KY) only during June, because natural seed dispersal occurs only during late spring. We dispersed seeds in Rhode Island (RI) during June and November because plants mature and disperse seeds during both seasons in RI. From this design, the effects of maternal photoperiod can be determined within each site and season of dispersal. The effects of site and its interaction with maternal photoperiod can be determined by comparing the seeds dispersed during June in KY and RI. The effects of season of dispersal and its interaction with maternal photoperiod can be determined by comparing the seeds dispersed during June and November in RI.

The design includes some natural combinations of maternal photoperiod and dispersal season and some combinations that do not occur in nature (cf. solid and dashed lines in Fig. 1). Long photoperiod with June dispersal and short photoperiod with November dispersal are natural combinations, but long photoperiod with November dispersal is not. While it is possible that seeds could be matured under short days if plants flower in late winter, the combination of short photoperiod and June dispersal is not as typical as a long photoperiod with June dispersal. If maternal photoperiod influences offspring in an adaptive manner in these lines, plants in treat-

ments with properly matched photoperiod and season should have higher fitness than plants with mismatched treatments.

Plants were grown in two batches to provide seeds to be dispersed into the field during June and November. In each planting, six replicates (distributed over two blocks) of each of the recombinant genotypes 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., Pembina, ND) growth chambers at 22°C. The short-day photoperiod is the shortest photoperiod that plants are likely to experience in our study sites when they flower in the autumn, and the long-day photoperiod resembles the photoperiod under which plants are likely to mature seeds during the early summer. Seeds were collected from each plant as they matured, and seeds were pooled across replicates. Ten seeds from a given genotype and maternal photoperiod combination were put into a separate microcentrifuge tube and kept at 23°C until planting in the field, with each tube corresponding to one replicate pot for the field study. See Donohue et al. (2005) for more details on the culturing of the seed parents.

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 with 5 cm between them. Because the same soil was used to fill pots in both sites, comparisons across sites control for differences in soil quality. In addition, weed density within the plots was low at this early successional stage, so the primary contrast between sites was due to climatic factors. Three blocks were established in KY, and three

replicates of each genotype and maternal 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. One replicate of each genotype and maternal 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. 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. See Donohue et al. (2005) for more details on the field setup. The entire experiment included 16,686 pots, or approximately 150,000 seeds (KY: 110 lines × 2 maternal photoperiods × 3 germination cohorts × 9 replicates = 5940; RI, June dispersal: 110 lines × 2 maternal photoperiods × 3 germination cohorts × 9 replicates = 5940; RI November dispersal: 120 lines × 2 maternal photoperiods × 2 germination cohorts × 9 replicates = 4320; total = 16,200 plus 18 control pots per block).

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 classified as summer (June to August), autumn (August to January), and spring (after February). Two germination cohorts existed for seeds dispersed in November: winter (November to January) and spring (after February). The germination cohort treatments therefore enabled us to follow individuals that germinated during the summer, autumn, winter, or spring under noncompetitive conditions. Because the presence of a rosette very likely alters the germination behavior of ungerminated seeds, only the spring germination pots, which had no rosettes during most of the experiment, were used to determine germination patterns of each genotype and photoperiod combination. In these spring germination pots, the total number of germinants in each pot (10 maximum) was recorded, as was the date on which each seed germinated. From these data, the total proportion of seeds that germinated in each season and the average day of germination (in Julian days) were calculated for each genotype (for analyses see Donohue et al. 2005).

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 in this highly selfing plant. The genotypic mean fitness was estimated as follows. Germination was classified into five different seasons, depending on when the peaks in germination occurred (see Donohue et al. 2005). The five seasons were early summer, midsummer, autumn, winter, and spring. For each genotype, the proportion of its seeds that germinated in each season was determined, and the mean fruit production of plants that germinated in each season was calculated. The genotypic mean fitness was

calculated as the mean lifetime fruit production of seeds that germinated in each season multiplied by the proportion of seeds that germinated in each season, summed over seasons. This estimate of fitness includes only those seeds that germinated, because our primary objective was to compare the fitness of genotypes that expressed the phenotype of germination.

Statistical Analyses

To determine the effects of maternal photoperiod, site, and season of seed dispersal on fitness components, the total lifetime fruit production, the proportion of plants that survived to initiate reproduction ("bolt"), and the fruit production of individuals that survived to bolt were analyzed using analysis of variance based on genotypic means pooled across blocks for each combination of maternal photoperiod, site, and dispersal season (Proc GLM; SAS Institute 1990). Genotypic means were used instead of individual phenotypes because they more accurately reflect the natural distribution of germination cohorts for each genotype and thereby more accurately reflect the natural fitness of each genotype. Individual values, in contrast, intentionally had a more even representation of the different germination cohorts, imposed by the experimental design that was used to monitor the fitness of each germination cohort separately. Pooling over individual values therefore would inflate the representation of rare germination cohorts in the sample. To compare between the two sites, the seeds dispersed in KY and RI during June were analyzed, with photoperiod and site as fixed factors. To compare between the two dispersal seasons, the seeds dispersed in RI during June and November were analyzed in a separate model, with maternal photoperiod and season as fixed factors. Any significant interactions between maternal photoperiod and other factors were investigated by testing for effects of photoperiod within each treatment separately. The residuals of some analyses of fitness were not normally distributed, so main effects were also tested using nonparametric Kruskal-Wallis tests when appropriate.

To test for environment-dependent natural selection on germination timing, phenotypic (Lande and Arnold 1983; Arnold and Wade 1984) and genotypic selection analyses (Mitchell-Olds and Shaw 1987; Rausher 1992) were performed. In the genotypic analysis, we also estimated selection on the standard deviation of germination timing using genotypic standard deviations. Relative fitness within each site and dispersal season was calculated as the total lifetime fruit production divided by the mean fruit production within that treatment. The date of germination (and its SD in the genotypic analysis) was standardized within each treatment to have a mean of zero and standard deviation of one. Linear and quadratic selection differentials were calculated through regression analysis. Because residuals were sometimes leptokurtic, jackknife resampling was performed to calculate standard errors using the program Freestat (Mitchell-Olds 1989). To test for significant differences in selection across treatments, analysis of covariance was performed to test for differences in the slopes of the regression of germination timing on fitness between sites or seasons. Traits were standardized, and fitness was relativized within each treatment, as before. Interactions

TABLE 1. Results of analysis of variance to test for effects of maternal photoperiod and site on the fitness of seeds dispersed during June in Kentucky and Rhode Island (A) and to test for effects of maternal photoperiod and dispersal season on fitness of plants grown in Rhode Island (B). Genotypic means were used. Residuals of analyses of total fruits and proportion survived to bolt were non-normal in ANOVA, but a significant effect in ANOVA was also significant in a nonparametric Kruskal-Wallis. Fruits produced by reproductive plants was natural-log transformed so that residuals were normal. In (A) $N = 423$ for total fruit production and survival; $N = 254$ for fruit production by survivors. In (B) $N = 488$ for total fruit production and survival; $N = 376$ for fruit production by survivors.

A. Comparing sites	Total fruits		Proportion survived to bolt		Fruits produced by reproductive plants	
Source	df	F	df	F	df	F
Photoperiod	1	0.56	1	1.41	1	0.32
Site	1	30.39***	1	117.27***	1	77.95***
Photoperiod × site	1	0.73	1	0.01	1	1.32
B. Comparing dispersal seasons	Total fruits		Proportion survived to bolt		Fruits produced by reproductive plants	
Source	df	F	df	F	df	F
Photoperiod	1	0.87	1	0.53	1	2.69
Season	1	21.08***	1	474.61***	1	36.07***
Photoperiod × season	1	0.86	1	0.33	1	3.21†

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

between germination day and site or dispersal season would indicate that the influence of germination timing on fitness varied across site or dispersal season.

To test for natural selection on the ability of germination timing to be plastic, independent of selection on germination timing itself (Van Tienderen 1991; DeWitt et al. 1998; Scheiner and Berrigan 1998), we estimated the strength of selection on genotypic plasticity. Plasticity to site was calculated as the mean germination day expressed in RI (in June only) minus the mean germination day expressed in KY. Plasticity to season was calculated as the mean number of days between seed dispersal in June and germination (in RI only) minus the mean number of days between seed dispersal in November and germination. We included both the genotypic mean germination day and genotypic plasticity in a regression on genotypic fitness in each treatment. To interpret results of these selection analyses, we calculated Spearman rank correlations between genotypic mean germination timing expressed in each treatment and genotypic plasticity to site and season. We also conducted an analysis of the total selection acting on plasticity at different simulated frequencies of environments (Donohue et al. 2000); we calculated genotypic mean fitness for each simulated frequency of environments by multiplying the fitness observed in each environment by the simulated frequency of that environment and summing over environments. Genotypic plasticities were then regressed against genotypic mean fitness.

RESULTS

Treatment Effects on Fitness

Maternal photoperiod did not significantly influence any fitness component in KY or RI (Tables 1A, B). In KY, where seeds are naturally matured and dispersed in June under long days, one would predict that seeds matured under long days would have higher fitness than seeds matured under short days (Fig. 2). While fruit production of reproductive plants was slightly (but nonsignificantly) greater when seeds were matured under long days in KY, survival to reproduction was also less for these seeds (also nonsignificantly), so there was

no overall effect of photoperiod on total lifetime fruit production in KY (Table 1A). For seeds dispersed in RI during June, total fitness and fruit production of survivors was somewhat higher for seeds matured under long days, but these effects were also not significant. Similarly, for seeds dispersed in November in RI (Table 1B, Fig. 2) no significant effects of maternal photoperiod were observed, and seeds matured under short days did not have higher fitness. In summary, we found no evidence for adaptive maternal photoperiod effects.

Germinants from seeds dispersed in RI had much higher total lifetime fitness than seeds dispersed in KY (Fig. 2, Table 1A). Survival to reproductive stage was extremely low for plants in KY, and fruit production of those plants was also lower, leading to lower overall fitness of KY plants compared to RI plants.

Germinants from seeds dispersed in June had higher fitness than those from seeds dispersed in November in RI (Fig. 2, Table 1B). Although survival to reproductive age was higher for germinating seeds dispersed in November, fruit production by those that survived was much higher for seeds dispersed in June. This led to total lifetime fitness being higher for seeds dispersed in June. A nearly significant interaction between dispersal season and maternal photoperiod for fruit production by survivors was due to a slightly greater effect of dispersal season for seeds matured under long days ($F_{\text{long days}} = 31.84$, $P < 0.0001$; $F_{\text{short days}} = 8.57$, $P = 0.004$).

Transgressive segregation of germination timing created recombinant lines that germinated either earlier or later than either of the parental lines (Figs. 3–5; Donohue et al. 2005). However, we found limited transgressive segregation for fitness of seeds dispersed in June in KY (Fig. 3). The Cal parental line had a fitness of zero in KY, so no recombinant lines could exhibit transgressive fitness that was lower than either parent. Four recombinant genotypes exceeded the fitness of the parental lines, but only if they were matured under long days. For seeds dispersed in RI during June (Fig. 4), Cal parental lines had zero fitness, but 14 genotypes had higher fitness than the Tac parental line if they matured under
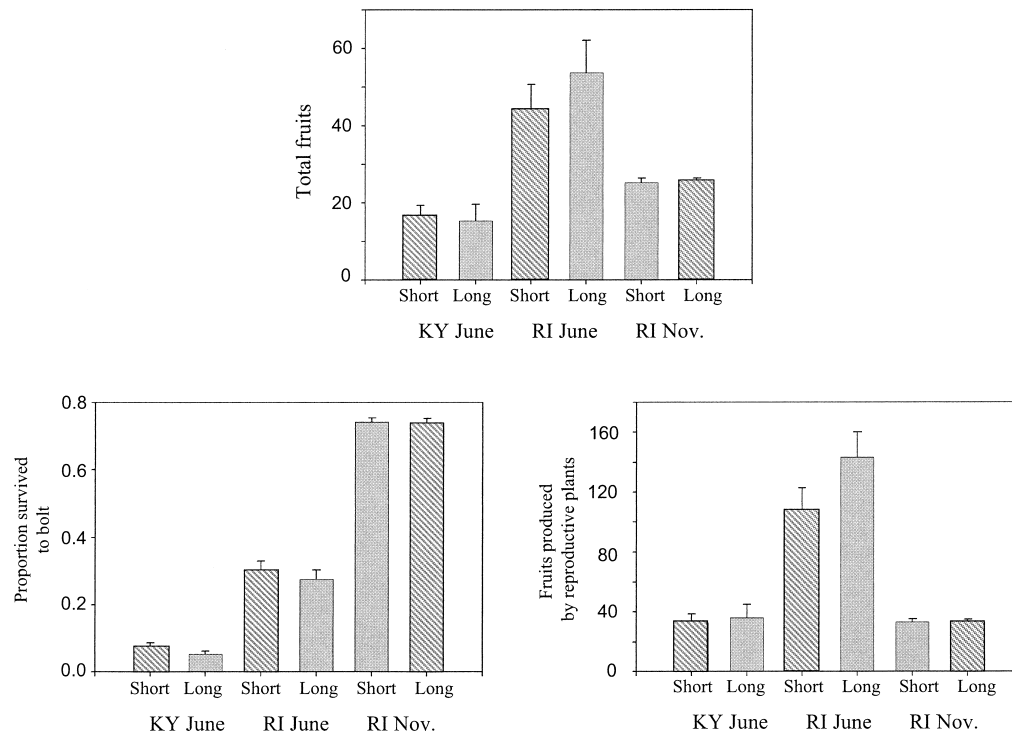
FIG. 2. Total number of fruits produced (top), proportion of plants that survived to bolt (lower left), and number of fruits produced by those that survived to bolt (lower right) for plants grown in each treatment. ''Long'' and ''short'' indicate the maternal photoperiod. Solid bars indicate properly matched dispersal seasons and maternal photoperiod. Striped bars indicate mismatched dispersal seasons and maternal photoperiod. Error bars indicate untransformed standard errors. Values are based on genotypic means.

short days, and 58 had higher fitness than Tac if they matured under long days. Transgressive segregation for fitness was also observed in seeds dispersed in November (Fig. 5). In contrast to seeds dispersed in June, the Cal parental line had higher fitness than the Tac line. Twenty three recombinant genotypes had mean fitness between the means of the parental lines, one had higher fitness, and 98 had lower fitness if they matured under long days. No Cal parental lines that were matured under short days germinated, so we could not assess transgressive segregation for fitness in that treatment. In general, transgressive segregation caused lower fitness of seeds dispersed in November, but some recombinant lineages had higher fitness than either parental line when dispersed in June.

Plasticity of Germination Timing

A companion paper reports germination patterns from this experiment (Donohue et al. 2005), but we summarize here the overall patterns of plasticity of germination in response to the experimental treatments. Germination timing was delayed very slightly (3.2 days) in seeds matured under short days when they were dispersed in KY ($F = 7.73$, $P < 0.01$, df = 1,40). Photoperiod did not significantly influence germination timing in the other treatments. Germination timing depended strongly on geographic location, with germination proceeding much earlier in KY than in RI (mean Julian germination day in KY = 170, SE = 0.5; mean in RI = 273, SE = 2; $F = 193.34$, $P < 0.001$, df = 1,6). While seeds were deposited approximately two weeks earlier in KY than in RI, the average day of germination was 103 days earlier

in KY. Germination timing also depended strongly on the season of dispersal. Germination was later (in Julian days) for seeds that were dispersed during November (mean Julian germination day for June-dispersed seeds = 273, SE = 2; mean for November-dispersed seeds = 368, SE = 1; $F = 409.44$, $P < 0.001$, df = 1,14), but they germinated much sooner after they were dispersed than seeds dispersed during June, many of which remained dormant for some months (mean number of days between dispersal and germination: June-dispersed seeds = 104, SE = 2; November-dispersed seeds = 61, SE = 1).

Natural Selection on Germination Timing

In KY, strong stabilizing selection favored an intermediate germination date in mid-October (Table 2, Fig. 3). Germinating too early in the summer was fatal for most seeds, and germinating in the winter or spring resulted in either very low or no fruit production. The slight acceleration in germination of seeds matured under long days (the more typical photoperiod) was in the maladaptive direction, but accelerating germination by only 3.2 days was not enough to influence fitness. The slight increase in fruit production by plants from seeds that were matured under long days therefore cannot be attributable to adaptive photoperiod effects on germination, because photoperiod effects actually caused a germination response in the maladaptive direction. In a genotypic analysis, total selection favored increased variation (SD) in germination timing. Those genotypes with greater variation in germination timing had later mean germination.
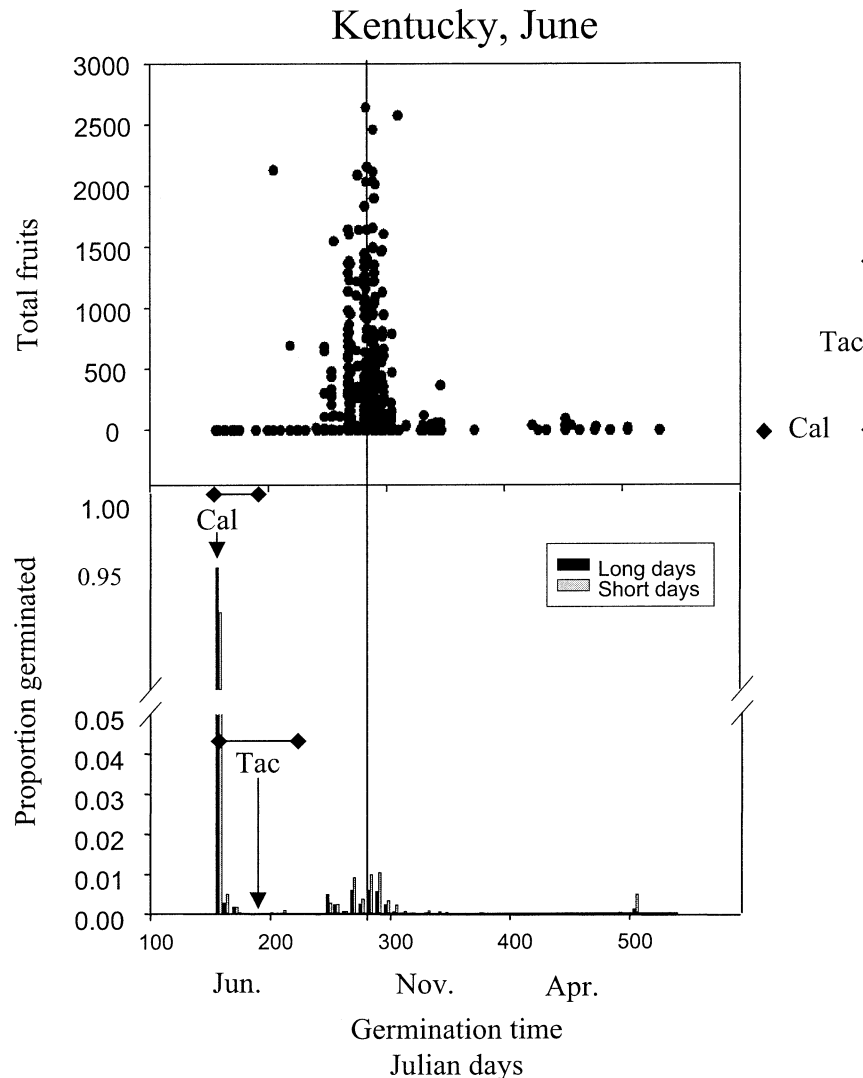
FIG. 3. Relationship between germination timing and fitness in seeds dispersed during June in Kentucky. Scatter-plot of germination timing (*x*-axis) and total number of fruits produced (*y*-axis) is shown in the upper graph. Each point represents the value for a given pot. The lower graph shows the schedule of germination in Julian days. The vertical line indicates the point in time at which maximum fitness was observed. Black bars represent seeds matured under long days, and gray bars represent seeds matured under short days. The range of fitness (upper) and germination time (lower) for each parental genotype is indicated by bars bracketed by diamonds. Arrows in the lower panel indicate the genotypic mean germination time for each parental line. All replicates of the Cal parental line had zero fitness.

When mean germination date was controlled for, selection favored less variable germination, so variation in germination per se was disadvantageous unless it led to a more adaptive mean germination timing. Germination timing explained 26% of the variance in fitness among individuals and 72% of the variance in fitness among genotypes in KY.

In RI, seeds that were dispersed in June also experienced strong stabilizing selection, favoring germination in early October, approximately 2 weeks earlier than in KY (Table 2, Fig. 4). Unlike the case in KY, germination in the summer in RI was not always fatal and occasionally led to high fitness. Later germination in the autumn was associated with a decrease in fitness, but not as rapid a decrease as in KY. Spring germination was associated with very low or no fruit production, as in KY. Selection favored less variation in ger-

mination timing when mean germination timing was controlled for. Germination timing explained only 4% of the variance in fitness among individuals, but 30% of the variance in fitness among genotypes. Both directional (phenotypic analysis: $F = 1453.62$, $P < 0.001$; genotypic analysis: $F = 14.88$, $P < 0.001$) and stabilizing (phenotypic analysis: $F = 135.79$, $P < 0.001$; genotypic analysis: $F = 4.48$, $P < 0.05$) selection on germination timing were significantly stronger in KY than in RI.

Seeds dispersed in November in RI did not experience stabilizing selection, and earlier germination was favored (Table 2, Fig. 5). Therefore, those seeds that germinated immediately after being dispersed in mid-November had the highest fitness. No selection on the mean or standard deviation of germination timing was detected in the genotypic
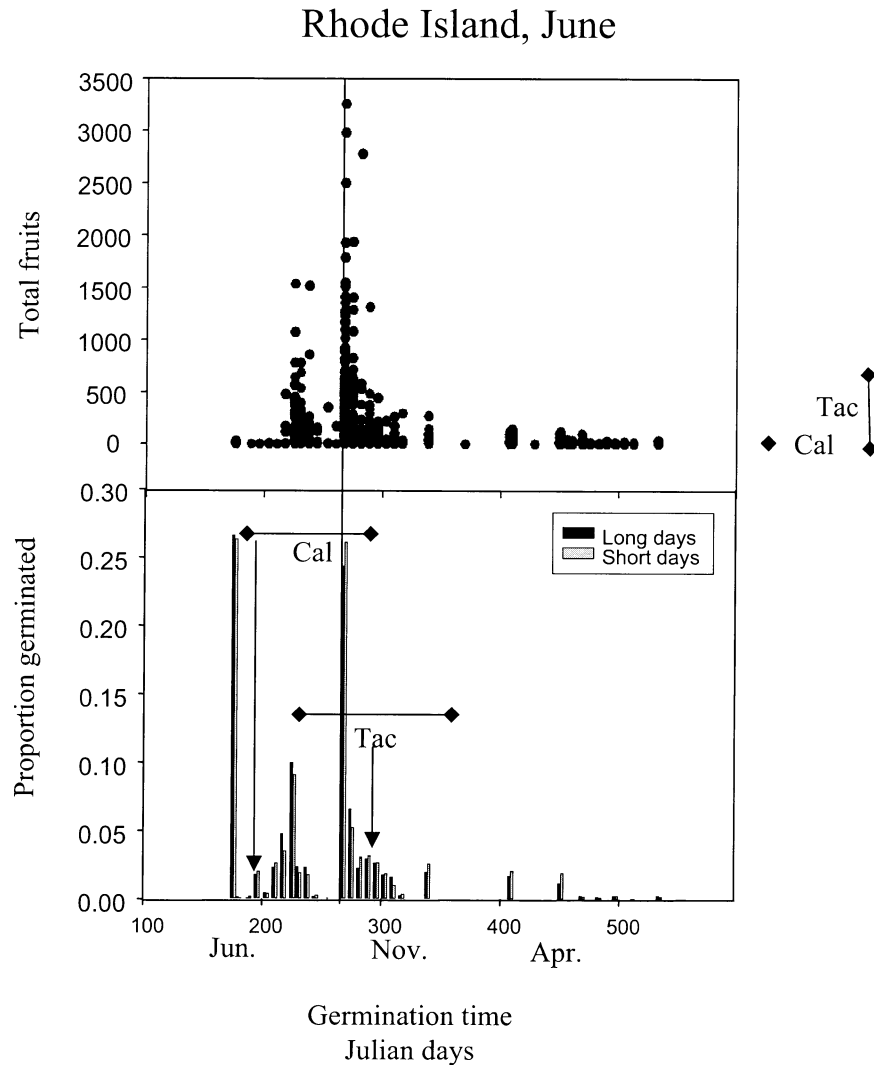
Rhode Island, June

FIG. 4. Relationship between germination timing and fitness in seeds dispersed during June in Rhode Island. See Figure 3 for explanation. All replicates of the Cal parental line had zero fitness. Adapted from Donohue (2005) with the permission of the New Phytologist Trust.

selection analysis. Germination timing explained only 2% of the variance in fitness among individuals and only 4% of the variation in fitness among genotypes. Such weak selection is due in large part to the extreme synchrony of germination in November-dispersed seeds and the corresponding lack of opportunity for selection. Directional (phenotypic analysis: $F = 81.99$, $P < 0.001$; genotypic analysis: $F = 27.48$, $P < 0.001$) and stabilizing (phenotypic analysis: $F = 69.39$, $P < 0.001$; genotypic analysis: $F = 0.60$, $P > 0.05$) selection on germination was significantly stronger in seeds dispersed in June than in seeds dispersed in November. When pooled over both sites and dispersal seasons, stabilizing selection favored an intermediate germination date, but no selection was detected on the variation (SD) in germination timing (Table 2).

If the observed plasticity to site were adaptive, then early germination would be favored in KY and later germination would be favored in RI. This was not observed. Comparing the observed germination schedules to the optimal germination timing, we see that seeds in KY germinated much earlier than the optimum (Fig. 3), and in fact had their peak

of germination during a time when the fitness was minimal. A second, much smaller pulse of germination occurred very close to the optimum. While seeds in RI also had a large pulse of germination too early, which resulted in zero fitness, a second pulse of comparable magnitude was observed very near the optimum germination time (Fig. 4). Therefore, more seeds in RI germinated at an appropriate time than the seeds in KY. Clearly, the plastic response of germinating earlier in KY was highly maladaptive, although postponing germinating in RI was adaptive. Therefore, plasticity to site was not adaptive, and a less plastic, later-germinating genotype would have performed better in both locations.

If plasticity of germination timing in response to season of dispersal were adaptive, then accelerated germination after dispersal in the autumn and postponed germination of seeds dispersed in June would be adaptive. This was observed, with the earliest germinants having the highest fitness in November-dispersed seeds, and seeds that germinated in early October having the highest fitness in June-dispersed seeds (Figs. 4, 5). The peak of germination of seeds dispersed in Novem-
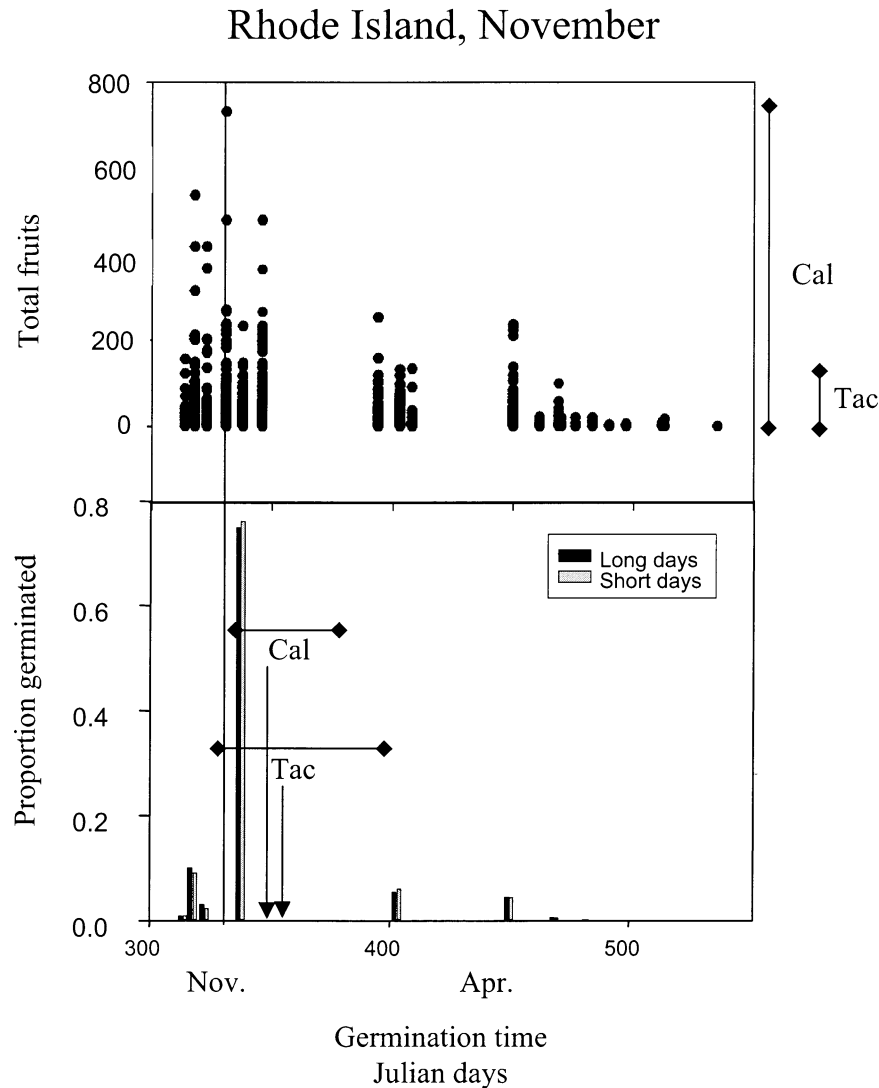
Rhode Island, November

FIG. 5. Relationship between germination timing and fitness in seeds dispersed during November in Rhode Island. See Figure 3 for explanation. Adapted from Donohue (2005) with the permission of the New Phytologist Trust.

ber was slightly later than the optimum timing, suggesting some degree of delay that was maladaptive. However, the overall pattern of plasticity to dispersal season was adaptive.

Costs or Benefits of Plasticity

A positive value of plasticity to site indicates later germination in RI than in KY. Such plasticity was positively associated with fitness for seeds dispersed in KY, independently of the germination timing expressed in KY (Table 3). Therefore in KY, there appears to be a benefit of having the ability to be plastic, despite the observation that the phenotype resulting from that plasticity was highly maladaptive. We detected no selection on the ability to be plastic to site in seeds dispersed in RI in either season. The total selection (direct plus indirect selection) on plasticity to site was positive over all frequencies of sites (Fig. 6A), and plasticity increased in adaptive value as the frequency of the RI environment increased. The result that total selection favored increased plasticity can be explained by the following ob-

servations. First, plasticity to site caused a favorable delay of germination in RI. Second, the ability to be plastic (independently of the phenotype) was favored in KY. Finally, the ability to be plastic was positively correlated with the ability to have later germination in both KY and RI (Table 3). Selection acting on germination timing would favor postponed germination in both sites and thereby indirectly favor increased plasticity, leading to total selection favoring increased plasticity. While variable frequencies of geographic locations experienced by a lineage are not especially biologically realistic, comparable environmental variation may be experienced as either microenvironmental variation or as temporal variation in environmental conditions.

A positive value of plasticity to season indicates later germination in June than in November. This ability to be plastic had a positive effect on fitness in seeds dispersed in KY, independent of the germination timing expressed in KY. It had no measurable effect on fitness in the other treatments. The result for seeds in KY is due to the strong positive cor-

TABLE 2. Selection differentials (S), selection gradients (β), quadratic selection coefficients (γ), and R^2 for germination timing in each treatment. Jackknife standard errors (SE) are in parentheses. Results from a phenotypic selection analysis are in the upper table, and results from a genotypic selection analysis using genotypic means and genotypic standard deviation (SD) are in the lower table. Significance indicates significant differences from zero based on jackknife standard errors. The selection differential (S) for SD measures total selection on the SD of germination date, not controlling for mean germination date. r (mean, SD) measures the correlation between the mean germination timing and the SD in germination timing. R^2 is the total variance in fitness among individuals explained by directional and stabilizing selection on germination timing in the phenotypic analysis. In the genotypic analysis, R^2 is the total variance in fitness among genotypes explained by directional and stabilizing selection on germination time and selection on the SD of germination time. No correlational selection was found between the mean and SD of germination timing.

Phenotypic selection analysis

Treatment	Mean germination S (SE)	Mean germination γ (SE)	R^2
Kentucky, June	2.78 (0.24)***	−0.67 (0.05)***	0.26
Rhode Island, June	−0.03 (0.03)	−0.60 (0.05)***	0.04
Rhode Island, November	−0.22 (0.02)***	−0.03 (0.03)	0.02

Genotypic selection analysis

Treatment	Mean germination β (SE)	Mean germination γ (SE)	SD germ β (SE)	SD germination S (SE)	r (mean, SD)	R^2
Kentucky, June	4.07 (0.72)**	−0.43 (0.15)**	−1.21 (0.35)***	1.16 (0.54)*	0.83***	0.72
Rhode Island, June	0.76 (0.13)***	−0.12 (0.09)	−0.34 (0.11)**	0.00 (0.11)	0.46***	0.30
Rhode Island, November	0.02 (0.03)	−0.04 (0.03)	0.10 (0.05)	0.06 (0.04)	0.48***	0.04
Pooled	0.19 (0.18)	−0.38 (0.07)*	−0.81 (0.32)	−0.53 (0.41)	−0.89***	0.26

* $P < 0.0.5$, ** $P < 0.01$, *** $P < 0.001$.

relation between plasticity to site and plasticity to season ($r = 0.96$, $P < 0.001$). Plasticity to site was favored (see preceding paragraph), so plasticity to season was favored indirectly in KY. Total selection on plasticity to season favored higher plasticity across all frequencies of seasons (Fig. 6B), except when no seeds were dispersed in June (a highly unlikely condition). As the frequency of dispersal in June increased, total selection favoring plasticity to season was stronger. This is due entirely to correlated selection acting on germination timing itself, as the correlation between plasticity and germination timing was exceedingly strong in June-dispersed seeds; selection for delayed germination of seeds dispersed in June would cause correlated selection for increased plasticity.

DISCUSSION

Natural selection on germination timing was extremely strong under some conditions, indicating that variation in germination can be an important selective sieve that eliminates many genotypes at the earliest life stage. Bet hedging in germination timing was favorable in KY; genotypes with more variation in germination timing had higher fitness because greater variation was associated with postponed germination. We found no evidence of adaptive maternal photoperiod effects. In contrast, we found strong effects of geographic location and season of dispersal on fitness. The observed direction of plasticity of germination timing in response to site was maladaptive, with the acceleration of germination in KY being fatal in most cases. The observed plasticity of germination in response to season of dispersal, however, was adaptive, with selection favoring the accelerated germination of seeds dispersed during November. Total selection on plasticity to site and plasticity to season favored increased plasticity, despite the maladaptive phenotype expressed in KY resulting from plasticity to site. The strong response of germination timing to postdispersal seasonal factors and the evidence of adaptive plasticity for such responses suggests that appropriate germination responses to postdispersal seasonal conditions can be stronger influences on the ability of plants to establish themselves in new locations than responses to maternal photoperiod.

The Strength of Natural Selection on Germination

Natural selection on germination timing was very strong in seeds dispersed in June. In KY, germination timing alone accounted for 72% of the variation in fitness among genotypes. Many studies have demonstrated strong natural selection on naturally segregating germination variants (Miller 1987; Kalisz 1989; Biere 1991; Gross and Smith 1991; Masuda and Washitani 1992; Donohue 2002; Griffith et al. 2004). This study shows just how strong natural selection on germination timing can be on a range of potential segregants derived from natural genotypes. Many of these segregants are likely to be eliminated almost immediately after their

TABLE 3. Selection on plasticity in response to site and season of dispersal. The strength of selection on plasticity independently of selection on germination timing, or the selection gradient, β, is shown with jackknife standard errors. The correlation, r, between plasticity and germination date is based on genotypic means.

Treatment	Plasticity to site β (SE)	r (plasticity, germination)	Plasticity to season β (SE)	r (plasticity, germination)
Kentucky, June	0.28 (0.19)**	0.46***	0.30 (0.20)**	0.58***
Rhode Island, June	0.38 (0.47)	0.96***	−0.53 (0.96)	0.99***
Rhode Island, November	−0.03 (0.03)	0.15	0.02 (0.03)	0.15

** $P < 0.01$, *** $P < 0.001$.
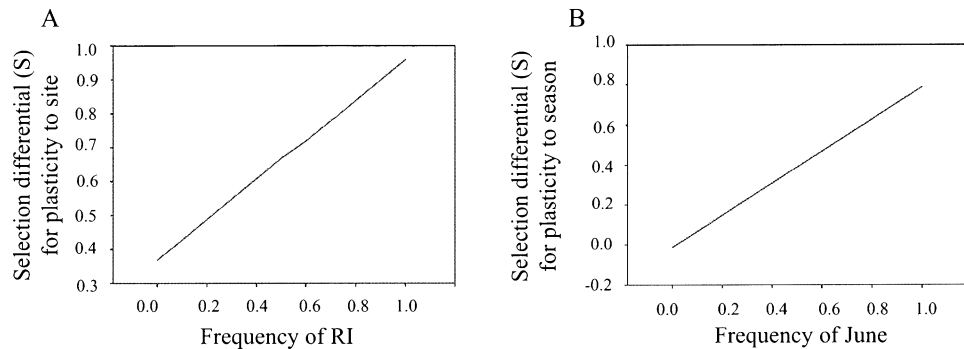
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FIG. 6.   Selection differentials for plasticity to site (A) and plasticity to season (B) at different simulated frequencies of environments.

creation. Thus, the strength of natural selection on germination may be cryptic because it can occur so quickly and with such strength, leaving only the better-adapted genotypes for us to study.

In mobile species such as *A. thaliana*, germination timing is likely to be an important factor that determines the rate of range expansion and population growth. In this study, germination timing was inappropriately altered in response to geographic location, demonstrating an inability to adjust germination to different locations. In addition to finding strong natural selection on germination timing, we previously found a genetically based trade-off across sites in germination percentage (Donohue et al. 2005); genotypes with high germination success in RI had low germination success in KY and vice versa. Thus, selection acting at the earliest life stages is likely to be an extremely important filter. Genotypes that may otherwise be adaptive can be eliminated in this earliest episode of natural selection because they must survive through germination to express any adaptive postgermination trait. Therefore, linkage to germination loci may be a constraint on the evolution of postgermination traits, and the evolution of germination may be the first necessary condition for successful range expansion of certain weedy species.

In addition, with global warming, more variable soil moisture conditions, warmer drier conditions are likely to become more common (Intergovernment Panel on Climate Change 2002), including warmer, drier conditions like those in KY. The inappropriate germination response of *A. thaliana* in this environment and the extremely strong selection on this response suggest that global warming will exert strong selection on germination timing.

Natural Selection on Dormancy Induction and Dormancy Breakage

Both dormancy induction and dormancy breakage were subjected to strong natural selection in this study. An especially intense episode of selection eliminated June-dispersed seeds that had weak primary dormancy. In contrast, nondormant seeds were favored if they were dispersed in November. Therefore, selection on after-ripening requirement and innate dormancy appears to depend strongly on the season of seed dispersal, and natural selection on dormancy induction or maintenance was especially intense in seeds dispersed in June.

The timing of dormancy breakage of seeds with innate dormancy was also under selection. In particular, seeds that postponed germination until spring had low fitness, and this was true regardless of the dispersal season. A previous study (Griffith et al. 2004) found evidence that spring germination could be caused by a cumulative cold requirement for dormancy breakage, and that seeds that postponed germination too long in the autumn experienced prohibitively cold temperatures that prevented germination until the warmer temperatures of spring. Their evidence for this was that those genotypes that germinated late in the autumn also had a high percentage of spring germination. We found the same in this study (KY: $r = 0.19$, $P < 0.001$; RI, June-dispersed seeds: $r = 0.53$, $P < 0.001$; RI, November-dispersed seeds: $r = 0.82$, $P < 0.001$; based on genotypic means). Under the conditions of this experiment, a requirement for too many days of cold for dormancy breakage was maladaptive. However, a requirement for some cold was adaptive for June-dispersed seeds, as indicated by the stabilizing selection on germination timing, favoring seeds that germinate early in autumn. In these seeds, the optimal germination timing was expressed by a balance between dormancy induction (or maintenance) and dormancy breakage, which led to germination in the autumn. In short, both dormancy induction and dormancy breakage are under strong selection that depends on geographic location and season of dispersal. Genes associated with natural variation in both processes have been identified through analysis of quantitative trait loci (Alonso-Blanco and Koornneef 2000; Alonso-Blanco et al. 2003). Our results suggest that such genes are likely to be under environment-dependent natural selection in the field.

Plasticity to Season of Dispersal

In contrast to the plastic response of germination to geographic location, plasticity to season of dispersal was adaptive. It is interesting that these recombinant lines exhibited adaptive plasticity, as recombination is expected to have disrupted adaptive gene combinations. The result suggests that both parental lines have evolved the same adaptive plasticity to dispersal season, although significant variation for plasticity to dispersal season (Donohue et al. 2005) indicates that not all alleles associated with plasticity to dispersal season are shared between the parental lines. The ability to germinate immediately under cool conditions and short photoperiods may be adaptive even in late winter-flowering plants, so ger-

mination responses to such seasonal cues may be adaptive over a broad range of geography and life-history schedules.

Transgressive Segregation for Fitness

Transgressive segregation created novel genotypes that exhibited germination timing and fitness that was beyond the range of variation of the parental lines. Hybridization between inbred lineages appears to have broken up combinations of alleles that were adaptive in most treatments, as many recombinants had lower fitness than either parent. However, transgressive segregation for higher fitness also was observed, especially if seeds were dispersed in June and especially if they were matured under long days. Therefore, hybridization does have the potential to contribute to adaptive evolution (Lexer et al. 2003; Rieseberg et al. 2003a,b), and its ability to do so depends on seasonal conditions of seed maturation and dispersal.

Conclusions

Germination timing was under intense natural selection in some locations, and the observed plasticity in germination was not always adaptive. An inappropriate germination response to a potentially novel environment caused a significant reduction of fitness in KY. Therefore, germination may be a critical stage that strongly influences the ability of immigrant genotypes to establish in new locations. Germination responses to dispersal season, however, were shown to be adaptive, and they may even be adaptive for plants with diverse life-history schedules. The abundant genetic variation in germination responses to geographic location and seasonal environmental factors (Donohue et al. 2005), and the transgressive segregation of both germination and fitness, indicate that germination timing has much evolutionary potential, even if the responses of most genotypes to novel locations are not adaptive. Because survival through germination may be a prerequisite for adaptation of any subsequent life stage, especially when linkage disequilibrium is pronounced, evolving appropriate germination responses to new environments is necessary for establishing in these new environments. Therefore, the rate of evolution of germination timing may strongly influence the rate at which mobile species can expand their ranges and adapt to new locations.

Literature Cited

Abbott, R. J., and M. F. Gomes. 1989. Population genetic structure and outcrossing rate of *Arabidopsis thaliana* L. Heynh. Heredity 62:411–418.

Alonso-Blanco, C., and M. Koornneef. 2000. Naturally occurring variation in *Arabidopsis*: an underexploited resource for plant genetics. Trends Plant Sci. 5:22–29.

Alonso-Blanco, C., L. Bentsink, C. J. Hanhart, H. Blankestijn-de Vries, and M. Koornneef. 2003. Analysis of natural allelic variation at seed dormancy loci of *Arabidopsis thaliana*. Genetics 164:711–729.

Arnold, S. J., and M. J. Wade. 1984. On the measurement of natural and sexual selection: applications. Evolution 38:720–734.

Baskin, C. C., and J. M. Baskin. 1998. Seeds: Ecology, biogeography and evolution of dormancy and germination. Academic Press, San Diego.

Baskin, J. M., and C. C. Baskin. 1972. Ecological life cycle and physiological ecology of seed germination of *Arabidopsis thaliana*. Can. J. Bot. 50:353–360.

———. 1983. Seasonal changes in the germination responses of buried seeds of *Arabidopsis thaliana* and ecological interpretation. Bot. Gaz. 144:540–543.

Berge, G., I. Nordal, and G. Hestmark. 1998. The effect of inbreeding systems and pollination vectors on the genetic variation of small plant populations within an agricultural landscape. Oikos 81:17–29.

Bergelson, J., E. Stahl, S. Dudek, and M. Kreitman. 1998. Genetic variation within and among populations of *Arabidopsis thaliana*. Genetics 148:1311–1323.

Bernardo, J. 1996. Maternal effects in animal ecology. Am. Zool. 36:83–105.

Bewley, J. D. 1997. Seed germination and dormancy. Plant Cell 9:1055–1066.

Bewley, J. D., and M. Black. 1994. Dormancy and the control of germination. Seeds: physiology of development and germination. 2nd ed. Plenum, New York.

Biere, A. 1991. Parental effects in *Lychnis flos cuculi*. II. Selection on time of emergence and seedling performance in the field. J. Evol. Biol. 3:467–486.

Chouard, P. 1960. Vernalization and its relations to dormancy. Ann. Rev. Plant Physiol. 11:191–237.

DeWitt, T. J., A. Sih, and D. S. Wilson. 1998. Costs and limits of phenotypic plasticity. Trends Ecol. Evol. 13:77–81.

Donohue, K. 2002. Germination timing influences natural selection on life-history characters in *Arabidopsis thaliana*. Ecology 83:1006–1016.

———. 2005. Niche construction through phenological plasticity: life history dynamics and ecological consequences. New Phytol. Special issue 166:83–92.

Donohue, K., and J. Schmitt. 1998. Maternal environmental effects: Adaptive plasticity? Pp. 137–158 *in* T. A. Mousseau and C. W. Fox, eds. Maternal effects as adaptations. Oxford Univ. Press, Oxford, U.K.

Donohue, K., D. Messiqua, E. Hammond-Pyle, S. M. Heschel, and J. Schmitt. 2000. Evidence of adaptive divergence in plasticity: density- and site-dependent selection on shade avoidance responses in *Impatiens capensis*. Evolution 54:1956–1968.

Donohue, K., L. A. Dorn, C. Griffith, E. Kim, A. Aguilera, and J. Schmitt. 2005. Environmental and genetic influences on the germination of *Arabidopsis thaliana* in the field. Evolution 59:740–757.

Effmertova, E. 1967. The behaviour of ''summer annual,'' ''mixed,'' and ''winter annual'' natural populations as compared with early and late races in field conditions. Arabidopsis Information Service 4.

Ellstrand, N. C., and K. A. Schierenbeck. 2000. Hybridization as a stimulus for the evolution of invasiveness in plants? Proc. Natl. Acad. Sci. 97:7043–7050.

Evans, A. S., and R. J. Cabin. 1995. Can dormancy affect the evolution of post-germination traits? The case of *Lesquerella fendleri*. Ecology 76:344–356.

Evans, J., and D. Ratcliffe. 1972. Variation in ''after-ripening'' of

seeds of *Arabidopsis thaliana* and its ecological significance. Arabidopsis Information Service 9:3–5.

Foley, M. E. 2001. Seed dormancy: an update on terminology, physiological genetics, and quantitative trait loci regulating germinability. Weed Sci. 49:305–317.

Galloway, L. F. 2001. Parental environmental effects on life history in the herbaceous plant *Campanula americana*. Ecology 82:2781–2789.

———. 2002. The effect of maternal phenology on offspring characters in the herbaceous plant *Campanula americana*. J. Ecol. 90:851–858.

Griffith, C., E. Kim, and K. Donohue. 2004. Life-history variation and adaptation in the historically mobile plant *Arabidopsis thaliana* in North America. Am. J. Bot. 91:837–849.

Gross, K. L., and A. D. Smith. 1991. Seed mass and emergence time effects on performance of *Panicum dichotomiflorum* Michx. across environments. Oecologia 87:270–278.

Gutterman, Y. 1992. Maternal effects on seeds during development. Seeds: the ecology of regeneration in plant communities. M. Fenner, ed. C. A. B. International, Wallingford, U.K.

———. 1993. Seed germination in desert plants. Springer, Berlin.

Hoffmann, M. H. 2002. Biogeography of *Arabidopsis thaliana* (L.) Heynh. (Brassicaceae). J. Biogeogr. 29:125–134.

International Panel on Climate Change. 2002. Climate change and biodiversity. H. Gitay, A. Suárez, R. T. Watson, and D. J. Dokken, eds. IPCC, Geneva, Switzerland.

Jordan, N. 1991. Multivariate analysis of selection in experimental populations derived from hybridization of two ecotypes of the annual plant *Diodia teres* W. (Rubiaceae). Evolution 45:1760–1772.

Kalisz, S. 1989. Fitness consequences of mating system, seed weight, and emergence date in a winter annual, *Collinsia verna*. Evolution 43:1263–1272.

Kalisz, S., and G. M. Wardle. 1994. Life history variation in *Campanula americana* (Campanulaceae): population differentiation. Am. J. Bot. 81:521–527.

Lande, R., and S. J. Arnold. 1983. The measurement of selection on correlated characters. Evolution 37:1210–1226.

Lexer, C., M. E. Welch, O. Raymond, and L. H. Rieseberg. 2003. The origin of ecological divergence in *Helianthus paradoxus* (Asteraceae): selection on transgressive characters in a novel hybrid habit. Evolution 57:1989–2000.

Masuda, M., and I. Washitani. 1992. Differentiation of spring emerging and autumn emerging ecotypes in *Galium spurium* var. *echinospermon*. Oecologia 89:42–46.

Miller, T. 1987. Effects of emergence time on survival and growth in an early old-field plant community. Oecologia 72:272–278.

Mitchell-Olds, T. 1989. Free-stat users manual. Tech. Bull. 101. Division of Biological Sciences, University of Montana, Missoula, MT.

Mitchell-Olds, T., and R. G. Shaw. 1987. Regression analysis of natural selection: statistical inference and biological interpretation. Evolution 41:1149–1161.

Mousseau, T. A., and C. W. Fox, eds. 1998. Maternal effects as adaptations. Oxford Univ. Press, Oxford, U.K.

Munir, J., L. Dorn, K. Donohue, and J. Schmitt. 2001. The influence of maternal photoperiod on germination requirements in *Arabidopsis thaliana*. Am. J. Bot. 88:1240–1249.

Nordborg, M., and J. Bergelson. 1999. The effect of seed and rosette cold treatment on germination and flowering time in some *Arabidopsis thaliana* (Brassicaceae) ecotypes. Am. J. Bot. 86:470–475.

Nordborg, M., J. O. Borevitz, J. Bergelson, C. C. Berry, J. Chory, J. Hagenblad, M. Kreitman, J. N. Maloof, T. Noyes, P. J. Oefner, E. A. Stahl, and D. Weigel. 2002. The extent of linkage disequilibrium in *Arabidopsis thaliana*. Nat. Genet. 30:190–193.

Ratcliffe, D. 1976. Germination characteristics and their inter- and intra-population variability in *Arabidopsis*. Arabidopsis Information Service no. 13.

Rausher, M. D. 1992. The measurement of selection on quantitative traits: biases due to environmental covariances between traits and fitness. Evolution 46:616–626.

Rieseberg, L. H., O. Raymond, D. M. Rosenthal, Z. Lai, K. Livingstone, T. Nakazato, J. L. Durphy, A. E. Schwarzbach, L. A. Donovan, and C. Lexer. 2003a. Major ecological transitions in wild sunflowers facilitated by hybridization. Science 301:1211–1216.

Rieseberg, L. H., M. A. Arntz, and J. M. Burke. 2003b. The genetic architecture necessary for transgressive segregation is common in both natural and domesticated populations. Philos. Trans. R. Soc. Lond. 358:1141–1147.

SAS Institute. 1990. SAS/STAT user's guide. SAS Institute, Cary, NC.

Scheiner, S. M., and D. Berrigan. 1998. The genetics of phenotypic plasticity. VIII. The cost of plasticity in *Daphnia pulex*. Evolution 52:368–378.

Schemske, D. W., and H. D. J. Bradshaw. 1999. Pollinator preference and the evolution of floral traits in monkeyflowers (*Mimulus*). Proc. Natl. Acad. Sci. 96:11910–11915.

Schmitt, J. 1995. Genotype-environment interaction, parental effects, and the evolution of plant reproductive traits. Pp. 1–16 *in* P. Hoch, ed. Experimental and molecular approaches to plant biosystematics. Missouri Botanical Garden, St. Louis, MO.

Schmitt, J., J. Niles, and R. D. Wulff. 1992. Norms of reaction of seed traits to maternal environments in *Plantago lanceolata*. Am. Nat. 139:451–466.

Sharbel, T. F., B. Haubold, and T. Mitchell-Olds. 2000. Genetic isolation by distance in *Arabidopsis thaliana*: biogeography and postglacial colonization of Europe. Mol Ecol. 9:2109–2118.

Simons, A. M., and M. O. Johnston. 2000. Variation in seed traits of *Lobelia inflata* (Campanulaceae): sources and fitness consequences. Am. J. Bot. 87:124–132.

Thompson, L. 1994. The spatiotemporal effects of nitrogen and litter on the population dynamics of *Arabidopsis thaliana*. J. Ecol. 82:63–68.

Todokoro, S., and R. K. Terauchi. 1995. Microsatellite polymorphisms in natural population of *Arabidopsis thaliana* in Japan. Jpn. J. Genet. 70:543–554.

Vander Zwan, C., S. A. Brodie, and J. J. Campanella. 2000. The intraspecific phylogenetics of *Arabidopsis thaliana* in worldwide populations. Syst. Bot. 25:47–59.

van Tienderen, P. H. 1991. Evolution of generalists and specialists in spatially heterogeneous environments. Evolution 45:1317–1331.

Venable, D. L. 1985. The evolutionary ecology of seed heteromorphism. Am. Nat. 126:577–595.

Vleeshouwers, L. M., H. J. Bouwmeester, and C. M. Karssen. 1995. Redefining seed dormancy: an attempt to integrate physiology and ecology. J. Ecol. 83:1031–1037.

Weinig, C. 2000. Differing selection in alternative competitive environments: shade-avoidance responses and germination timing. Evolution 54:124–136.

Weinig, C., J. R. Stinchcombe, and J. Schmitt. 2003. QTL architecture of resistance and tolerance traits in *Arabidopsis thaliana* in natural environments. Mol. Ecol. 12:1153–1163.

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