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ENVIRONMENTAL AND GENETIC INFLUENCES ON THE GERMINATION OF *ARABIDOPSIS THALIANA* IN THE FIELD

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Abstract.—Seasonal germination timing strongly influences lifetime fitness and can affect the ability of plant populations to colonize and persist in new environments. To quantify the influence of seasonal environmental factors on germination and to test whether pleiotropy or close linkage are significant constraints on the evolution of germination in different seasonal conditions, we dispersed novel recombinant genotypes of *Arabidopsis thaliana* into two geographic locations. To decouple the photoperiod during seed maturation from the postdispersal season that maternal photoperiod predicts, replicates of recombinant inbred lines were grown under short days and long days under controlled conditions, and their seeds were dispersed during June in Kentucky (KY) and during June and November in Rhode Island (RI). We found that postdispersal seasonal conditions influenced germination more strongly than did the photoperiod during seed maturation. Genetic variation was detected for germination responses to all environmental factors. Transgressive segregation created novel germination phenotypes, revealing a potential contribution of hybridization of ecotypes to the evolution of germination. A genetic trade-off in germination percentage across sites indicated that determinants of fitness at or before the germination stage may constrain the geographic range that a given genotype can inhabit. However, germination timing exhibited only weak pleiotropy across treatments, suggesting that different sets of genes contribute to variation in germination behavior in different seasonal conditions and geographic locations. Thus, the genetic potential exists for rapid evolution of appropriate germination responses in novel environments, facilitating colonization across a broad geographic range.

Key words.—Dormancy, life history, maternal effects, phenotypic plasticity, pleiotropy, seasonal cues.

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Germination timing can determine the environmental conditions experienced by plants for the rest of their lives. It can directly influence seedling survival (Biere 1991; Gross and Smith 1991), as well as influence the phenotypic expression of postgermination characters and alter the strength and mode of natural selection on those life histories (Evans and Cabin 1995; Donohue 2002). In *Arabidopsis thaliana*, variation in germination timing alone accounted for 72% of the variation in fitness among genotypes in one location (Donohue et al. 2005). Determining causes of variation in germination timing can consequently identify causes of mortality, range limits, and life-history variation in general.

Geographic variation in germination timing has been attributed to genetic differentiation among populations in some cases, but geographically variable environmental factors are known to influence germination to a large extent (Cruden 1974; Van der Vegte 1978; Hacker et al. 1984; Hacker and Ratcliff 1989; Meyer and Monsen 1991; Meyer 1992; Kalisz and Wardle 1994; Meyer et al. 1997; Munir et al. 2001; Griffith et al. 2004). In particular, environmental conditions experienced during seed maturation, such as photoperiod (Gutterman and Evanari 1972; Gutterman 1978, 1992; Pourrat and Jacques 1978), have been shown to alter germination behavior and determine the conditions that break dormancy (Roach and Wulff 1987; Biere 1991; Platenkamp and Shaw 1993; Lacey 1996; reviewed in Gutterman 1992; Schmitt et al. 1992; reviewed in Baskin and Baskin 1998). Frequently, conditions during seed maturation interact with conditions experienced after dispersal to determine germination timing (Alexander and Wulff 1985, Platenkamp and Shaw 1993; Lacey 1996; Munir et al. 2001). Thus, germination is known to be highly plastic in response to environmental conditions experienced both during seed maturation and after dispersal.

In plant species that are distributed widely across latitudes and that show geographic variation in flowering time, seasonal maternal effects are likely to be especially important influences on germination phenology. Seasonal variation in flowering time influences the conditions during seed maturation, such as temperature and photoperiod, and determines the environmental conditions experienced by seeds immediately after dispersal. Therefore, seasonal maternal effects imposed by variation in flowering time can operate through determining both pre- and postdispersal seasonal conditions experienced by seeds. Here we examined how such seasonal maternal effects influence germination phenology in *A. thaliana*, a plant that has a wide geographic distribution and that exhibits pronounced variation in flowering time throughout its range.

For colonizing species like *A. thaliana*, and especially for species that are expanding their geographic or ecological range, an appropriate germination response to varied environmental conditions is the first requirement for successful

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establishment in a new location. Natural selection on germination can be a strong force that filters out many genotypes at the earliest stage of colonization (Donohue et al. 2005). The ability to evolve flexible germination responses to variable seasonal environments requires adequate genetic variation for germination timing in each environment and weak genetic correlations across different environments (Via and Lande 1985). Pleiotropic constraints are among the most severe constraints to evolving flexible responses to variable environments. If a gene that controls germination in one environment also controls germination in another environment, evolutionary responses to selection that occur in the first environment would cause correlated evolution of germination expressed in the alternate environment, even if the response is maladaptive in the alternate environment. In contrast, if different genes control germination in different environments, then adaptive evolution of germination in one environment would not compromise the ability to evolve adaptively in other environments. Identifying pleiotropic effects on germination under different geographic and seasonal environments would identify significant constraints on the adaptive evolution of a critical life-history character of colonizing plants. In this study, we characterize the response of germination to seasonal environmental factors and geographic location and test for genetic constraints on the adaptive evolution of flexible germination responses in *A. thaliana*.

Arabidopsis thaliana is an annual, weedy mustard (Brassicaceae) that was introduced into North America about 200 years ago (Sharbel et al. 2000; Vander Zwan et al. 2000; Hoffman 2002). With its numerous genetic tools, it is a uniquely appropriate ecological system (Mitchell-Olds 2001) for investigating the biology of range expansion of introduced species. Its pronounced variation in flowering time, moreover, makes it an ideal species in which to examine how seasonal maternal effects on germination influence life-history properties of mobile, introduced plants.

Arabidopsis thaliana ecotypes express different life histories that vary in the timing of germination and flowering (Ratcliffe 1976; Effmertova 1967; Evans and Ratcliffe 1972; Napp-Zinn 1976; Nordborg and Bergelson 1999). Winter annuals, the most typical life history, germinate in autumn; overwinter as vegetative rosettes; and flower, mature, disperse seeds, and die in the following spring or early summer (Baskin and Baskin 1974, 1983). Spring annuals germinate, flower, mature, disperse seeds, and die in spring or early summer. Plants in some populations have been observed to flower and disperse seeds in autumn (Thompson 1994; Griffith et al. 2004).

The seasonal conditions during seed maturation differ among these life histories. Winter and summer annuals mature seeds in late spring or summer, under long photoperiods and warm temperatures. Their seeds experience warm summer conditions immediately after dispersal. Seeds from typical spring-flowering winter annuals have been shown to require warm summer after-ripening to germinate in autumn (Baskin and Baskin 1983). In contrast, autumn-flowering plants mature seeds during autumn or winter under short days and low temperatures, and the seeds experience cold winter conditions immediately after dispersal. Seeds from autumn-flowering plants may not share the same germination requirements as seeds from summer-flowering plants, and they may consequently germinate during different seasons. Munir et al. (2001) showed that, indeed, seeds that were matured under short days and then experienced cold temperatures (such as seeds matured on autumn-flowering plants) germinated to high percentages under controlled conditions, while seeds matured under long days (as in summer) did not respond to cold as strongly. Thus, the photoperiod during seed maturation on the maternal plant altered germination responses to seasonal cues experienced by seeds in ways that could promote germination in the spring (as opposed to autumn) of seeds that matured during the autumn. Such maternal effects on germination may influence entire life-history schedules, possibly enabling two generations to be completed within a single year (Donohue 2005). Therefore, characterizing seasonal environmental effects on germination in *A. thaliana* pertains directly to the population demography of weedy species.

Like many weeds, *A. thaliana* is highly selfing (Abbott and Gomes 1989), and successive generations of selfing create genomes that are primarily homozygous (Todokoro and Terauchi 1995; Berge et al. 1998; Bergelson et al. 1998). *Arabidopsis thaliana* is also a highly mobile species. Its tiny seeds apparently are quite efficient at moving long distances through soil transport, air, or other means. Populations that are geographically close are therefore not necessarily closely related genetically (Sharbel et al. 2000; Hoffmann 2002). Rare outcrossing events between such populations can create diverse recombinant genotypes, potentially providing diverse genetic material that can contribute to evolutionary responses to natural selection (Ellstrand and Schierenbeck 2000; Lexer et al. 2003; Rieseberg et al. 2003a,b; Weinig et al. 2003; Griffith et al. 2004).

Experimental outcrossed segregants offer an experimental tool that is valuable for characterizing the genetic basis and adaptive value of germination. In particular, because selection on germination can be such an early and strong selective filter (Donohue et al. 2005), it is necessary to create recombinant lineages experimentally to discover a representative array of possible natural germination phenotypes; standing adult populations would already have been depleted in variation for germination. Recombinant inbred lines, moreover, disrupt linkage disequilibrium more completely than F_2 hybrids and thereby enable us to distinguish genetic constraints on phenotypes that are due to linkage disequilibrium as opposed to pleiotropy (although very tight linkage would not be detected). Recombinant inbred lines also enable replicates of essentially identical genotypes to be grown under diverse environmental conditions for the direct evaluation of environment-dependent genetic expression and the genetic basis of phenotypic plasticity. In this study, we used recombinant inbred lines derived from two natural populations that differ strongly in their germination responses to the seasonal cues of photoperiod during seed maturation and temperature experienced after dispersal (Munir et al. 2001). These lines therefore provide ideal material for investigating the genetic basis and adaptive value of germination responses to seasonal environmental factors.

In this study, we manipulated seasonal environmental factors experienced by phenotypically diverse recombinant in-

bred lines to identify mechanisms of seasonal maternal effects on germination timing in the field and to quantify the role of pleiotropy in constraining the evolution of germination responses to seasonal environments. We manipulated, under controlled conditions, the photoperiod under which seeds were matured to represent the photoperiod during summer and autumn when plants in the field are maturing seeds. We also manipulated the postdispersal seasonal conditions experienced by seeds by depositing them into the field during late spring and autumn (see also Galloway 2001, 2002) in two sites. We then monitored the germination of these seeds to determine the effects of geographic location, maternal photoperiod, and postdispersal seasonal conditions on germination and on the expression of genetic variation for germination. Through these experimental manipulations, we addressed the following questions. Do maternal seasonal effects influence germination in the field? If so, do maternal effects operate more strongly by determining the photoperiod during seed maturation or by determining the postdispersal seasonal environment? Does the expression of genetic variation for germination depend on geographic location and seasonal conditions? Does transgressive segregation occur for germination, and does it depend on seasonal environmental factors and location? Finally, is pleiotropy a strong constraint on the evolution of plasticity of germination to different seasonal cues?

Materials and Methods

The Genetic Material

We used seeds from two natural ecotypes, one from Calver, England (Cal) and one from Tacoma, Washington (Tac) to create a set of recombinant inbred lines. The natural phenology of these ecotypes is unknown in the field, but experiments conducted under laboratory conditions indicate that they differ in flowering and germination and that their recombinant progeny exhibit variation in germination responses to maternal photoperiod and to seed cold stratification (Munir et al. 2001). Our site in Rhode Island, which is near the coast like the parental sites, is likely to be more similar to the native sites of both ecotypes than is our site in Kentucky.

Cal seeds were acquired through the Arabidopsis Biological Resource Center at Ohio State University (Stock CS1062). Tac seeds were collected by T. Mitchell-Olds in 1990, and this stock was maintained through single-seed descent for two generations before crossing. Recombinant inbred lines were created by crossing one genotype from Cal to one from Tac, with Tac as the maternal line. One hundred twenty lineages were maintained for six to eight generations by single-seed descent, resulting in 98.4–99.6% homozygosity. Stocks were maintained on a 12-h photoperiod at 22°C with no vernalization. Seeds were planted after two weeks of cold stratification at 4°C for all generations prior to this experiment. 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 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 and the season during which seeds were dispersed, and we dispersed seeds in two locations: Rhode Island (RI) and Kentucky (KY). In KY, natural populations flower only during the spring, so we dispersed seeds in KY only during the late spring. In RI, populations flower both during spring and autumn, so we dispersed seeds in RI both during late spring and autumn. From this design, the effect of maternal photoperiod can be determined within each site and season of dispersal. The effect of site and its interaction with maternal photoperiod on germination can be determined by comparing the seeds dispersed in KY and RI during June. The effect of season of dispersal on germination and its interaction with photoperiod can be determined by comparing the seeds dispersed in RI during June and November. Long photoperiod with June dispersal and short photoperiod with November dispersal are natural combinations, whereas the other combinations, are not natural or are atypical.

Plants were grown in two batches to provide seeds for dispersal into the field during June and November. In each temporal planting, six replicates of each recombinant genotype were grown in a short-day treatment and a long-day treatment in Conviron E7/2 growth chambers (Controlled Environment Ltd., Pembina, ND) at 22°C. The replicates were distributed over two blocks (three replicates per block), with each growth chamber compartment serving as a block. The short-day photoperiod consisted of 10 hours of full-spectrum light (followed by 14 hours of darkness), which is the shortest photoperiod that plants are likely to experience when they flower in the autumn. The long-day photoperiod consisted of 14 h of full-spectrum light (followed by 10 h of darkness), which resembles the photoperiod under which plants are likely to mature seeds during late spring in North America. To synchronize flowering time across the two treatments, the long-day treatment was planted one month after the short-day treatment.

Seeds were collected from each plant as they matured, and seeds were pooled across replicates. A growth chamber malfunction during the first planting imposed near-freezing conditions on one of the blocks of each treatment during the seed collection period, and a second malfunction occurred in one of the long-day blocks in the second planting. Seeds from these cold-shocked plants were not used whenever possible, but a few late-flowering genotypes did not have enough seeds from the normal block alone, so some seeds from the cold-shocked plants were used. We tested for effects of cold shock on germination in a separate experiment (details available upon request), but we found none except for a significant genotype $\times$ cold-shock interaction for seeds dispersed in KY ($F = 2.25$, df $= 14$, $P = 0.01$).

After seeds from all genotypes were collected, 10 seeds from a given genotype and maternal photoperiod combination were put into separate microcentrifuge tubes. Some genotypes did not have enough seeds for 10 seeds per tube. Fewer seeds were used per tube for these genotypes, and these ge-

notypes were not used to assess germination if they had fewer than seven seeds per tube. Seed collection and processing took three to four weeks, during which time seeds were maintained in the centrifuge tubes at room temperature (23°C). Seeds were deposited into the field as soon after collection as was possible to provide the seeds with some natural seasonal conditions for after-ripening. While this period of dry after-ripening in the laboratory reduced dormancy before seeds were deposited into the field, it was necessary for all genotypes to receive the same treatment to conduct a genetic analysis. It should be noted that those seeds that germinated soon after dispersal into the field are likely to be a combination of congenitally nondormant seeds, seeds that lost dormancy during dry after-ripening, and seeds that retained weak primary dormancy.

In both KY and RI, experimental gardens were prepared in old-field sites by plowing in the autumn and discing in the spring as soon as the ground thawed. 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). Because the same soil was used to fill pots in both sites, comparisons across sites control for differences in soil quality, although some drainage differences would remain. In addition, the vegetation canopy was minimal at this early successional stage in both sites. The primary difference between sites, therefore, was climatic. The pots were planted into blocks with approximately 5 cm between them and were covered with mason jar lid rims fitted with wire mesh screen and transparent organdy cloth to prevent seed contamination from surrounding plants. The screens and cloth did not significantly alter the temperature (average over a two-week period in early June: with screen and cloth = 21°C, without = 20°C) or light levels (with = 312 μmoles f^{-2}, without = 328 μmoles f^{-2}) significantly, but relative humidity was slightly higher with the screen and cloth (with = 33% without = 26%; $F = 41.27$, df = 1, $P < 0.001$). Control pots were planted to assess background seed contamination and possible misidentification of very young seedlings. These pots were covered and uncovered at the same time as the experimental pots, and they were censused at the same time as the experimental pots. Background contamination was low, with an average of 0.13 seeds germinating in the control pots in RI and 0.24 seeds germinating in control pots in KY. Seeds were deposited in KY from 1 to 4 June (photoperiod = 14.5 h, mean daily high temperature in June = 27.6°C, mean daily low = 16.0°C, maximum temperature = 32.8°C, minimum temperature = 8.3°C, precipitation = 6.6 cm), and they were deposited in RI from 20 to 23 June (photoperiod = 15 h, mean daily high temperature within a month of dispersal = 25.3°C, mean daily low = 16.7°C, maximum temperature = 33.3°C, minimum temperature = 10.5°C, precipitation = 4.3 cm). The second batch of seeds was deposited in RI from 3 to 6 November (photoperiod = 10 h, mean daily high temperature in November = 14.2°C, mean daily low = 3.2°C, maximum temperature = 21.7°C, minimum temperature = −6.7°C, precipitation = 1.0 cm).

Three blocks were established in KY, each consisting of a 24 × 92 pot array with access strips every six pots. This array included pots that were used for purposes other than for the analysis presented here (for more details see Donohue et al. 2005). For this analysis, three replicates of each genotype and photoperiod combination were randomly positioned within each block. In RI, nine blocks were established in 7 × 103 pot arrays for each season of dispersal (again, including pots not analyzed here). June-dispersal blocks alternated with November-dispersal blocks on the site. One replicate of each genotype and photoperiod combination was randomly positioned within each block. To compare KY to RI, blocks were combined to give three blocks of the same size as the KY blocks.

Because the presence of a rosette very likely alters the germination behavior of ungerminated seeds, all seedlings that germinated before March were plucked from the pots after recording their date of germination. 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 and the average day of germination were calculated for each pot. The total number of pots used in this analysis was 6120 pots or approximately 60,000 seeds (seeds dispersed in June: 110 lines × 2 maternal photoperiods × 2 sites × 9 replicates = 3960; seeds dispersed in November: 120 lines × 2 maternal photoperiods × 9 replicates = 2160; total = 6120). Not all pots had enough seeds or germinants for accurate germination estimates, and the total number of pots available for analysis was 4027.

Statistical Analyses

To determine the effects of photoperiod, site, and season of seed dispersal on germination, the average germination day (Julian day) and total proportion germination were analyzed using mixed-model analysis of variance (Proc GLM, SAS Institute 1990). We also analyzed germination timing as the number of days from seed dispersal into the field until germination, but the results did not differ unless indicated. To compare germination between the two sites, the seeds dispersed in KY and RI during June were analyzed, with photoperiod and site as fixed factors and genotype and block as random factors. Block was nested within site. The effect of photoperiod was tested over its interactions with block and genotype, and the effect of site was tested over its interactions with genotype. Results did not differ when we pooled the interactions with block with the error. To compare germination between the two dispersal seasons, the seeds dispersed in RI during June and November were analyzed in a separate model, with photoperiod and dispersal season as fixed factors and genotype and block as random factors. Block was nested within season. The effect of photoperiod was tested over its interactions with block and genotype, and the effect of season was tested over its interactions with genotype. Again, results did not differ if interactions with block were pooled with the error. Because of significant three-way interactions in these analyses, the effect of photoperiod was also tested separately within each site and dispersal season treatment. The residuals of all analyses were somewhat leptokurtic regardless of transformation, so probabilities from these analyses are only approximate.

We examined whether germination during different seasons was independent, or whether genotypes that germinated

TABLE 1. Results of analysis of variance comparing seeds dispersed in Kentucky and Rhode Island during June, based on Type III sums of squares. ndf, numerator degrees of freedom; ddf, denominator degrees of freedom.

Source	Day of germination			Total proportion germination		
	ndf/ddf	*F*	*P*	ndf/ddf	*F*	*P*
Site	1/6.6	193.34	<0.001	1/5.6	400.46	<0.0001
Photoperiod	1/50	5.45	0.024	1/21	0.32	0.576
Genotype	112/91	1.71	0.004	112/112	0.54	0.999
Block (site)	4/7	15.39	0.001	4/4	16.93	0.007
Site × photoperiod	1/32	0.59	0.446	1/6	0.02	0.892
Site × genotype	111/116	1.93	<0.001	112/83	2.51	<0.0001
Photoperiod × genotype	100/89	1.45	0.038	102/99	1.72	0.004
Photoperiod × block (site)	4/366	0.63	0.641	4/402	0.57	0.683
Genotype × block (site)	427/351	1.37	0.001	446/400	1.09	0.197
Site × photoperiod × genotype	93/440	2.22	<0.0001	97/398	1.16	0.164

in one season tended to germinate in another season more frequently than expected. This analysis also identified genotypes that germinated in only one season. For each genotype, we determined whether seeds germinated during each pulse of germination in the early summer (immediate), midsummer, autumn, winter, and spring, and categories were assigned based on the pulses observed in each treatment. We then tallied the number genotypes that germinated in each combination of seasonal class. Using χ^2, we tested whether germination occurred more frequently in certain combinations of classes than would be expected by the marginal frequencies of the germination classes. For example, we tested whether spring-germinating genotypes were more likely to comprise those that also germinated in autumn or those that also germinated during the previous summer. In this manner, we could identify the extent to which seasons are most similar with respect to eliciting germination for our set of genotypes.

Genetic correlations across environments were calculated based on genetic variance components using maximum-likelihood analysis (Proc Mixed, SAS Institute 1990; Fry 1992). Correlations across photoperiod were estimated within each site and dispersal season combination. Correlations across site were estimated for each photoperiod by comparing seeds dispersed in KY and RI during June. Correlations across dispersal season were estimated for each photoperiod by comparing seeds dispersed in RI during June and November. Significant differences from zero or one were tested using restricted maximum likelihood (REML), and the hypothesis of equal variances across environments was also tested. Differences between the -2 log residual likelihood were compared to a χ^2 distribution with one degree of freedom. Because germination data were not normally distributed, Spearman rank correlations across environments were also calculated based on genotypic means. It should be noted that, because Spearman rank correlations based on genotypic means do not include within-genotype variance, significance levels of Spearman correlations tend to be higher than for estimates based on individual phenotypes (such as the REML method used here).

RESULTS

Environmental and Genotypic Effects on Germination

Germination timing and proportions depended strongly on the site in which seeds were dispersed (Table 1, Fig. 1), with the mean germination timing being faster and the proportion of germination being higher in KY. Most seeds dispersed in KY germinated almost immediately (Fig. 2A) possibly because they experienced unusual rain and cool weather as soon as they were deposited. Another pulse of germination occurred in autumn, and a small number germinated the following spring. In contrast, several seeds that were dispersed in RI during June germinated immediately, while others germinated throughout the summer and autumn (Fig. 2B). A small number of seeds even germinated in the winter, and another small pulse of germination occurred in spring, as in KY. The slightly longer period of dry after-ripening before seeds were deposited in the field in RI is expected to decrease dormancy, so the differences in germination schedules across sites are unlikely to be due to the difference in after-ripening before dispersal. In RI, a much higher proportion of seeds did not germinate than in KY (Fig. 1B) due to long-term dormancy, seed mortality, or seed loss. We were not able to distinguish these alternative fates because seeds were deposited onto soil, which made them nearly invisible and prevented us from recovering ungerminated seeds. Seeds dispersed in KY had a slightly lower proportion of spring germination (<1%, SD = 3%) than seeds dispersed in RI (6%, SD = 20%). The effect of site on germination timing and proportions depended strongly on genotype, as indicated by the significant genotype-by-site interaction (Table 1; Figs. 3, 4). Significant genetic variation for plasticity to geographic location therefore is expressed in this sample of recombinant lineages derived from only two inbred parents.

The season of seed dispersal strongly altered germination timing and proportions in RI (Table 2; Figs. 1, 2), with later (in Julian days) but more rapid (sooner after dispersal in the field) germination and a higher proportion of germination of seeds dispersed in November. The effect of dispersal season was due mostly to the germination during summer of seeds dispersed in June (Fig. 2B) and to the much broader range of germination timing in June-dispersed seeds. Seeds dispersed in November germinated much more synchronously than those dispersed in June (Fig. 2C). Seeds dispersed in November had a similar proportion of spring germinants (8%, SD = 10%) as seeds dispersed in June (6%, SD = 20%). Therefore, dispersal in November did not promote spring germination more than dispersal in June. The effect of dispersal season on germination time and proportion depended strongly on genotype, as indicated by the significant inter-

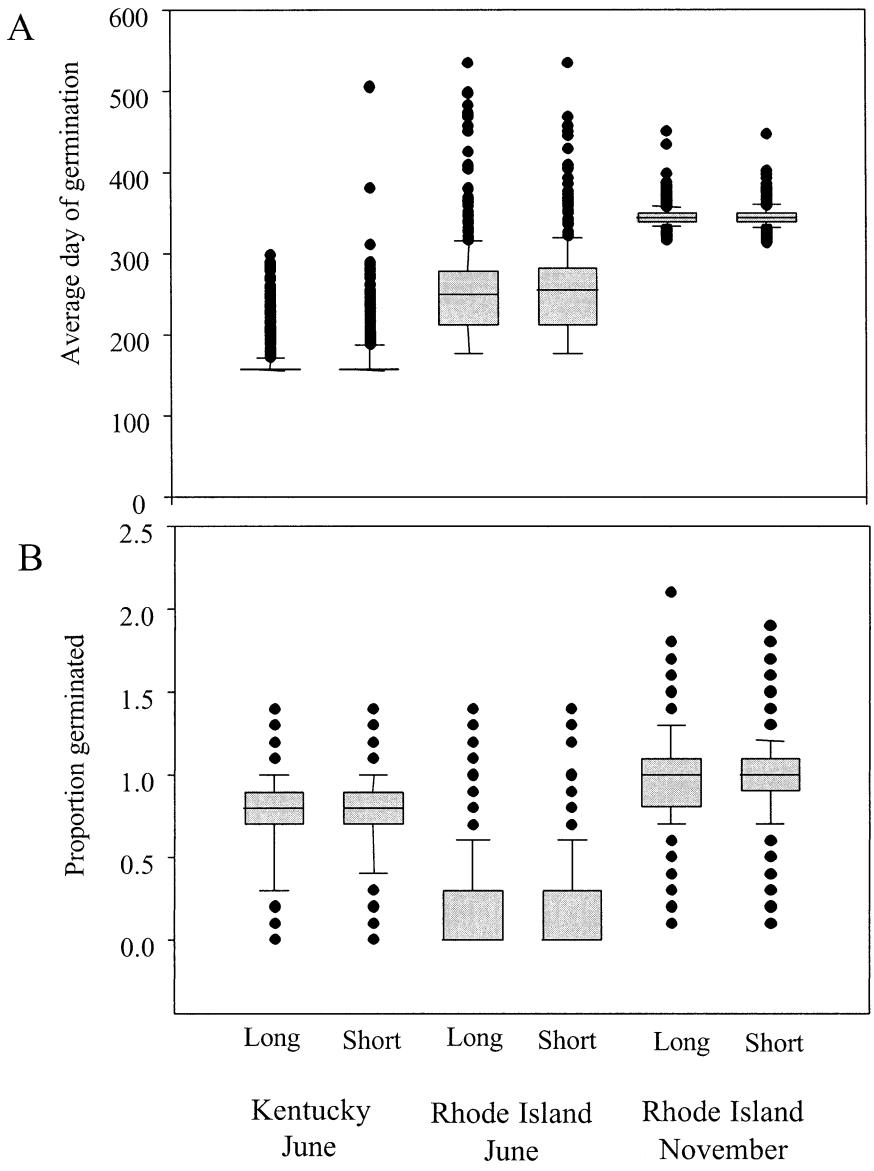

FIG. 1. Average day of germination in Julian days (A) and germination proportions (B) in the different treatments, based on values of individual pots. Box plots show mode, quartiles, and range for seeds matured in long and short days and dispersed in Kentucky during June, Rhode Island during June, and Rhode Island during November. Proportions above 1.0 are due either to extra seeds of a given genotype and treatment accidentally being deposited, background contamination, or misidentification of seedlings. The greater number of pots that appear to have contamination (i.e., above 100% germination) in the November treatment is only because germination proportions were already close to 1.0. The number of outliers is similar across treatments. Therefore, the effect of contamination is randomly distributed over the experiment, so there was no need to adjust proportions for analysis.

action between genotype and dispersal season (Table 2; Fig. 3, 4). Therefore, significant genetic variation was detected for plasticity of germination to season of dispersal.

A significant effect of maternal photoperiod on germination timing was observed in seeds dispersed in June (Table 1). While there was no significant interaction between site and photoperiod, a main effect of the maternal photoperiod detected only in KY (Table 3). In KY, seeds matured under short days had slightly later germination than seeds matured under long days (Fig. 1A). No significant effect of maternal photoperiod was detected for proportion germination when the whole sample was analyzed. When considering only those seeds that did not germinate immediately (i.e., more strongly dormant seeds), however, seeds matured under short days germinated to a significantly higher percentage in KY (12% of those remaining) than seeds matured under long days (6%; $F = 122.59$, df = 1,2, $P = 0.008$, $N = 1621$). Therefore, at least under KY conditions, short days during seed maturation promoted the germination of dormant seeds although photoperiod did not affect the germination proportion of nondormant or weakly dormant seeds.

The effect of photoperiod depended on the particular combination of site and genotype (Table 1) or dispersal season and genotype (Table 2), as indicated by the significant three-way interactions. When data were analyzed separately within each site and dispersal season, significant genetic variation

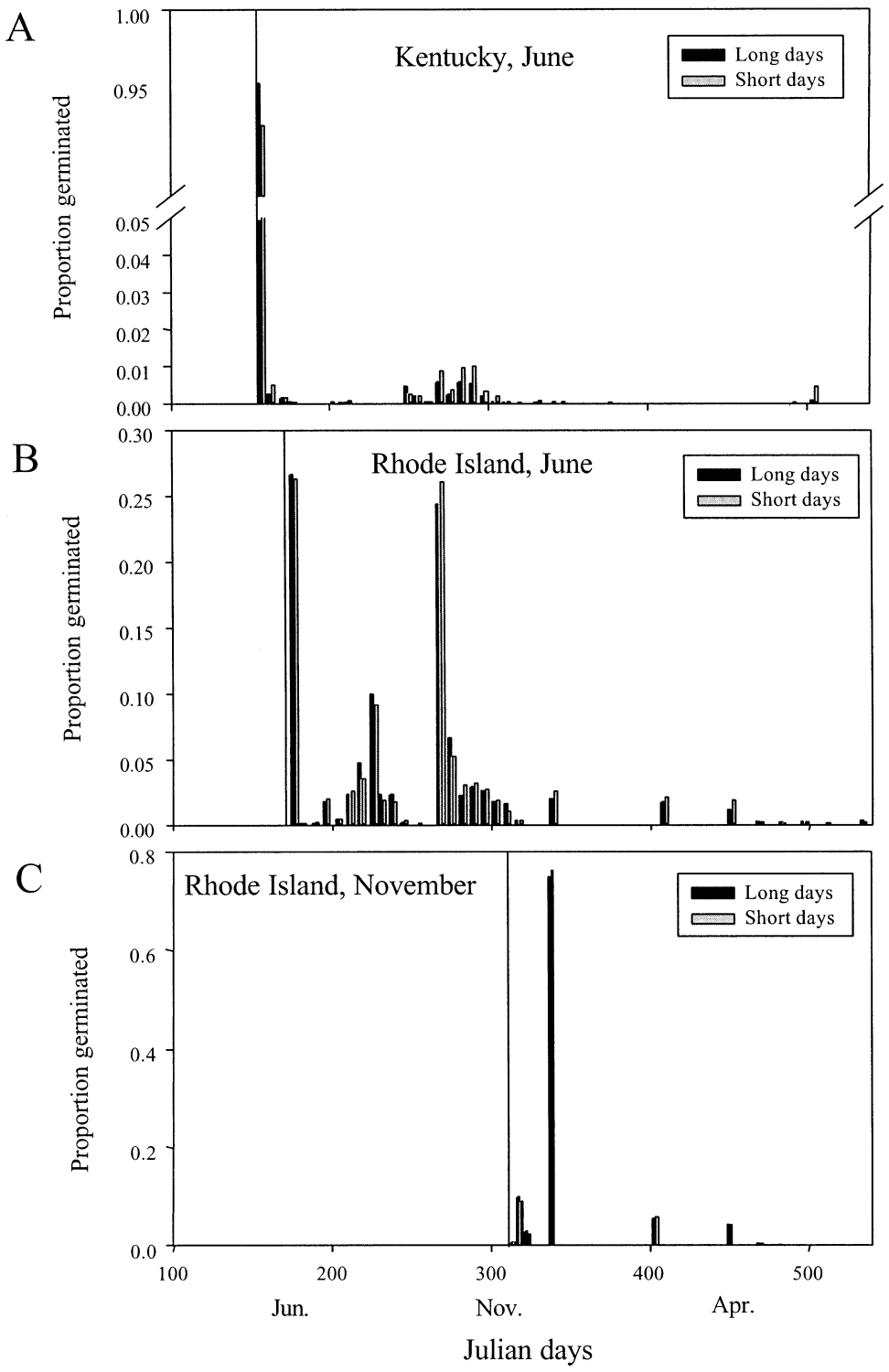

FIG. 2. Germination schedule of seeds dispersed in Kentucky during June (A), Rhode Island during June (B), and Rhode Island during November (C). Proportion of all seeds that germinated (*y*-axis) over time (*x*-axis). Days are in Julian days, with 1 January, 2001 being the first day. Vertical line indicates the day on which seeds were deposited in each treatment. Black bars represent seeds matured under long days, and gray bars represent seeds matured under short days.

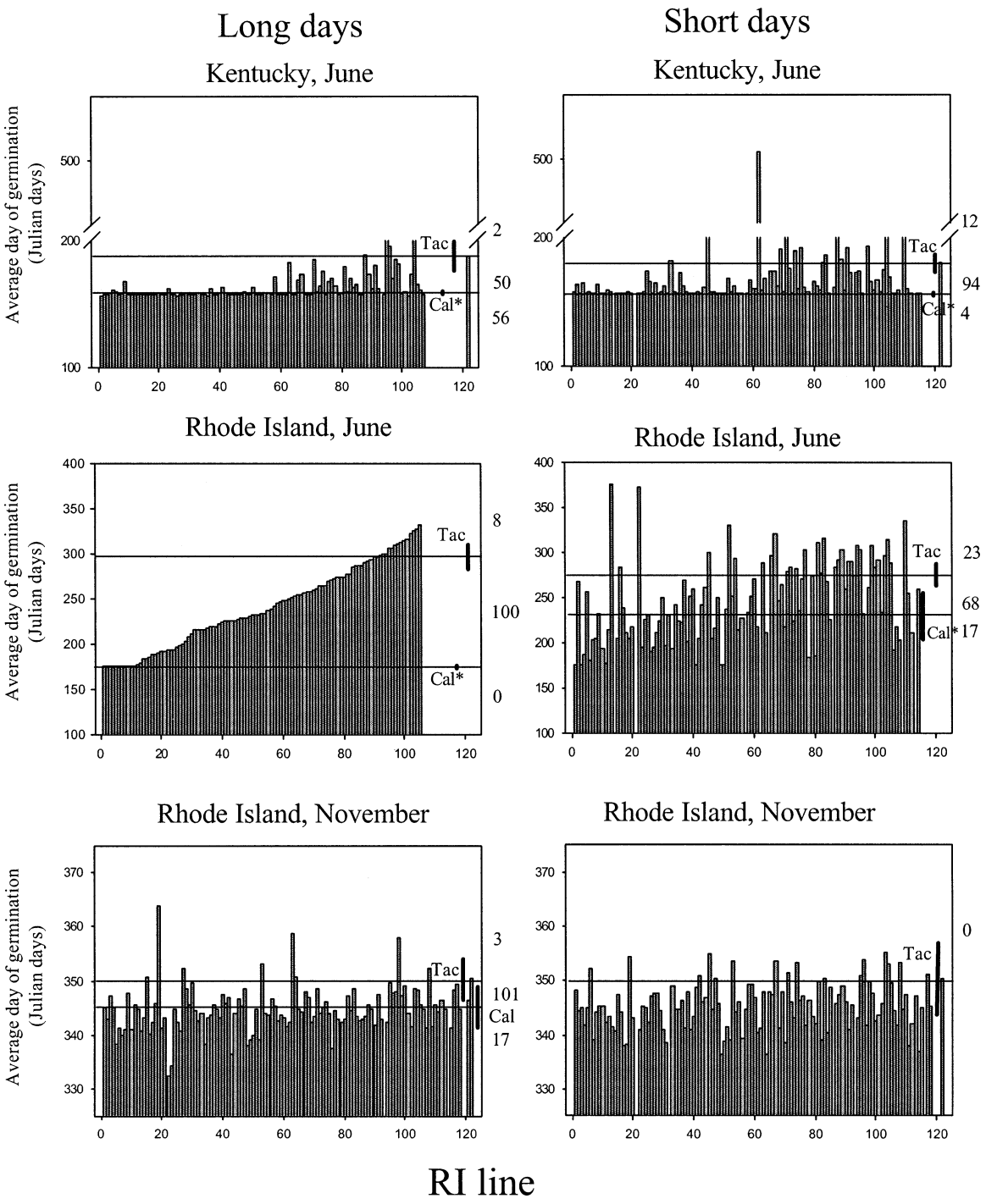

FIG. 3. Average day of germination of seeds matured under short days and long days in each treatment. Each bar represents the mean phenotype of a separate recombinant inbred line. The order of the genotypes is the same in all graphs and is based on their rank in the Rhode Island, June treatment. Horizontal lines indicate the mean phenotype of the parental lines (Cal and Tac), and black bars indicate the standard error of parental means. The numbers to the right indicate the number of genotypes with mean phenotypic values above, between, or below those of the parental lines. Cal* indicates that the phenotype for the Cal parent was based on fewer than seven seeds per pot because of limited seed stock. Adapted from Donohue (2005) with the permission of the New Phytologist Trust.

for plasticity to photoperiod was detected for germination timing of seeds dispersed in June in both KY and RI but not of those dispersed in November in RI (Table 3). The interaction between genotype and photoperiod was most pronounced in KY, in which the main effect of photoperiod was strongest. Main effects of genotype were significant in all treatments, indicating genetic differences in germination timing across both photoperiods.

Significant genetic variation for plasticity to maternal photoperiod was detected for germination proportion in both KY

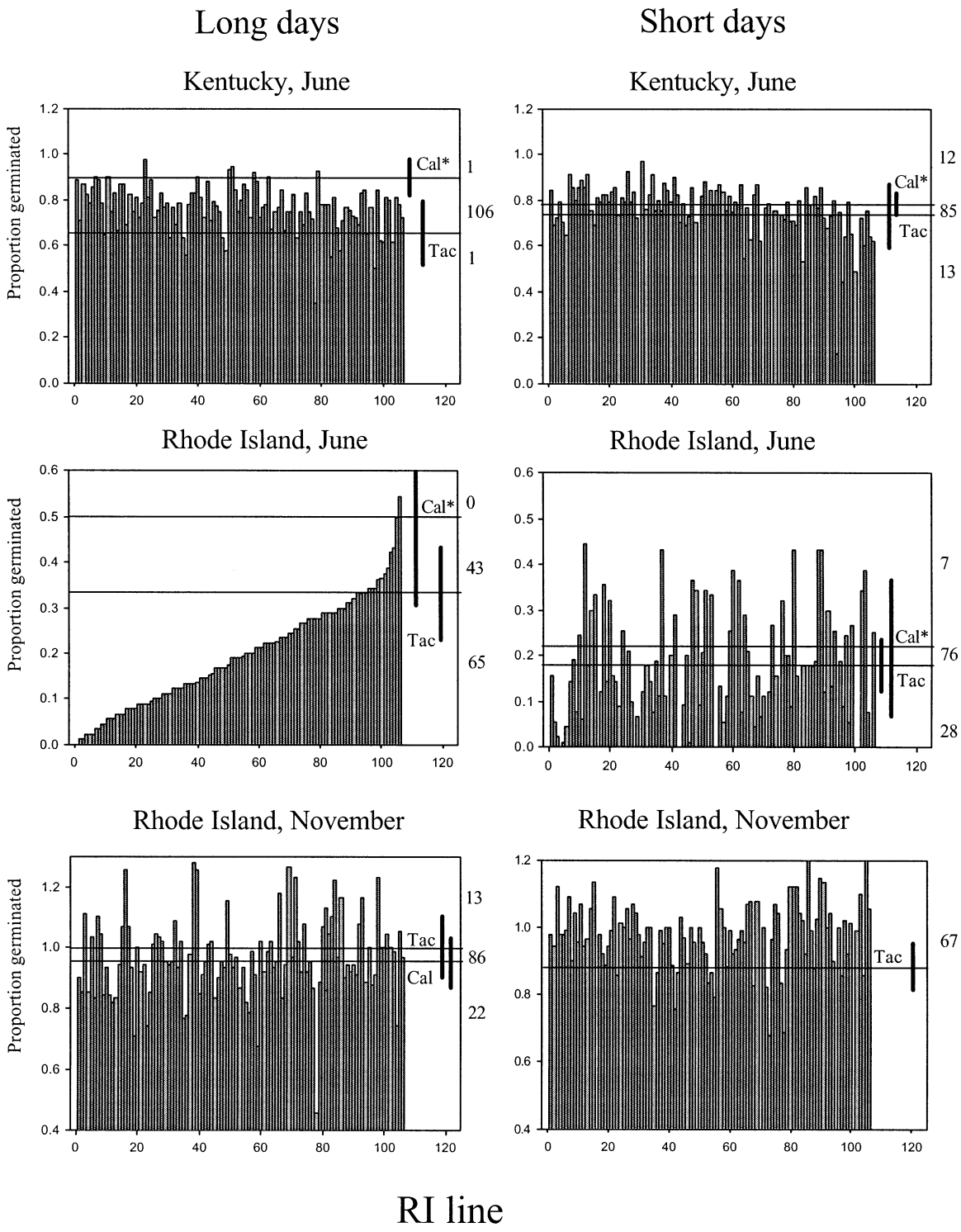

FIG. 4. Proportion of seeds that germinated in each treatment. Each bar represents the mean phenotype of a separate recombinant inbred line. The order of the genotypes is the same in all graphs and is based on their rank in the Rhode Island, June treatment. See Figure 3 for further details.

and RI, although it was only nearly significant for seeds dispersed in June in RI (Table 3). Main effects of genotype were significant or nearly significant (in KY) in all treatments, indicating genetic variation in germination proportions across both maternal photoperiods.

In summary, environmental effects on germination were strong, with maternal effects operating much more strongly through effects of dispersal season than through effects of maternal photoperiod. Maternal photoperiod effects were only expressed in seeds dispersed in June (and significantly

TABLE 2. Results of analysis of variance comparing seeds dispersed during June and November in Rhode Island, based on Type III sums of squares. ndf, numerator degrees of freedom; ddf, denominator degrees of freedom.

Source	Day of germination			Total proportion germination		
	ndf/ddf	*F*	*P*	ndf/ddf	*F*	*P*
Season	1/14	409.44	<0.0001	1/5	547.70	<0.0001
Photoperiod	1/28	2.62	0.117	1/13	0.5	0.492
Genotype	121/70	1.03	0.459	121/81	1.07	0.382
Block (season)	4/7	8.72	0.008	4/1	138.22	0.158
Season × photoperiod	1/44	1.18	0.284	1/11	2.85	0.119
Season × genotype	111/106	2.56	<0.0001	112/63	2.48	<0.0001
Photoperiod × genotype	116/85	0.71	0.959	116/98	1.10	0.315
Photoperiod × block (season)	4/414	0.86	0.489	4/432	0.32	0.868
Genotype × block (season)	447/391	1.39	0.0005	464/430	0.84	0.967
Season × photoperiod × genotype	93/509	2.95	0.0001	98/429	1.30	0.040

only in KY). Genetic variation was pronounced in these recombinant lines for germination and for responses of germination to pre- and postdispersal environmental factors and geographic location.

Transgressive Segregation of Germination

Novel germination phenotypes that exceeded those of either parental line were created through hybridization of the Cal and Tac ecotypes (Figs. 3, 4). Transgressive segregation of both germination timing and germination proportion occurred in most treatments. In KY, recombinant genotypes tended to germinate earlier than either parental line if they were matured under long days (Fig. 3). Transgressive segregation for germination proportion, in contrast, was somewhat more pronounced in seeds matured under short days in KY (Fig. 4). Recombinant lineages germinated to both higher and lower proportions than both parental lines.

For seeds dispersed in RI during June, little transgressive segregation was observed for germination timing when seeds were matured under long days, with a small number germinating later than both parental lines (Fig. 3). Some recombinant lineages matured under short days, however, germinated earlier than either parental line, and others germinated later. For germination proportion, a large number of recombinant genotypes matured under long days had lower germination proportions than both parents. Some transgressive genotypes matured under short days had higher germination proportions than both parents, but most of them had lower germination proportions (Fig. 4).

For seeds dispersed in RI during November, seeds of the Cal genotype that were matured under short days did not germinate, so transgressive segregation could not be evaluated in that treatment. For seeds matured under long days, transgressive segregation for germination time tended to create genotypes that germinated earlier than either parental line, as in KY (Fig. 3). Transgressive segregation for germination proportion created genotypes that germinated to both lower and higher proportions than either parental line (Fig. 4).

Restricted Versus Variable Germination of Genotypes: Similarity of Seasons as Perceived by Seeds

In KY, most genotypes were restricted to germinating immediately after dispersal, but one genotype germinated only in autumn, and some genotypes germinated in more than one season (Table 4). The genotypes with variable seasons germinated in the different combinations of seasons in proportion to their marginal frequencies. That is, they did not detect similarities in the different seasons but germinated in each season with a frequency that was independent of their tendency to germinate in any other season.

Very few genotypes dispersed in RI during June germinated only during a single season (Table 4), but 75 of them germinated only in early summer and midsummer. Genotypes with variable seasons of germination and that germinated in autumn had more than expected germination in the winter and spring. Genotypes that germinated in winter had more than expected germination in autumn and less than expected germination in other classes. Thus, genotypes reacted to autumn and winter in a similar manner, and the reaction to summer was dissimilar to all other seasons.

Nearly all genotypes that were dispersed in RI during November germinated in all seasons, and genotypes exhibited completely independent germination in the different classes (Table 4). For seeds dispersed in November, therefore, the genotypes reacted similarly to all seasons.

In summary, for seeds dispersed in June, many genotypes germinated only in the summer and only one genotype germinated only in autumn. For seeds dispersed in November, genotypes were not restricted to germinating in only one season but had more variable germination.

Seeds dispersed during different seasons displayed completely independent germination from one another ($\chi^2 < 0.02$, $P \gg 0.05$ for all comparisons). For example, genotypes that were dispersed in June and germinated in winter did not germinate in winter if they were dispersed in November at a frequency higher than expected based on independence. Therefore, the June and November postdispersal environments appear to be distinct based on the reaction of genotypes.

Genetic Correlations across Environments

The significant genotype-by-environment interactions that were observed could be due to differences in the amount of genetic variation expressed in each environment and to differences in the ranking of genotypes in the different environments. We tested whether genetic variances for germination timing and proportion were the same in the different environments and estimated the strength of genetic correla-

TABLE 3. Results of analysis of variance to test for effects of photoperiod and genotype on germination in each treatment, based on Type III sums of squares. *F*-ratios are given for analyses of germination day and proportion germination (Prop.). ndf, numerator degrees of freedom; ddf, denominator degrees of freedom.

Source	Kentucky, June				Rhode Island, June				Rhode Island, November			
		Germination day				Germination day				Germination day		
	ndf/ddf		ndf/ddf	Prop.	ndf/ddf		ndf/ddf	Prop.	ndf/ddf		ndf/ddf	Prop.
Photoperiod	1/40	7.73**	1/8	0.03	1/5	1.97	1/5	0.13	1/2	0.39	1/9	2.82
Genotype	112/117	1.39*	112/105	1.31†	111/63	3.08***	112/50	2.29***	121/37	2.89***	121/68	2.73***
Block	2/6	35.45***	2/4	6.98†	2/1	14.36	2/2	111.57	2/2	5.48	2/4	188.6
Photoperiod × genotype	99/198	4.17***	100/200	1.89***	94/143	1.44*	99/198	1.30†	115/230	0.92	115/230	1.46**
Photoperiod × block	2/199	0.59	2/201	0.84	2/146	0.51	2/201	0.33	32/231	2.76†	2/230	0.28
Genotype × block	222/198	1.56***	224/200	1.39**	205/135	1.09	222/200	0.81	242/230	0.81	242/230	0.87
Photoperiod × genotype × block	196/1258	1.41***	198/1329	0.93	125/473	1.04	198/1312	1.02	230/1450	0.85	230/1450	0.96

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

tions across environments. Genetic correlations that are not significantly different from 1 if positive, or −1 if negative, represent significant genetic constraints on the evolution of plasticity; selection in one environment would cause strong correlated selection of that trait expressed in the alternate environment.

For day of germination, genetic correlations across maternal photoperiod, site, and dispersal season were often significant, indicating some common genetic basis of germination timing in the different environments (Table 5). Many were also significantly different from one, however, indicating some independence of the genetic basis of germination in different environments. Genetic correlations were significantly positive across maternal photoperiod in all treatments. In the two treatments in which we detected significant main effects of maternal photoperiod or significant interactions between maternal photoperiod and genotype, namely the KY and RI-June treatments, this correlation was significantly different from one, indicating that the genetic constraint on the evolution of plasticity to maternal photoperiod is not absolute. In KY, genetic variation for germination timing was significantly greater in seeds matured under short days (the novel environment) than in seeds matured under long days (the typical environment).

Correlations across site for germination timing (Fig. 5A) were significantly greater than zero for seeds matured under both photoperiods (Table 5). However, these correlations were also significantly different from one, indicating that the genetic constraints across sites are not absolute. Genetic variation for germination timing was greater in RI than in KY.

Correlations across dispersal season for germination timing (Fig. 5A) were weak but significant for seeds matured under long days. Although weak, the estimates were also not significantly different from one at either maternal photoperiod (Table 5). Genetic variation was greater in seeds dispersed during June than for seeds dispersed during November.

For germination proportion, correlations across many environments were weak, indicating independent genetic determination of germination proportion in the different environments (Table 6). Correlations across maternal photoperiod were significant in RI but not in KY (significant when based on Spearman correlations). In KY, the correlation was also significantly different from one, and genetic variation was slightly greater in seeds matured under short days (the novel environment). In RI-June seeds, the correlation across maternal photoperiod was significantly different from one, but genetic variances were the same in both photoperiods. In RI-November seeds, the correlation was not significantly different from one, but genetic variation for proportion germination was greater for seeds matured under long days (the novel environment) than under short days. Therefore, the maternal photoperiod that enabled the expression of more genetic variation depended on the geographic location and season of dispersal; in both cases in which photoperiod-dependent genetic expression was observed, genetic variation was greater in the novel, or mismatched, photoperiod.

The genetic correlations for germination proportion across site (Table 6, Fig. 5B) were significantly negative for seeds matured under long days and not significantly different from −1. Therefore, those genotypes that germinated to high pro-

TABLE 4. Number of genotypes that had germinants in each combination of seasons for each site and dispersal season. Numbers are corrected for background germination by contaminants. Numbers in the diagonal classes are the numbers of genotypes that had germinants only in that season. Total genotypes indicates the total number of genotypes that had any germinants in each season. χ^2 tests the hypothesis that certain combinations of germination seasons occur more frequently than expected based on the marginal frequencies of germination seasons. Bold indicates cells that show significant deviations from expectation; (<) indicates that the observed number is less than expected, and (>) indicates that the observed number was greater than expected.

Kentucky, June	No. immediate within 3 weeks	No. summer 25 Jun–21 Aug	No. autumn 30 Aug–31 Oct	No. winter 7 Nov–25 Jan	No. spring 1 Mar and later
No. immediate	70				
No. summer	3	0			
No. autumn	37	1	1		
No. winter	1	0	1	0	
No. spring	3	0	0	0	0
Total genotypes	112	3	38	1	3
χ^2	0.00	0.11	2.36	0.83	1.09

Rhode Island, June	No. immediate within 3 weeks	No. summer 24 Jul– 27 Aug	No. autumn 3 Sep– 31 Oct	No. winter 7 Nov–6 Dec	No. spring 15 Feb and later
No. immediate	3				
No. summer	72	0			
No. autumn	74	77	0		
No. winter	**34 (<)**	**34 (<)**	**40 (>)**	0	
No. spring	**39 (<)**	**46 (<)**	**52 (>)**	**34 (<)**	0
Total genotypes	92	89	94	44	56
χ^2	0.60	0.15	29.23***	25.32***	16.21***

Rhode Island, November	No. autumn 11 Nov–20 Nov	No. winter 28 Nov–13 Dec	No. spring 15 Feb and later
No. autumn	0		
No. winter	120	0	
No. spring	120	122	0
Total genotypes	120	122	122
χ^2	0.0	0.0	0.0

portions in KY germinated to low proportions in RI. For seeds matured under short days, the correlation across site was weakly negative and nonsignificant (significant when based on Spearman correlations), and the correlation was significantly different from -1. Therefore, the trade-off in germination across sites was somewhat stronger when seeds were matured under long days. Genetic variance was the same in the two sites, indicating that any genotype-by-environment interactions were due more to changes in the ranks of the genotypes rather than changes in the degree of genetic variation.

Correlations across dispersal season for proportion germination (Table 6, Fig. 5B) were not significant and were significantly different from one, but genetic variance was the same in both dispersal seasons. The genotypes are therefore changing rankings in the different dispersal seasons. Germination proportion of a genotype in one dispersal season is therefore not predictable from its germination proportion in the other dispersal season.

DISCUSSION

The germination behavior of a single genotype depended on the photoperiod during seed maturation, the season of dispersal, and the geographic location to which it was dispersed. Seasonal maternal effects on germination operated more strongly by determining which season the seeds will experience immediately after dispersal (Galloway 2001, 2002) rather than by determining the photoperiod during seed maturation. Such extreme plasticity of germination as observed in this experiment indicates that germination behavior may be a major influence on variation in performance (Donohue et al. 2005) and variation in life histories (Donohue 2002; Weinig et al. 2003) of plants that inhabit variable seasonal environments over broad geographic ranges. Extensive environment-dependent transgressive segregation for germination occurred. While we found evidence for a genetically based trade-off in germination success across sites, we also found that pleiotropy was not a strong constraint on the evolution of flexible germination responses to seasonal environments. Therefore, these highly plastic germination responses are likely to have high evolutionary potential.

Plasticity of Germination

The season of seed dispersal strongly influenced germination phenology. Seeds dispersed in late spring had a much broader range of germination timing than seeds dispersed in autumn, and spring-dispersed seeds also exhibited either greater mortality or stronger between-year dormancy. Therefore, the ability of some maternal plants to mature and disperse seeds in autumn is expected to change population phenology and increase seed survivorship to germination, all else being equal. The dependence of germination timing on the

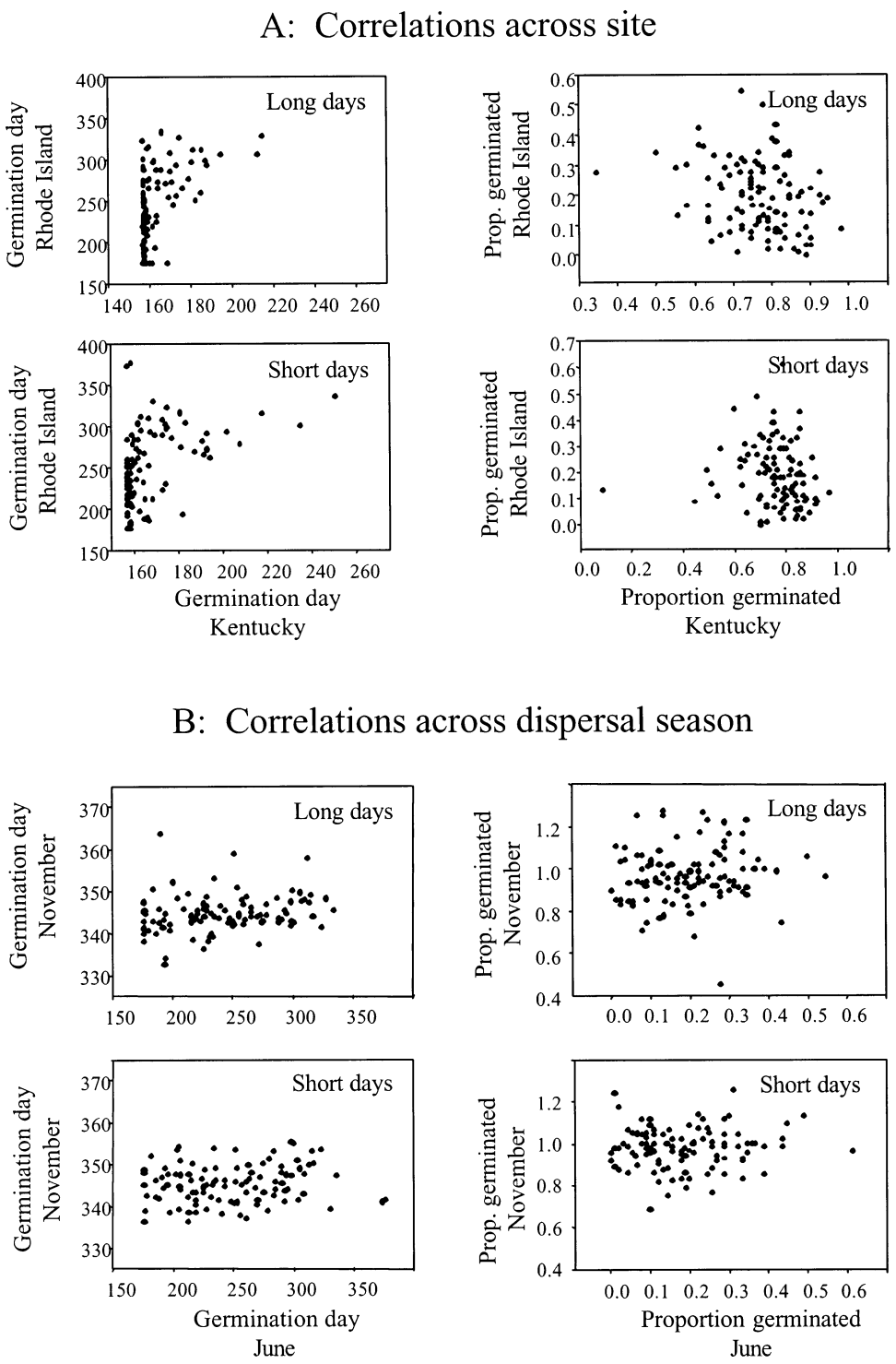

FIG. 5. Genetic correlations across sites (A) and dispersal seasons (B) for germination day (left) and germination proportion (right) of seeds matured under long days (upper) and short days (lower). Each point represents the mean phenotype for one genotype. Correlations across sites are based on seeds dispersed during June. Correlations across dispersal seasons are based on seeds dispersed in Rhode Island.

season of dispersal has been documented in *Campanula americana* as well (Galloway 2001, 2002), with seeds from early-flowering plants having a higher probability of germinating in autumn. In that species, the timing of germination, in turn, determines whether an annual or biennial life history is expressed. Seasonal dependence of germination timing can therefore strongly influence the basic life history of plants.

Maternal photoperiod effects on germination were not apparent in RI, but they were detected in KY. Munir et al. (2001) found that seeds matured under short days were more re-

TABLE 5. Genetic correlations across environments for germination day. ''Environment'' indicates the environments to be compared. Correlations across photoperiod are shown for seeds dispersed in Kentucky during June, Rhode Island during June, and Rhode Island during November. Correlations across site are shown for long-day and short-day seeds dispersed during June. Correlations across season are shown for long-day and short-day seeds dispersed in Rhode Island. ''Spearman *r*'' indicates the Spearman rank correlation based on genotypic means; asterisks in this column indicate that the correlation is significantly greater than zero. *N* ranges from 100 to 116 for Spearman *r*. ''REML *r*'' indicates the genetic correlation based on restricted maximum likelihood; asterisks in this column indicate that the correlation is significantly greater than zero. *N* ranges from 1987 to 2167 for REML *r*. ''Vg(1)'' indicates the genetic variance expressed in long days, Kentucky, or June. ''Vg(2)'' indicates the genetic variance expressed in short days, Rhode Island, or November. ''−2L(unres)'' indicates the −2 log likelihood ratio in an unrestricted model. ''−2L($r = 1$)'' indicates the −2 log likelihood ratio in a model in which the correlation across environments is constrained to be 1 if the unrestricted estimate was positive and −1 if it was negative; asterisks in this column indicate that the estimate was significantly different from 1 or −1. ''−2L(g1 = g2)'' indicates the −2 log likelihood ratio in a model in which the genetic variance is constrained to be equal in both environments; asterisks in this column indicate that the genetic variances were not equal in the two environments.

Environment	Spearman *r*	REML *r*	Vg(1) (SE)	Vg(2) (SE)	−2L(unres)	−2L($r = 1$)	−2L(g1 = g2)
Correlations across maternal photoperiod							
Kentucky	0.30***	0.30*	79.3 (16.1)	552.8 (133.0)	16,931.7	16,991.0***	16,967.0***
Rhode Island June	0.57***	0.84***	1438.7 (282.5)	1159.9 (246.8)	11,101.6	11,106.1*	11,102.4
Rhode Island November	0.37***	1.0***	6.4 (2.4)	6.1 (2.5)	16,708.4	16,708.4	16,708.4
Correlations across site							
Long days	0.49***	0.76***	79.3 (16.1)	1438.7 (282.5)	13,676.1	13,688.0***	13,749.3***
Short days	0.55***	0.81***	552.8 (133.0)	1159.9 (246.8)	14,334.9	14,361.3***	14,351.7***
Correlations across dispersal season							
Long days	0.23**	0.46*	1438.7 (282.5)	6.4 (2.4)	13,828.3	13,832.0	13,916.3***
Short days	0.14	0.37	1159.9 (246.8)	6.1 (2.5)	14,028.8	14,032.1	14,103.6***

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

sponsive to cold stratification and germinated to higher proportions than seeds matured under long days. They hypothesized that the increased germination of short-day seeds exposed to cold would promote germination of autumn-matured seeds during the following spring in the field. Contrary to that prediction, we did not observe in RI that a short photoperiod during seed maturation and dispersal in autumn promoted spring germination of seeds. Rather, most seeds that were dispersed in autumn (97% of those that did not germinate immediately) germinated quickly regardless of the photoperiod under which they were matured. Therefore, in the geographic location in which plants mature seeds under different photoperiods, photoperiod had a negligible effect. In KY, short days promoted the germination of dormant seeds, thereby increasing germination in autumn. This result is consistent with those of Munir et al. (2001), as short days promoted germination after seeds had begun to experience cold conditions in autumn. If seeds are matured during short days early in spring, then such seeds may germinate to higher proportions in autumn. Thus, maternal photoperiod may effectively influence seed germination under conditions when seeds are dormant, with short days alleviating some requirements to break dormancy. Alternatively, dormant seeds may have increased survivorship when matured under short days, as we could not distinguish dormancy from seed mortality in this experiment.

Geographic location also had large effects on germination. Germination behavior expressed in a new location therefore is not necessarily predictable from the behavior in the site of origin or in another site within the range. Geographic variation in germination phenology is not uncommon (e.g., Cruden 1974; van der Vegte 1978; Hacker et al. 1984; Hacker and Ratcliff 1989; Meyer and Monson 1991; Meyer et al. 1997). This study indicates that such variation could be in-

TABLE 6. Genetic correlations across environments for proportion germination. *N* ranges from 101 to 116 for Spearman *r*. *N* ranges from 1987 to 2167 for REML *r*. Column headings are as in Table 5.

Environment	Spearman *r*	REML *r*	Vg(1) (SE)	Vg(2) (SE)	−2L(unres)	−2L($r = 1$)	−2L(g1 = g2)
Correlations across maternal photoperiod							
Kentucky	0.26**	0.32	0.007 (0.002)	0.009 (0.002)	350.8	367.0***	369.9***
Rhode Island June	0.44***	0.71***	0.006 (0.002)	0.008 (0.002)	136.2	140.2*	137.0
Rhode Island November	0.36***	0.77***	0.010 (0.002)	0.005 (0.001)	−252.4	−249.0	−244.5**
Correlations across site							
Long days	−0.29*	−0.68**	0.007 (0.002)	0.006 (0.002)	360.7	364.3	360.7
Short days	−0.24*	−0.29	0.009 (0.002)	0.008 (0.002)	134.1	155.2***	134.1
Correlations across dispersal season							
Long days	0.15	0.17	0.006 (0.002)	0.010 (0.002)	76.2	103.8***	78.3
Short days	0.04	0.12	0.008 (0.002)	0.005 (0.001)	−154.3	−131.4***	−150.7

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

duced environmentally in addition to being determined genetically. The large difference in germination phenology in different geographic locations, coupled with the strong natural selection on germination timing (Donohue et al. 2005), indicates that germination responses to novel geographic location can be a strong influence on the ability of *A. thaliana* to establish in new locations.

Germination Timing as a Composite Trait

Germination timing is a composite trait that results from mechanisms of dormancy induction and maintenance, dormancy breakage, and germination after dormancy is broken (Léon-Kloosterzeil et al. 1996; Bewley 1997; Foley 2001). In those seeds that germinated very soon after dispersal, dormancy induction during seed development and/or the maintenance of dormancy after imbibition appear to have been deficient. Dormancy could also have been very weak, requiring only very short periods of cold to break it (as occurred in KY). Those seeds that were dispersed during June but that did not germinate until spring, in contrast, were strongly dormant; they experienced similar temperatures and photoperiod during autumn, but they did not germinate until after a period of winter stratification. Mechanisms of dormancy breakage appear to be important for determining the timing of germination of those seeds. For all seeds, mechanisms that determine permissive conditions for the germination of nondormant seeds also likely contributed to determining the seasonal timing of germination.

The geographical and seasonal contingency of germination schedules observed in this study indicates that genetic mechanisms of dormancy and germination operate differently in different conditions. The strong geographic and seasonal influence on the frequency of seeds that germinated immediately after dispersal suggests that physiological mechanisms of dormancy induction and maintenance are environmentally responsive. Transgressive segregation tended to create more early germinating, nondormant or weakly dormant genotypes, suggesting that dormancy maintenance requires the coordinated activity of combinations of genes. In addition, the variation in the frequency of autumn, winter, and spring germination indicates that the facility of dormancy breakage, most likely in response to cold, varied across genotypes. Recent evidence indicates that, in *A. thaliana*, both dormancy induction and breakage are genetically variable among natural populations, with novel genes associated with both processes being identified through analysis of quantitative trait loci (Alonso-Blanco and Koornneef 2000; Alonso-Blanco et al. 2003). A major challenge is to interpret germination timing in the field in terms of the underlying genetic mechanisms of dormancy induction, dormancy maintenance, and germination.

Generalist versus Specialist Germination

Several genotypes germinated only in summer when dispersed in June, but others germinated during different seasons. When seeds were dispersed in November, all genotypes germinated from autumn through spring. Genotypes that are limited to germinating only during one season will experience only one seasonal environment as adults, whereas those that germinated during several seasons will experience variable seasonal environments. In principle, specialized germination behavior can promote specialized life histories that are adapted to the particular seasonal environment following restricted germination (Evans and Cabin 1995; Donohue 2003), whereas more variable germination over different seasons may promote the evolution of generalist life histories. In this study, the ''specialized'' germination in summer is not an adaptation but a consequence of transgressive segregation that caused weak or no dormancy. Nevertheless, the observation that only some genotypes exhibited germination that was restricted to one seasonal condition indicates that it is possible for natural genotypes to vary in the frequency of seasonal environments that they experience. In addition, the contrast between seeds dispersed in June having more seasonally restricted germination versus seeds dispersed in November having more variable germination suggests that autumn-flowering genotypes could be selected to exhibit more generalist, or plastic, postgermination characters than strictly spring-flowering genotypes.

Genetic Constraints on the Evolution of Flexible Germination Responses

We found a genetically based trade-off in germination proportion across geographic location. Genotypes that germinated to high proportions in KY germinated to low proportions in RI, and this effect was stronger for seeds matured under long days. The result indicates that the same genes or closely linked genes are associated with germination proportion in the different locations; one allele of a gene may increase germination in KY but the same allele may decrease germination in RI. Therefore, pleiotropy or close linkage could be an important constraint on the evolution of germination success in different geographic locations. If germination proportion indicates seed viability, then this trade-off would directly limit the ability of a genotype to have high seed viability over a broad range of environments. The evidence for maternal photoperiod effects on this trade-off also suggests that such constraints may depend on flowering time. The fact that a significant trade-off in performance operates at such an early life stage (just at or even before germination) indicates that the very first stages of colonization, which are typically invisible to researchers, are likely to be especially important for determining which genotypes can establish in a given location in this sort of weedy species.

While we found trade-offs in germination proportions, we found no trade-offs for germination timing. Genetic correlations across environments for germination timing were less than one, and correlations across dispersal season were especially weak. This result indicates that some genes appear to control germination in one environment, while other genes control it in alternate environments; pleiotropy of genes controlling germination under different conditions is therefore not a severe constraint on the evolution of germination responses. While pleiotropy was not strong, correlations across some environments were often significantly different from zero, indicating some common genetic basis of germination in the different environments. Therefore, some genes appear

to control germination in multiple environments, while other genes control germination only under specific conditions.

We found abundant genetic variation for germination in some treatments, as well as genetic variation for germination responses to our experimental treatments. Genetic variation for germination timing was greatest in seeds that were dispersed in RI during June, and it was least for seeds that were dispersed in RI during November. Thus, the evolutionary potential of germination timing depends strongly on the particular set of seasonal conditions and geographic location that seeds experience. In particular, the evolution of germination timing of seeds that are dispersed in autumn is likely to be strongly constrained due to lack of genetic variation, even if those same genotypes express abundant genetic variation for germination timing when they are dispersed in spring. Seasonal changes in the expression of genetic variation have the potential to strongly influence the evolution of basic phenology and life-history expression.

Substantial transgressive segregation of germination timing and proportion occurred, and the degree of transgressive segregation depended on all environmental factors. In most treatments, transgressive segregation for germination proportion resulted in genotypes with both higher and lower germination success than either parent. The exception was those seeds matured under long days and dispersed in June in RI, in which transgressive segregation caused most genotypes to have lower germination success than either parent.

Transgressive segregation also created genotypes that germinated both earlier and later than either parental line. In several treatments, a large number of transgressive segregants germinated earlier and exhibited very weak or no dormancy. For seeds dispersed in June, this reduction in dormancy was disadvantageous, as many seedlings died before reproducing in the summer (Donohue et al. 2005). For seeds dispersed in November, in contrast, seeds with early germination had higher fitness. Therefore, transgressive segregation actually created genotypes that had more adaptive germination timing than either parent under some conditions. In naturally hybridizing populations, such transgressive segregation would quickly provide substantial genetic variation for evolutionary responses to natural selection on germination. The effectiveness of hybridization in creating genetic variation, moreover, will depend on seasonal conditions and geographic location.

Summary and Conclusions

Germination timing was highly responsive to seasonal environmental factors, with seasonal maternal effects operating more strongly through effects on postdispersal seasonal environment than through effects of the photoperiod during seed maturation. The large degree of genetic variation in germination and the transgressive segregation of germination indicate that the two populations that were used to create the recombinant inbred lines differed substantially in germination at more than one locus and that hybridization between ecotypes can effectively release genetic variation for germination behavior. There was a high degree of genotype-by-environment interaction for all environmental factors and weak genetic correlations across environments. The weak correlations indicate that germination responses to seasonal cues are evolutionarily labile and that pleiotropic constraints are not likely to inhibit the evolution of germination in response to selection in different environments. However, the expression of genetic variation and transgressive segregation depended on the environmental conditions, indicating that the evolutionary potential of germination depends on seasonal environmental factors—especially season of dispersal.

In conclusion, this study demonstrated that germination was highly responsive to season, especially postdispersal seasonal environment. A trade-off in germination success in different sites indicates that ecologically significant constraints on establishing over a broad geographic range can occur at extremely early stages. Germination timing, however, exhibited weak pleiotropic constraints. This crucial life-history stage, therefore, appears to have substantial evolutionary lability that is likely to facilitate evolutionary responses to selection on plasticity to diverse seasonal cues. While the trade-off in germination success across geographic locations would limit the range expansion of this species, the extreme evolutionary lability of germination responses to different seasonal environments could facilitate the colonization of new environments by enabling appropriate germination responses to evolve.

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