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THE EFFECT OF MATERNAL PHOTOPERIOD ON SEASONAL DORMANCY IN *ARABIDOPSIS THALIANA* (BRASSICACEAE)[1]

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The maternal photoperiod at the time of seed maturation can predict the seasonal conditions of newly dispersed seeds. We investigated the effects of maternal photoperiod on seasonal dormancy in *Arabidopsis thaliana* using a set of F6 recombinant inbred lines derived from a cross between individuals from two natural populations (Cal-0 and Tac-0) differing in cold requirements for germination. We grew 40 Cal × Tac lines in a long-day photoperiod (8 h of full spectrum light plus 8 h of low-fluence incandescent light) and a short-day photoperiod (8 h full spectrum light). We then exposed seeds from each family and maternal photoperiod to either a cold stratification treatment (4°C, 21 d) or no cold stratification. Both maternal photoperiod and progeny stratification influenced the percentage of seeds that germinated and the speed of germination. The short-day photoperiod caused increased responsiveness to stratification, with higher germination percentages and speeds in stratified seeds. Stratification influenced the expression of maternal photoperiod effects, such that short days increased germination percentage and speed in stratified seeds but inhibited germination in unstratified seeds. Families differed significantly in their plasticity to maternal photoperiod and stratification, but genetic variation for plasticity to maternal photoperiod was expressed only in unstratified seeds. Because the expression of maternal photoperiod effects and genetic variation for photoperiod effects depended on progeny stratification, the evolution of these maternal effects will depend on the seasonal environment experienced by progeny.

Key words: dormancy; genetic variation; maternal effect; photoperiod; plasticity; recombinant inbred; stratification.

In many plant species, seasonal seed dormancy may strongly influence fitness by delaying germination until conditions are appropriate for growth. Autumn germination may provide a selective advantage when the risk of winter mortality is low by enabling the plant to flower earlier in the spring or at a larger size (Silvertown, 1988; Masuda and Washitani, 1992). However, it may be advantageous to delay germination until spring if the risk of winter mortality is high. Breakage of seasonal dormancy often depends on specific environmental cues experienced by seeds after dispersal from the maternal plant. For example, seeds of many species commonly break dormancy only after being cooled for weeks or months at temperatures of 1°–5°C. Since these temperatures usually occur only during winter, seeds that require such stratification usually will not germinate until after the winter season (Bewley and Black, 1994; Vleeshouwers, Bouwmeester, and Karssen, 1995). Within a species or population, seeds can have different requirements for dormancy breakage and therefore may often vary in response to the same seasonal cues (reviewed in Baskin and Baskin, 1998, Chapter 8). Geographic or ecotypic variation in seasonal dormancy has been observed in several species (e.g., Meyer, McArthur, and Jorgensen, 1989; Meyer, 1992; Kalisz and Wardle, 1994). Understanding both the genetic basis and environmental determinants of such variation is important for understanding how seasonal dormancy may evolve.

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Variation in seasonal dormancy may be strongly influenced by genes expressed in the maternal parent. Maternal control of germination can operate through the seed coat, endosperm, or maternal provisioning of resources and hormones (Dobrovolna and Cetl, 1966; Goto, 1982; Roach and Wulff, 1987; Biere, 1991; Platenkamp and Shaw, 1993; Léon-Kloosterziel, Keijzer, and Koornneef, 1994; Lacey, Smith, and Case, 1997; Baskin and Baskin, 1998). The seed coat, composed of maternal tissue, mediates the environment that the embryo experiences (Schmitt, Niles, and Wulff, 1992; Platenkamp and Shaw, 1993; Donohue and Schmitt, 1998). It imposes mechanical constraints to germination (Kugler, 1951; Dobrovolna and Cetl, 1966; Goto, 1982; Biere, 1991; Platenkamp and Shaw, 1993; Léon-Kloosterzeil, Keijzer, and Koornneef, 1994) and can determine permeability (Baskin and Baskin, 1998) and alter light environments experienced by the embryo (Botto, Sanchez, and Casal, 1995). Thus, the evolution of seasonal dormancy may involve selection on genetic variation in germination responses to offspring environments among maternal parents, as well as among individual seeds (e.g., Donohue and Schmitt, 1998).

The environment experienced by the maternal plant prior to seed dispersal may also strongly influence seasonal dormancy and germination timing. In particular, the photoperiod during seed maturation is a reliable indicator of season. Maternal photoperiod influences germination and dormancy in several species (Gutterman, 1992, 1993; Baskin and Baskin, 1998). For example, in the desert annual *Ononis sicula*, day-length variation caused changes in the development of the seed coat and its surface structure, modifying the permeability of the seed coat, fungal resistance of the seed coat, and seed longevity (Gutterman, 1992). Through these morphological and physiological changes in maternal tissue, day length altered the timing of germination (see also Gutterman, 1972, 1978, 1993;

Gutterman and Evanari, 1972; Pourrat and Jacques, 1978). Such maternal environmental effects have been viewed as a form of potentially adaptive cross-generational phenotypic plasticity (Schmitt, Niles, and Wulff, 1992; Schmitt, 1995; Bernardo, 1996; Donohue and Schmitt, 1998; Mousseau and Fox, 1998). The evolution of adaptive maternal effects requires selection to discriminate among maternal genotypes that differ in effects of the maternal environment on seasonal dormancy of progeny.

Thus, the evolution of seasonal dormancy can be viewed as the evolution of plasticity of seed traits to both the maternal and the progeny environment. For plastic dormancy and germination responses to evolve in response to natural selection, genetic variation for plasticity of germination traits to maternal and/or offspring environments must exist (e.g., Via and Lande, 1985; Schlichting, 1986; Van Tienderen, 1991; Scheiner, 1993). The expression of such variation and therefore the potential for response to selection may depend upon the environmental context. For example, expression of genetic variation in effects of parental photoperiod on dormancy breakage might differ between autumn and spring offspring environments or among sites differing in the length of the winter cold period experienced by seeds. Only a few studies have simultaneously examined genetic variation in germination responses to both maternal and offspring environments or tested for environment-specific expression of such variation (Schmitt, Niles, and Wulff, 1992; Wulff, Caceres, and Schmitt, 1994).

In this study, we investigated the effects of maternal and progeny environments on seed dormancy in the genetic model species *Arabidopsis thaliana* (Brassicaceae), a primarily selfing, weedy annual. Seasonal dormancy varies among known *A. thaliana* ecotypes, as well as among natural populations in North America (personal observation), Europe (Ratcliffe, 1965, 1976; Effmertova, 1967; Evans and Ratcliffe, 1972), and worldwide (Nordberg and Bergelson, 1999). Most populations in North America have been characterized as winter annuals that germinate only in autumn (Baskin and Baskin, 1983; Nordberg and Bergelson, 1999), whereas Rhode Island populations can germinate either in autumn or in spring (personal observation). Populations also differ in season of flowering, with Rhode Island populations flowering in both autumn and spring (personal observation), and southern populations (Baskin and Baskin, 1983), flowering only in spring. As a consequence, seeds can be matured in different seasons and under different day lengths in some natural populations. In populations that experience harsh winter conditions then selection may favor maternal genotypes that produce seeds whose germination is enhanced by cold stratification when seeds are matured in a short-day photoperiod (autumn). A requirement for cold stratification in these seeds could prevent them from germinating too late in the autumn and could effectively postpone germination until after the winter cold. Conversely, seeds that matured under a long-day maternal photoperiod (spring) may have higher fitness if they do not have a requirement for cold stratification. A lack of a cold requirement would enable them to germinate early in the autumn. In populations where winter conditions are not severe the maternal photoperiod may still be an important predictor of offspring conditions but not of winter cold. Therefore, all plants are expected to germinate without stratification in autumn and flower under long days in spring, so selection is less likely to act upon variation in maternal photoperiod effects. The observed variation in seasonal dormancy and associated life-history characters among natural populations of *A. thaliana* provides a useful system for investigating the role of maternal effects on germination requirements in contributing to variation in and evolution of these important life-history characters. *Arabidopsis thaliana*'s highly selfing nature relieves this system of the complex issue of maternal versus embryonic determination of dormancy, since the genotype of the maternal parent is generally equivalent to the genotype of the offspring.

We used F6 seeds of recombinant inbred lines derived from two natural populations, Cal-0 (Calver, UK) and Tac-0 (Tacoma, Washington, USA), differing in their cold requirement for germination. We allowed these seeds to mature on maternal plants under either long or short days and then determined the effect of cold stratification on germination. We addressed the following questions: (1) What is the effect of maternal photoperiod on the responsiveness of germination to stratification, that is, on the likelihood of fall vs. spring germination? (2) What is the effect of progeny stratification on the responsiveness of germination to maternal photoperiod, that is, can seasonal cues typical of fall or spring germination environments affect the expression of maternal photoperiod effects? (3) Is the germination response to maternal photoperiod and progeny stratification genetically variable in this sample? (4) How does maternal photoperiod influence the expression of genetic variation for germination requirements, and conversely, how does progeny cold treatment influence the expression of genetic variation for maternal photoperiod effects on germination?

MATERIALS AND METHODS

This experiment used 40 inbred families of *A. thaliana* that were in the fifth generation of selfing by single-seed descent following a cross between an individual from Calver, UK (Cal-0) and an individual from Tacoma, Washington, USA (Tac-0) (L. A. Dorn and T. Mitchell-Olds, unpublished data). Germination trials of seeds descended from the Tac-0 individual found 96% of seeds germinated following 2 wk of cold stratification but 0% germinated without stratification (L. A. Dorn and K. Donohue, unpublished data). Therefore Tac-0 seeds may be most likely to germinate in the spring under field conditions. In a subsequent experiment (N. Kane and L. A. Dorn, unpublished data) with seeds afterripened for <3 mo, some Tac-0 seeds germinated without stratification, suggesting that the stratification response may be reinforced by induction of secondary dormancy with afterripening. Seeds descended from the Cal-0 individual do not require cold to germinate and thus may be more likely to germinate in the autumn in the field.

Twelve replicates of these 40 families were grown in a short-day photoperiod and 12 replicates were grown in a long-day photoperiod, after which we collected their seeds (the F6) by family and photoperiod. The short-day maternal photoperiod consisted of 8 h of full-spectrum light in a compartment of a Conviron E-7 growth chamber (photosynthetic photon flux density or PPFD = 425 $\mu mol \cdot m^{-2} \cdot s^{-1}$). The long-day maternal photoperiod consisted of 8 h of full-spectrum light, followed by 8 h of low-fluence incandescent light (PPFD = 5 $\mu mol \cdot m^{-2} \cdot s^{-1}$). This treatment increased the perceived day length while maintaining daily PPFD at approximately the same level as in the short-day treatment (Lee and Amasino, 1995). Plants in both treatments were maintained at 24°C.

Seeds matured under these conditions were afterripened at room temperature for ~8 wk and then tested for germination as follows. Seeds from each family and maternal photoperiod were pooled across maternal replicates to ensure enough seeds for the experiment. Ten seeds from each combination of family and maternal photoperiod were placed on the surface of each of eight replicate petri dishes (50 × 9 mm) containing 5 mL of 0.5% agar dissolved in triple-distilled water. Four of the eight plates were given a cold and dark stratification treatment and four were not. This gave a total of 40 replicate seeds per family per photoperiod and stratification combination.

The plates that were subjected to the cold stratification treatment were

TABLE 1. Results of the four-way mixed-model ANOVA.

Source	Percentage germination				Germination speed		
	df	MS	MS error	*F*	MS	MS error	*F*
Block (MS Error)	1	0.04	0.03	1.53	0.05	0.02	2.65
Maternal photoperiod	1	0.11	0.16	0.70	0.04	0.09	0.50
Progeny stratification	1	0.17	0.14	1.21	0.66	0.07	8.54**
Family	25	0.18	0.23	0.79	0.14	0.12	1.11
Maternal photoperiod × Progeny stratification	1	1.33	0.07	18.06***	0.19	0.05	4.13*
Family × Maternal photoperiod	25	0.16	0.07	2.18***	0.09	0.05	2.04*
Family × Progeny stratification	25	0.14	0.07	1.99*	0.08	0.05	1.67
Family × Photoperiod × Stratification	25	0.07	0.03	2.92****	0.05	0.02	2.16***

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

sealed with plastic wrap, covered with aluminum foil (to ensure darkness), and placed at 4°C for 21 d. The number of seeds that had germinated in the dark was recorded for each plate immediately after removal from the dark stratification treatment. The plates were then divided equally between two Conviron E-7/2 growth chamber compartments (blocks) and were maintained at 24°C in a long-day photoperiod as described above. To minimize effects of potential environmental variation within chambers, the plates were randomly redistributed every 2 d. After 4 d, the number of germinants was recorded for each plate. Plates were then placed back into the growth chamber, and subsequent germinants were recorded after 10 d. Some seeds were obviously inviable as evidenced by severe fungal growth. Visual inspection of the remaining seeds (Baskin and Baskin, 1998) provided estimates of seed viability. The total number of viable seeds was recorded for each plate.

The plates that were not stratified were immediately divided between the growth chamber compartments and were censused as described above. To test for viability, those seeds that had not germinated after 14 d were placed in the cold for 4 d to break dormancy and then placed back in the growth chambers and recensused 7 d later. Those that had still not germinated were visually inspected for viability.

For stratified plates, germination percentage was calculated for each replicate as the total number of germinants 14 d after removal from the 21 d cold, dark stratification treatment divided by the total number of viable seeds in each plate. Germination speed was calculated for each replicate as the number of germinants 4 d after removal from the stratification treatment divided by the number of germinants 14 d after removal from the stratification treatment. The percentage of seeds germinating in the dark was calculated as the number of seeds that had already germinated upon removal from the dark, cold stratification treatment divided by the total number of germinants 14 d after removal from the stratification treatment.

For unstratified plates, germination percentage was calculated for each replicate as the total number of germinants 14 d after sowing in petri plates divided by the total number of viable seeds in each plate. Germination speed was calculated for each replicate as the number of germinants 4 d after sowing in petri plates divided by the number of germinants after 14 d. All measurements of germination in all treatments were transformed to normality using the arcsine-square root transformation.

To test for effects of maternal genotype (i.e., family), maternal environment, progeny environment, and their interactions on germination percentage and germination speed, we used a four-way mixed-model ANOVA (SAS PROC GLM). Block, maternal photoperiod, and progeny stratification were fixed effects, and family was a random effect. The *F* tests for fixed effects were calculated from the expected mean squares with the mean square for block tested over error, maternal photoperiod and progeny stratification mean squares were tested over their two-way interactions with family, all two-way interaction mean squares were tested over the three-way interaction that was tested over error. Interactions with block were pooled with the error term (Newman, Bergelson, and Grafen, 1997). Because we observed a significant three-way interaction between photoperiod, stratification, and family, we performed two separate three-way mixed-model ANOVAs, one with stratification and block as fixed main effects and family a random factor, within each maternal photoperiod treatment, and another with photoperiod and block as fixed main effects with family a random factor within each progeny stratification treatment. The effects of family and maternal photoperiod on the percentage of dark-germinating seeds were tested only for the stratified seeds in a mixed-model ANOVA, with block and photoperiod as fixed factors and family as a random factor. In all three of these analyses, the mean squares for block and family by treatment interaction were tested over error, while all family and treatment main effects mean squares were tested over the family by treatment interaction mean squares. Because some families performed poorly under some maternal photoperiods, there were only 26 families with seeds that had matured in both photoperiods. Seeds were collected from 27 families in the long-day photoperiod and 32 families in the short-day photoperiod. Therefore, only the 26 families common to both photoperiods were included in the four-way ANOVA and the three-way ANOVA performed within the two progeny stratification treatments. The three-way ANOVA performed within the long-day maternal photoperiod included 27 families, while the short-day ANOVA included 32 families.

Genetic correlations across maternal photoperiods within each stratification treatment and across stratification treatments within each maternal photoperiod were calculated by the method of Fry (1992) for unequal variances.

RESULTS

Interaction between maternal photoperiod and progeny stratification—Maternal photoperiod significantly influenced germination responses to stratification, as evidenced by significant interactions between maternal photoperiod and stratification for germination percentage and speed (Table 1). Specifically, maternal short days caused stratified seeds to germinate more rapidly and to higher percentages than unstratified seeds, as indicated by significant main effects of stratification in a three-way ANOVA for the short-day maternal photoperiod (Table 2; Fig. 1). In contrast, maternal long days caused stratified seeds to germinate to a slightly lower percentage than unstratified seeds on average, as indicated by a significant main effect of stratification in the long-day maternal photoperiod (Table 2; Fig. 1). No main effect of stratification was detectable for germination speed of seeds matured under long days, due to the large variation among families in response to stratification (Table 2; Fig. 1).

The effect of maternal photoperiod on response to stratification—The effects of maternal photoperiod on response to stratification differed substantially among families, indicated by highly significant family × photoperiod × stratification interactions for germination percentage and speed (Table 1). Although the germination percentage and speed of seeds matured under short days uniformly increased when stratified (Fig. 1), families differed substantially in degree of responsiveness to stratification, as indicated by significant family × stratification interaction terms for both traits in the short-

TABLE 2. Results of a three-way analysis of variance of percentage germination and germination speed for the two maternal photoperiods (long-day vs. short-day photoperiod).

Source	Long days						Short days					
	Percentage germination			Germination speed			Percentage germination			Germination speed		
	df	MS	MSE	MS	MSE	F	df	MS	MSE	MS	MSE	F
Block	1	0.01	0.02	0.18	0.02	8.76**	1	0.02	0.03	0.00	0.03	0.01
Family	26	0.22	0.09	0.16	0.06	2.54**	31	0.18	0.15	0.12	0.10	1.23
Progeny stratification	1	0.39	0.09	0.05	0.06	0.83	1	1.68	0.15	1.27	0.09	12.79***
Family × progeny stratification	26	0.09	0.02	0.06	0.02	3.12****	31	0.15	0.03	0.10	0.03	3.37****

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

day analysis (Table 2). This variation in degree of responsiveness to stratification was due to large differences in the expression of genetic variation across stratification treatments (Table 3). The germination percentages and speeds for stratified seeds matured under short days were uniformly high among families and therefore the percentage variance explained by family differences was small (Table 3; Fig. 1). For unstratified, short-day seeds, families differed significantly for these traits and therefore the percentage variance explained by family was large in this progeny environment (Table 3; Fig. 1). The cross-environment genetic correlation for germination percentage of short-day seeds across stratification treatments was positive but nonsignificant ($r_{GE} = 0.67$; $P < 0.3$), suggesting that the relative germination percentage of families could differ with the seasonal environment experienced by seeds. The cross-environment genetic correlation for germination speed of short-day seeds was not estimable because of a negative variance component in the cold stratification environment. However, the effect of family over all stratification treatments was not significant while the family by environment interaction was strongly significant, suggesting this trait was also not strongly correlated across stratification treatments for short-day seeds (Table 2). Although some families resembled the Cal-0 parent in their high germination regardless of stratification, no family displayed the strong response to stratification observed in the Tac-0 parent; at least 50% germination was observed for unstratified seeds of all families.

For seeds matured under long days, the influence of stratification on germination percentage differed in magnitude and direction among families, as indicated by significant family × stratification interactions (Fig. 1; Table 2). Stratification increased germination speed of all families, but those families differed in their degree of responsiveness to stratification (Fig. 1; Table 2). Consequently, greater genetic variation for germination speed of long-day seeds was expressed in unstratified seeds than in stratified seeds (Table 3). The genetic correlation across stratification environments was large and positive for the germination speed of long-day seeds ($r_{GE} = 1.00$; $P < 0.01$), whereas the genetic correlation was positive but less strong for percentage germination of long-day seeds ($r_{GE} = 0.54$; $P < 0.01$). Therefore, seasonal environment had some influence on the relative performance of families for the percentage germination of long-day seeds but there was little effect of seasonal environment on family rankings for speed of germination. Some families displayed consistently high or low germination percentages of long-day seeds regardless of stratification, some families had higher germination percentages of stratified long-day seeds, and other families had lower germination percentages of stratified seeds. Again, the high germination of unstratified seeds of several lines resembled the Cal-0 parent, but no line displayed the nearly absolute stratification requirement characteristic of Tac-0.

The effect of progeny stratification on the expression of maternal photoperiod effects—Stratification significantly influenced the effect of maternal photoperiod on germination percentage and speed (Table 1; Fig. 2). In stratified seeds, the short-day maternal photoperiod significantly increased mean germination percentage and speed (Table 4; Fig. 2). In contrast, in unstratified seeds, the short-day maternal photoperiod significantly *decreased* mean germination percentage, and there was no main effect of photoperiod on germination speed (Table 4; Fig. 2).

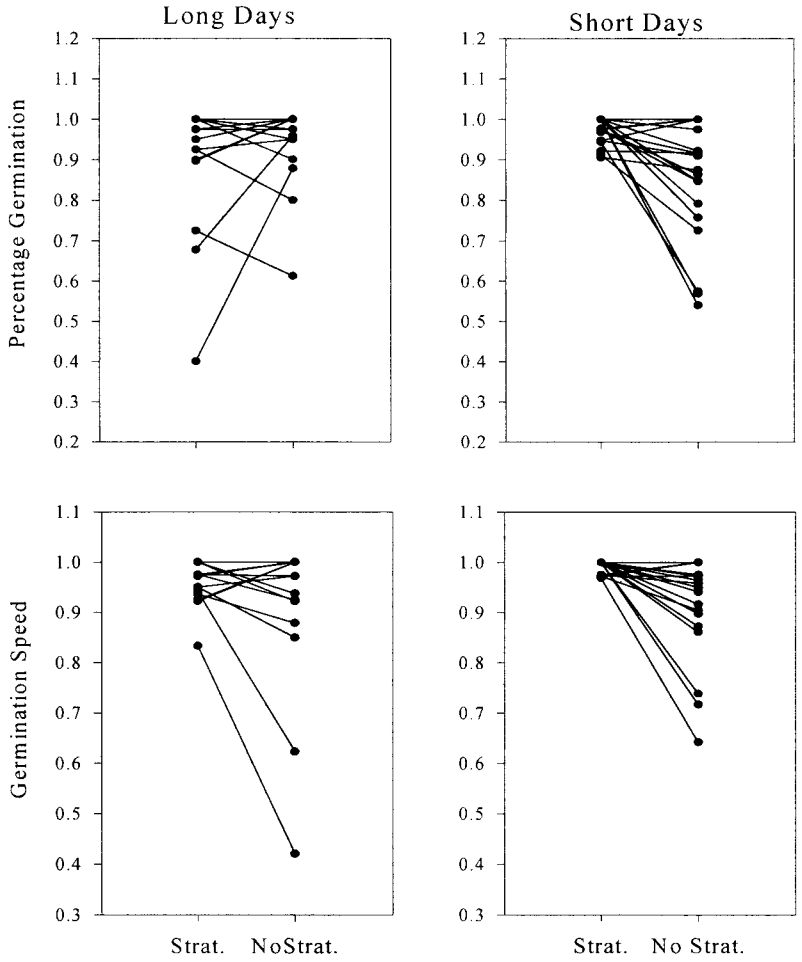

Fig. 1. Reaction norms of germination percentage and germination speed to progeny stratification treatment for seeds matured under long days and short days. Lines connect family means in each treatment.

Stratification also affected the expression of genetic variation for plasticity to maternal photoperiod, as indicated by the significant three-way interaction among family, maternal photoperiod, and progeny stratification (Table 1). When seeds were not stratified, simulating fall germination, families differed considerably in the magnitude and direction of their response to photoperiod indicated by the significant family × photoperiod interaction terms (Fig. 2; Table 4). Some families were unresponsive to photoperiod, other families had lower germination percentages in short days, and other families had higher germination percentages in short days. Likewise, the germination speed of many families was unresponsive to photoperiod, but some families accelerated their germination if seeds were matured under short days, while other families germinated more slowly if they were matured under short days (Fig. 2). Consequently, the genetic correlations of unstratified seeds across maternal photoperiods were significant but small for percentage germination ($r_{GE} = 0.44$; $P < 0.045$) and not significant for speed ($r_{GE} = 0.25$; $P < 0.16$), suggesting that different maternal photoperiods could cause different rankings of families in autumn-germinating seeds. When seeds were stratified, simulating spring germination, families differed significantly in the extent to which a short-day maternal photoperiod increased germination percentage as indicated by a significant family × photoperiod interaction for percentage germination (Table 4). The cross-environment genetic correlation for the percentage germination of stratified seeds across maternal photoperiods was negative but nonsignificant ($r_{GE} = -0.45$; $P < 0.67$). Thus, maternal photoperiod could also strongly affect the relative frequency of families in spring-germinating seeds. However, although families differed overall in germination speed of stratified seeds, indicated by a significant main effect of family, we did not detect significant genetic variation in response of this trait to maternal photoperiod, indicated by a nonsignificant family × maternal photoperiod interaction (Table 4).

The effect of maternal photoperiod on dark germination—Maternal short days significantly increased the mean percentage of stratified seeds germinating in the dark, simulating seed burial (Table 4; Fig. 3). However, the effect of maternal photoperiod on dark germination also differed significantly among families (Fig. 3; Table 4). Seed maturation under short days resulted in higher dark germination for some families, but lower dark germination for other families, relative to seeds matured under long days. For still other families, the maternal photoperiod had no effect on dark germination. However, a significant positive genetic correlation across maternal photoperiod environments indicated that the ranking of families in percentage of dark germination (that is, the propensity for ger-

TABLE 3. The F values for effect of family from ANOVAs performed within each combination of maternal photoperiod and progeny stratification and the broadsense heritability (H^2) calculated in each environment.

Germination characters	Stratified						Not Stratified					
	Short days			Long days			Short days			Long days		
	F	MS	MSE	H^2	F	MS	MSE	H^2	F	MS	MSE	H^2
Percentage germination	1.35 ns	0.04	0.034	0.14	6.54****	0.210	0.034	0.72	11.67****	0.465	0.039	0.84	7.56****	0.21	0.03	0.75
Germination speed	1.07 ns	0.008	0.007	0.03	1.18 ns	0.032	0.028	0.06	4.16****	0.211	0.051	0.61	13.67****	0.19	0.01	0.90

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

mination of buried seeds) was relatively unaffected by the maternal photoperiod ($r_{GE} = 0.80$; $P < 0.001$).

DISCUSSION

The results of this study illustrate the potential importance of maternal environmental effects for the expression and evolution of seasonal seed dormancy in *Arabidopsis*. Maternal photoperiod, an accurate cue of the seasonal environment during seed maturation, strongly influenced the sensitivity of dormancy breakage to cold stratification (another seasonal cue) and the dark germination of stratified seeds. Thus, maternal photoperiod effects may influence the proportion of progeny that germinate in autumn vs. spring, as well as the proportion allocated to the seed bank. Conversely, the expression of maternal photoperiod effects depended upon progeny stratification, suggesting that the importance of these effects may depend upon the season in which the progeny germinate. Germination percentage and speed were genetically variable in this sample, but the amount of genetic variation that was expressed depended on both maternal photoperiod and progeny stratification treatments. Consequently, the relative frequency of genotypes in a cohort of germinating seedlings may differ among seasonal environments and years. Moreover, the percentage of seeds germinating in the dark was also genetically variable and dependent on maternal photoperiod, suggesting that variation in the germination of buried seeds may also contribute to the genetic variation expressed in a single season. This observed variation among families in germination responses to maternal and progeny environments suggests a genetic basis for cross-generational plasticity. Such variation within natural populations would allow environmental maternal effects to evolve (e.g., Via and Lande, 1985; Schlichting, 1986; Van Tienderen, 1991; Scheiner, 1993) but may also contribute to within-population genetic variation for quantitative traits in general. Since the lines used in this experiment were recombinant inbreds from a cross between two natural populations that differed in seasonal dormancy, they will provide a useful tool for investigating the genetic basis of ecotypic differentiation of germination responses to seasonal cues.

The effect of maternal photoperiod on response to progeny stratification and seasonal timing of germination—In this study, maternal photoperiod influenced seed dormancy. In particular, seeds matured by parents under short days broke dormancy more readily when stratified, simulating spring conditions, but seeds matured under long days broke dormancy more readily on average when not stratified, simulating autumn conditions (Fig. 1). This observation is consistent with the natural history of *A. thaliana*. Most populations flower in the spring under long days, but some populations also have an autumn-flowering cohort. The photoperiod that maternal plants experience at the time of seed maturation may be a reliable predictor of progeny environmental conditions. Seeds matured under long days would be dispersed in late spring or early summer, toward the end of the growing season for *Arabidopsis* with the onset of hot weather in many regions. The observed maternal photoperiod effect would allow seeds matured under the longer days of spring to germinate in the autumn, without prolonged cold stratification. Those long-day seeds that did not germinate in the autumn and therefore do experience prolonged cold stratification may then enter a secondary dormancy, entering the seed bank, and may contribute to year to year

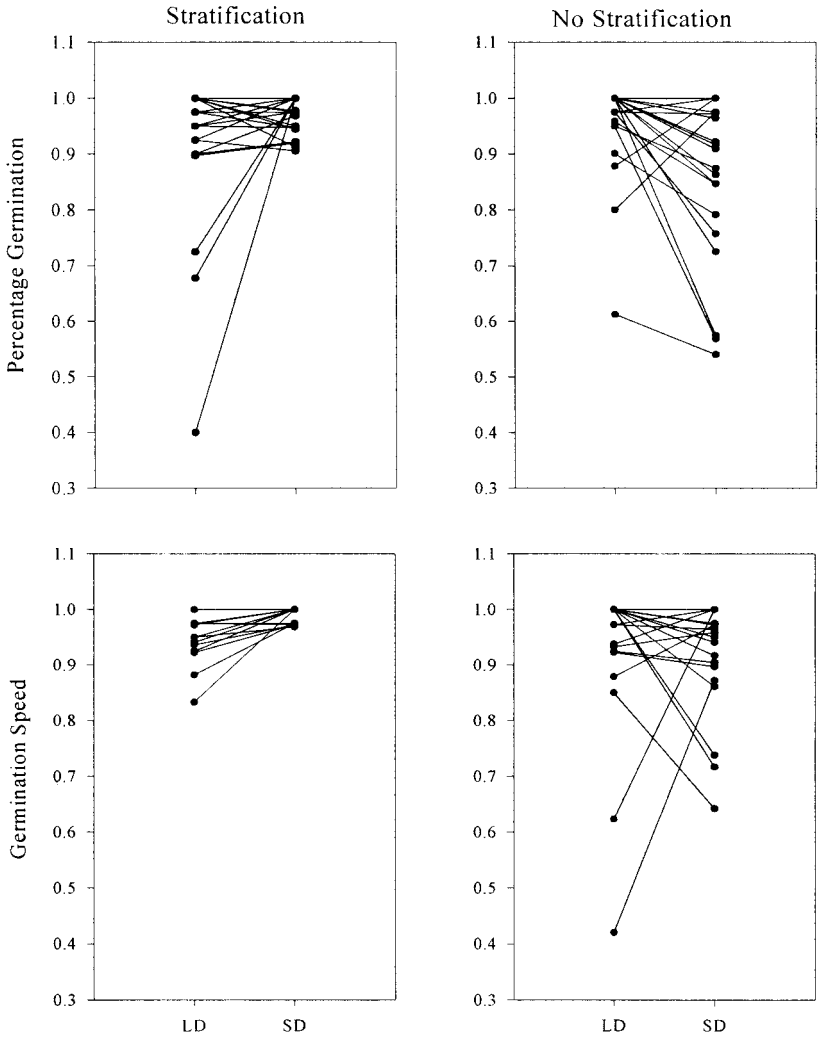

Fig. 2. Reaction norms of germination percentage and germination speed in response to maternal photoperiod for stratified and unstratified seeds. Lines connect family means in each treatment. LD = long day maternal photoperiod, SD = short-day maternal photoperiod.

variation among germinants. In populations with an autumn-flowering cohort, seeds would mature and disperse under short autumn days. A short-day maternal photoperiod would therefore predict imminent harsh winter conditions, which might prevent immediately germinating progeny from completing their life cycle. Induction of a stratification requirement for dormancy breakage by a short-day maternal photoperiod would allow autumn-matured progeny to overwinter safely as seeds and germinate under favorable conditions in spring. In such populations, maternal environmental effects on seasonal dormancy could thus result in two alternating generations per year.

The effect of progeny stratification on the expression of maternal photoperiod effects—The observation that expression of maternal photoperiod effects depended upon stratification suggests that the impact of these maternal effects may depend upon the seasonal environment experienced by the offspring. The effect of maternal photoperiod on germination percentage of seeds that had experienced cold, simulating spring germinating conditions, was in the opposite direction of the maternal photoperiod effect for those seeds that did not receive cold (Fig. 2). A main effect of maternal photoperiod on germination speed was detected only in stratified seeds. Other studies have also shown that the effect of the maternal environment on the phenotypic expression of progeny characters may depend on the progeny environment (Alexander and Wulff, 1985; Miao, Bazzaz, and Primack, 1991a, b; Schmitt, Niles, and Wulff, 1992; Platenkamp and Shaw, 1993; Lacey, 1996). Because *A. thaliana* seeds matured under short days were more likely to germinate following stratification while long-day seeds germinated more readily without stratification, maternal photoperiod may influence the season of germination, and therefore, the expression of maternal effects on germination. This is an important point for species like *A. thaliana* that are rapidly expanding their range. In some geographic locations seasonal differences in photoperiod may not necessarily be followed by drastically different temperature conditions, although other factors may vary. An invasive genotype that is plastic to maternal photoperiod may show no selective advantage or even a disadvantage if optimal germination conditions in the new location are different.

These interacting influences of maternal photoperiod and progeny stratification on germination may be important deter-

TABLE 4. Results of a three-way analysis of variance of percentage germination and germination speed for the two stratification treatments (stratified vs. not stratified).

Source	Stratified									Not stratified							
		Percentage germination			Germination speed			Percentage dark germination			Percentage germination			Germination speed			
	df	MS	MSE	*F*	MS	MSE	*F*	MS	MSE	*F*	MS	MSE	*F*	MS	MSE	*F*
Block	1	0.00	0.02	0.11	0.07	0.02	4.10*	0.17	0.07	2.40	0.05	0.03	1.86	0.00	0.03	0.22
Family	25	0.10	0.12	0.84	0.03	0.01	1.97*	0.78	0.18	4.26***	0.22	0.11	1.99*	0.19	0.13	1.48
Maternal photoperiod	1	0.33	0.12	2.74**	0.21	0.01	16.38***	1.72	0.18	9.34**	1.11	0.11	9.92**	0.03	0.13	0.23
Family × Maternal photoperiod	25	0.12	0.02	5.45****	0.01	0.02	0.78	0.18	0.07	2.64****	0.11	0.03	3.97****	0.13	0.03	4.93****

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

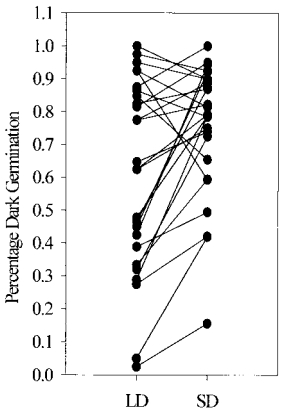

Fig. 3. Reaction norms of percentage of germination in the dark in response to maternal photoperiod. Lines connect family means in each treatment. LD = long day maternal photoperiod, SD = short-day maternal photoperiod.

minants of the variation in seasonal dormancy and other life-history traits that are well documented among geographically distinct populations of this species (Nordberg and Bergelson, 1999). The mechanism of ''summer annual'' vs. ''winter annual'' life histories in *A. thaliana*, however, is controversial (Nordberg and Bergelson, 1999). It is thought that these contrasting life histories are functions of both dormancy behavior and vernalization requirements for flowering (Napp-Zinn, 1976; Nordberg and Bergelson, 1999). Our results suggest that maternal photoperiod effects may also contribute to such variation. Because seeds are matured in both spring and autumn in some populations of *A. thaliana,* timing of germination and the photoperiod under which seeds are matured can vary in natural populations. This study suggests that the timing of seed maturation can influence the season of germination. If season of germination, in turn, influences the timing of reproduction and seed maturation, then variation in phenology may result. For example, some autumn germinants may flower in late autumn and mature their seeds under short days. Unlike their parents, these seeds are very likely to germinate in the spring rather than the autumn because of their increased requirement for stratification, leading to variation between generations in season of germination. Likewise, most plants that flower in the autumn are likely to be from seeds matured under long days, since seeds matured under short days will have flowered in the spring. This leads to variation in flowering time as well. In this manner, maternal effects on germination may contribute to ecologically important variation in life history and phenology.

Genetic variation for maternal photoperiod effects—The potential contribution of environmental maternal effects to life history variation does not exclude the potential importance of genetic variation for the same traits. For maternal photoperiod effects and seasonal dormancy to evolve, genetic variation must exist within natural populations (Schmitt, Niles, and Wulff, 1992; Donohue and Schmitt, 1998). We found strong evidence for such variation within our sample, which was derived from two natural populations. However, the expression of genetic variation for seasonal dormancy depended upon the maternal photoperiod, and conversely, the expression of genetic variation for the maternal effect depended on stratification. Similarly, Schmitt, Niles, and Wulff (1992) found that

the expression of genetic variation for germination responses of *Plantago lanceolata* to maternal light environment depended on the offspring's light environment. Thus, the genetic potential for evolutionary response to natural selection on maternal effects and/or seasonal dormancy will depend upon the seasonal environment. Moreover, the relative frequency of genotypes in a germinating cohort, and their relative position in the emergence hierarchy, may differ between spring and fall generations and be influenced by the maternal photoperiod.

Geographic variation in germination behavior has been documented in many species (e.g., Cruden, 1974; Van der Vegte, 1978; Hacker et al., 1984; Hacker and Ratcliff, 1989; Meyer and Monsen, 1991; Meyer, Allen, and Beckstead, 1997). Germination timing is an important contributor to life-history variation in other species with variation in seasonal dormancy. For example, in *Campanula americana*, germination timing strongly influences timing of reproduction and consequently the annual vs. biennial strategy (Kalisz and Wardle, 1994; Wardle, 1998). Our results suggest that maternal environmental effects, such as photoperiod effects, may contribute to such variation in dormancy in *A. thaliana*. Explicit investigations of parental effects on dormancy may reveal mechanisms for the maintenance of variation in dormancy and corresponding life-history characters within (Effmertova, 1967; Masuda and Washitani, 1990; Baskin and Baskin, 1998) and among natural populations.

Geographic variation in seasonal life histories is also likely to be strongly influenced by genetic variation in maternal effects and seasonal dormancy. We detected substantial genetic variation for maternal photoperiod effects and seasonal dormancy in recombinant inbred lines from a cross between natural ecotypes with different seasonal dormancy strategies. Since we did not observe segregation of the Tac-0 parental phenotype in any line, our results suggest genetic differentiation of the parental ecotypes at several loci. We plan to conduct quantitative trait loci (QTL) mapping of later generations of the Cal × Tac families to elucidate the genetic basis of the observed differentiation between the parental populations in seasonal dormancy behavior.

In conclusion, seasonal dormancy in *A. thaliana* results from interactions between environments experienced in two generations. Maternal environmental effects on germination were strong, but they depended on the progeny environment. Moreover, the expression of genetic variation for the maternal effect depended on the progeny environment. The evolution of maternal effects in general, and seasonal dormancy in particular, will therefore depend on the nature of selection in both maternal and progeny environments, as well as the expression of genetic variation in each of those environments (Donohue and Schmitt, 1998). Thus, a multigenerational perspective may be necessary in order to understand the origin and maintenance of the life-history variation observed in natural populations of *A. thaliana*.

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