SYMPOSIUM

Canalization of Seasonal Phenology in the Presence of Developmental Variation: Seed Dormancy Cycling in an Annual Weed

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Synopsis Variation in the developmental timing in one life stage may ramify within and across generations to disrupt optimal phenology of other life stages. By focusing on a common mechanism of developmental arrest in plants—seed dormancy—we investigated how variation in flowering time influenced seed germination behavior and identified potential processes that can lead to canalized germination behavior despite variation in reproductive timing. We quantified effects of reproductive timing on dormancy cycling by experimentally manipulating the temperature during seed maturation and the seasonal timing of seed dispersal/burial, and by assessing temperature-dependent germination of unearthed seeds over a seasonal cycle. We found that reproductive timing, via both seed-maturation temperature and the timing of dispersal, strongly influenced germination behavior in the weeks immediately following seed burial. However, buried seeds subsequently canalized their germination behavior, after losing primary dormancy and experiencing natural temperature and moisture conditions in the field. After the complete loss of primary dormancy, germination behavior was similar across seed-maturation and dispersal treatments, even when secondary dormancy was induced. Maternal effects themselves may contribute to the canalization of germination: first, by inducing stronger dormancy in autumn-matured seeds, and second by modifying the responses of those seeds to their ambient environment. Genotypes differed in dormancy cycling, with functional alleles of known dormancy genes necessary for the suppression of germination at warm temperatures in autumn through spring across multiple years. Loss of function of dormancy genes abolished almost all dormancy cycling. In summary, effects of reproductive phenology on dormancy cycling of buried seeds were apparent only as long as seeds retained primary dormancy, and a combination of genetically imposed seed dormancy, maternally induced seed dormancy, and secondary dormancy can mitigate variation in germination behavior imposed by variation in reproductive phenology.

Introduction

Different life stages frequently exhibit different environmental tolerances or optima. For instance, the eggs or seeds of many taxa are more resistant to freezing or desiccation than juvenile or reproductive stages, and insects enter diapause at specific life stages to escape environmental stresses (reviewed in Andrewartha 1952; Denlinger 1986, 2002; Sinclair et al. 2003; Baskin and Baskin 2014; Wilchesa et al. 2016). For this reason, the appropriate seasonal timing of developmental transitions (phenology)—such as hatch-out, diapause, or seed germination—is necessary to match each life stage to the seasonal conditions it can tolerate. The timing of prior life-stage transitions, however, can influence subsequent life stages, possibly disrupting optimal phenology in

subsequent stages. Organisms may be able to compensate for such variation, however, permitting individual life stages to counter effects of variation in prior stages through their own physiological responses to seasonal environments (Burghardt et al. 2015b, 2015c). To understand how organisms maintain adaptive phenology requires knowing the extent to which developmental variation in one life stage ramifies through subsequent life stages, and the extent to which the development of each life stage is controlled by environmental conditions experienced by that life stage as opposed to previous ones.

Prior life stages can influence subsequent life stages. In part, this is simply because later life stages cannot be expressed before previous ones, so a delay in early life stages can delay all subsequent ones unless compensatory developmental responses counteract it (Donohue 2014). Another manner through which prior life stages influence subsequent ones is through persistent effects of environmental conditions, such that environments experienced by one life stage have carry-over effects on later ones. These environmental effects can persist even across generations (Roach and Wulf 1987; Mousseau and Fox 1998). Such parental effects (frequently, maternal effects) may be adaptive, providing progeny with attributes to cope with specific environments before progeny are competent to respond to environments themselves (Mousseau and Fox 1998). Alternatively, parental effects may be neutral or maladaptive and simply result from environmental effects that cannot be mitigated in later developmental stages (Sultan 1995; Wright and McConnaughay 2002; van Kleunen and Fischer 2005; Valladares et al. 2007; reviewed in Auge et al. 2017b).

Developmental arrest is a general mechanism that may mitigate the effects of phenological variation in prior life stages on subsequent stages. Seasonally regulated developmental arrest is common across many taxa and is manifest as diapause in animals or seed or bud dormancy in plants (Andrewartha 1952, Denlinger 1986, 2002; Bewley 1997, Baskin and Baskin 2014). By inducing developmental arrest that is subsequently alleviated by certain environmental conditions, prior phenological variation may be dampened, leading to more canalized phenology subsequently.

In plants, seed dormancy is an important form of developmental arrest, and it suppresses the very first developmental transition in plants: seed germination. The timing of seed germination has been shown to be under extremely strong natural selection (reviewed in Donohue et al. 2010). In *Arabidopsis thaliana*, the focus of this study, germination timing has

been shown to contribute to local adaptation to diverse seasonal conditions across the geographic range of this species (Huang et al. 2010; Kronholm et al. 2012; Montesinos-Navarro et al. 2012; Akiyama and Ågren 2014; Postma and Ågren 2016; Vidigal et al. 2016; Marcer et al. 2017), and it has been shown to sometimes be under strong stabilizing selection, favoring germination in mid-autumn (Donohue et al. 2005). As such, seeds need to germinate within a specific, and narrow, time interval to maximize their probability of survival.

The timing of seed germination is determined by seed dormancy and the ability to germinate under specific environmental conditions as dormancy is lost. Seed dormancy is a state of developmental arrest during which seeds are not able to germinate under conditions that they could germinate under if they were not dormant (Bewley 1997; Baskin and Baskin 2014). "Primary seed dormancy" is imposed during the late states of seed maturation, and freshly shed seeds frequently exhibit strong primary dormancy. As seeds lose this primary dormancy, through a process referred to as "after-ripening," they acquire the ability to germinate over an increasingly wide range of environmental conditions. This phenomenon, termed "conditional dormancy," is manifest as an ability to germinate under some, but not all, conditions under which germination is possible. If non-dormant seeds fail to receive conditions conducive for germination—for instance, if they are denied light, adequate water, or a suitable temperature—they can enter "secondary dormancy." In *A. thaliana*, secondary dormancy can be induced by wet incubation at warm temperature, prolonged wet incubation at low temperature, and by low water potential, among other factors (Baskin and Baskin 1983; Auge et al. 2015; Coughlan et al. 2016; Edwards et al. 2016). Seeds in nature lose and regain dormancy over seasonal cycles ("dormancy cycling;" Baskin and Baskin 1972, 1983; Footitt et al. 2011, 2013, 2014).

The reproductive stage can influence subsequent life stages, including progeny stages, and such maternal effects are common across diverse taxa (Mousseau and Fox 1998; Miller 2008; van Asch et al. 2010; Carter et al. 2017). In plants, reproductive timing can influence seed germination time for two reasons. First, the timing of seed dispersal determines the seasonal timing and conditions that freshly dispersed seeds experience. All else being equal, seeds that are dispersed earlier have the opportunity to germinate earlier than late-dispersed seeds, and seeds dispersed at different times of year may experience different dormancy-breaking or

dormancy-inducing environmental conditions (Lacey and Pace 1983; Galloway 2001). Second, the environmental conditions experienced during seed maturation strongly influence the level of primary dormancy that is induced in seeds. In *A. thaliana*, the temperature experienced by maternal plants, both before and during seed maturation, influence seed dormancy and consequent germination behavior (Donohue et al. 2007; Kendall and Penfield 2012; Murphey et al. 2015; Auge et al. 2017a). In particular, cool temperature experienced during seed maturation imposes deeper primary dormancy than warmer temperatures, causing freshly dispersed seeds to have lower germination propensity. For both of these reasons, variation in the timing of reproduction can influence the germination of progeny. How then do plants accommodate variation in reproductive phenology to maintain adaptive germination time?

To investigate how developmental and environmental variation in prior life stages can be propagated across subsequent stages, or alternatively, how such variation may be mitigated to result in more canalized behavior subsequently, we studied how variation in reproductive timing influenced seed dormancy and germination behavior in the annual plant, *A. thaliana*. To express optimum germination timing, seeds need to germinate at a precise time of year, despite the influence of variation in reproductive timing. It is therefore pertinent to know how persistent the effects of reproductive timing on germination behavior are, and the mechanisms, such as developmental arrest via seed dormancy, whereby seeds may canalize their germination in the presence of variation in reproductive timing (Burghardt et al. 2015a). We first ask, how persistent are effects of differences in reproductive timing within seasons, across seasons, and across years; do effects of variation in reproductive timing dissipate as seeds lose and regain dormancy, and if so, how quickly? Second, do maternal effects on dormancy in any way compensate for temporal displacement caused by variation in dispersal time? That is, do seeds that are dispersed early versus late differ in dormancy, such that early-dispersed seeds are more dormant (and potentially later germinating) than late-dispersed seeds? Third, do certain dormancy genotypes exhibit more persistent effects of variation in reproductive timing than others? Otherwise put, are some dormancy genotypes more efficient than others at canalizing their germination behavior in the presence of variation in reproductive time? To answer these questions, we quantified how experimental variation in reproductive timing influenced seed germination and dormancy cycling, by examining the time course over which effects of reproductive-time
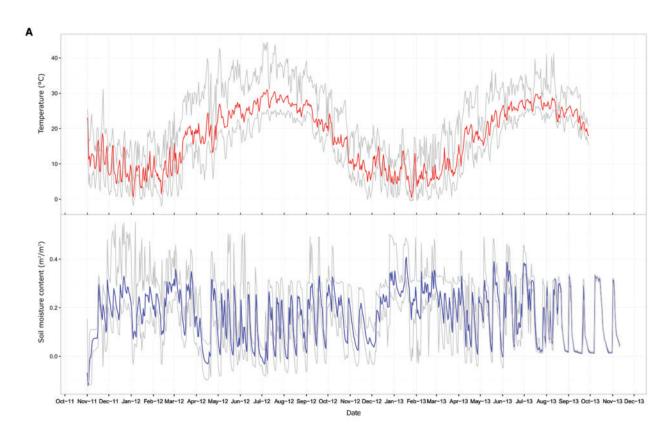
variation dissipated as buried seeds responded to their own environments under natural field conditions.

Methods

To test how the seasonal timing of reproduction influences germination, we experimentally manipulated reproductive timing by maturing plants under two temperatures and burying their fresh seeds at different times of year that correspond to natural seed-dispersal seasons. We then periodically unearthed these seeds and assessed their temperature-dependent germination as a measure of their depth of dormancy (Fig. 1).

We used six genotypes that differ in innate dormancy: two standard lab accessions, Landsberg *erecta* (L*er*) and Columbia (Col), and four contrasting genotypes that contain different natural variants of alleles at loci that influence seed dormancy. The genes *Delay of Germination-6* (*DOG6*) and *Delay of Germination-1* (*DOG1*) are major-effect loci in several quantitative trait locus (QTL) analyses of seed dormancy in *A. thaliana* (Alonso-Blanco et al. 2003; Bentsink et al. 2006, 2010), and *Flowering Locus C* (*FLC*) has been shown to promote seed germination (Chiang et al. 2009; Blair et al. 2017). We used near isogenic lines (NILs) that contain the active *FLC* allele or the dormant *DOG6*, or *DOG1* alleles from the Cape Verde Island (Cvi) accession, introgressed into the L*er* reference ecotype, such that all lines shared the L*er* genetic background but differed in the chromosomal region containing the dormancy locus (Alonso-Blanco et al. 2003). We also used a knock-out mutant of the highly dormant *DOG1*-Cvi allele derived from the $DOG1_{Cvi}$ NIL background ($dog1_{Cvi}$), isolated by Bentsink et al. (Bentsink et al. 2006), providing a contrast among the partially dormant L*er* reference accession, the highly dormant $DOG1_{Cvi}$ NIL, and the low-dormancy $dog1_{Cvi}$ knock-out, all on the same L*er* reference background. These lines were obtained from Maarten Koorneef and Leonie Bentsink

To simulate variation in the seasonal timing of reproduction, we manipulated the temperature and season of seed-maturation and dispersal. Specifically, we matured seeds at either $25\,^{\circ}C$ and $14\,^{\circ}C$, corresponding to late spring/early summer or early spring/autumn seed-maturation temperatures, respectively. We then buried seeds in the field at six different times. Seed burial times included burial in early and late autumn, early and late spring in the first year, and an additional autumn and spring burial in the second year. We subsequently un-earthed the seeds at regular intervals and assessed their
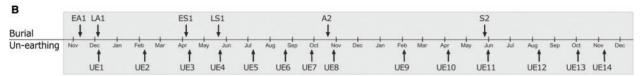
Fig. 1 Experimental design and environmental conditions during the experiment. (**A**) Hourly soil temperature (upper) and soil moisture (lower) over the course of the experiment (*x*-axis) averaged over all blocks. Temperature and moisture were smoothed (darker line). (**B**) Experimental schedule. Downward arrows indicate the timing of seed burial. Upward arrows indicate the timing of seed un-earthing (UE). Seed burial times are abbreviated as follows: EA1 = early autumn in the first year, LA1 = late autumn in the first year, ES1 = early spring in the first year, LS1 = late spring I the first year, A2 = autumn of the second year, S2 = spring of the second year.

temperature-dependent germination as a measure of the state of dormancy, or developmental arrest of the seeds expressed at a range of ecologically plausible temperatures that span the known range of permissive germination temperatures in *A. thaliana* (Burghardt et al. 2015a). Figure 1 shows the experimental schedule. Germination assays were conducted in the lab at 8 °C, 16 °C, 22 °C, and 31 °C. See Supplemental Text 1 for details of the experimental methods, including seed-production conditions, seed burial and un-earthing treatments, and germination assays.

Statistical analysis

The final proportion of seeds that germinated was analyzed with logistic regression (PROC LOGISTIC in SAS 9.4; SAS Institute) using Fisher's scoring optimization (ML) algorithm, Type-III likelihood ratio tests. The Firth's penalized likelihood was used to accommodate issues of quasi-separation caused by extreme germination proportions (0% or 100%) in some treatments. The total number of germinants (successes)/the total number of viable seeds (trials) was the dependent variable for all analyses.

We first implemented a full model that included all two-way, three-way, and four-way interactions, but the four-way interactions were not significant and therefore dropped from the model. Genotype (Geno), maternal plant temperature (Mat), Burial, Un-earthing (UE), and lab germination temperature (Temp) treatments were all fixed factors. When testing for differences between genotypes, each genotype was compared to the L*er* reference genotype. Because

of significant interactions with genotype, we then tested for effects of the treatments for each genotype separately. To evaluate interactions with genotype and to examine the treatments in which genotypic differences were expressed versus masked, we also tested for genotype differences in each combination of Mat, Burial, UE, and Temp treatments separately (Supplementary Fig. S1).

Because not all Burial cohorts were represented in each UE time point, we analyzed subsets of those cohorts separately. First, to test for persistent effects of burial time within a given season (either autumn or spring), we analyzed early versus late burial in autumn (EA1 and LA1) across a full year (UE1-UE8), and we analyzed early versus late burial in spring (ES1 and LS1) across a full year (UE4-UE11). Second, to compare patterns across seasons, we analyzed all Burial cohorts dispersed in the first year (EA1, LA1, ES1, LS1) for the UE time points that were represented in all those Burial cohorts, namely summer through autumn of the first year (UE4-UE8). Third, to test for between-year differences within each burial season, we compared germination of seeds that had been buried for a full year to that of freshly buried seeds. Specifically, we compared germination of seeds dispersed in autumn of 2011 versus autumn 2012 (EA1 vs. A2), from autumn to late summer of the second year (UE8-UE12). Likewise, we compared germination of 1-year old versus fresh seeds buried in late spring in 2012 versus 2013 (LS1 and S2), from late spring through autumn (UE11-UE14).

Mean hourly temperature and moisture readings were obtained from each of six weather stations. We considered the effects of three distinct temperature ranges hypothesized to influence germination in qualitatively different ways based on prior research: permissive temperatures ("optT" = between $6\,^\circ C$ and $27\,^\circ C$) that represent the range of temperatures under which germination is known to occur in non-dormant seeds, based on laboratory studies (Burghardt et al. 2015a), cold-stratifying temperatures ("cold" = $<6\,^\circ C$) that are known to break or induce dormancy depending on the duration of time spent at those temperatures (Coughlan et al. 2016), and supra-optimal temperatures ("supraT" = $>27\,^\circ C$) that are known to induce secondary dormancy in the lab (Auge et al. 2015; Coughlan et al. 2016). We also investigated the effects of environmental conditions during two time periods: 2 weeks before un-earthing ("2 weeks"), and the time from burial up to 2 weeks before unearthing ("prior"). We used the two burials that had the longest time series for analysis: EA1 (autumn) and ES1

(spring). The main text presents results of a logistic regression that tested for effects on germination of hydrothermal units accumulated (sum of temperature × soil moisture; a metric that captures the phenomenon that responses to temperature are more pronounced under more moist conditions) over the two time periods (2 weeks and prior). Mat, Temp, Genotype (fixed factors), and duration of burial (time from burial to unearthing, continuous) and their interactions with the environmental factors were included as co-variates. See Supplemental Text 2 for more details on the analysis of environmental data.

Results

Starting levels of primary dormancy

Seeds in each Burial cohort had some conditional dormancy at the time they were buried, indicated by a low propensity to germinate at the higher temperatures (Fig. 2). Seeds matured in cool temperature had greater dormancy (lower germination proportions) than those matured at higher temperature, with this effect being strongest for seeds imbibed at the highest temperature ($31\,^\circ C$). Temperature-dependent germination and the effect of seed-maturation temperature also varied among genotypes, as described below further.

Patterns of dormancy cycling in the field

Considering seeds buried in the first year (EA1, LA1, ES1, LS1), seeds buried in autumn lost dormancy over winter and spring, and were able to germinate to high percentages even at the highest temperature ($31\,^\circ C$) by mid-May (Fig. 2). Seeds buried in spring lost dormancy throughout the summer. By October, all seeds buried in both seasons in 2011–2012 were able to germinate to 100% at all temperatures. As winter progressed, seeds in all Burial cohorts entered secondary dormancy, as indicated by their decreased ability to germinate at the highest temperature. Seeds lost dormancy again by mid-May to autumn the following year, depending on the genotype (see below further), and re-entered dormancy again by autumn (seen in seeds of ES1, the only Burial cohort from that year remaining). Interestingly, this reduction of germination was manifest at the lowest imbibition temperature, not the highest imbibition temperature as was seen in prior un-earthing cohorts, and the same pattern was seen in seeds buried that same year (A2, S2). In summary, dormancy cycling was observed such that freshly dispersed seeds lost dormancy over their first summer, manifest as an ability to germinate at increasingly higher temperature,
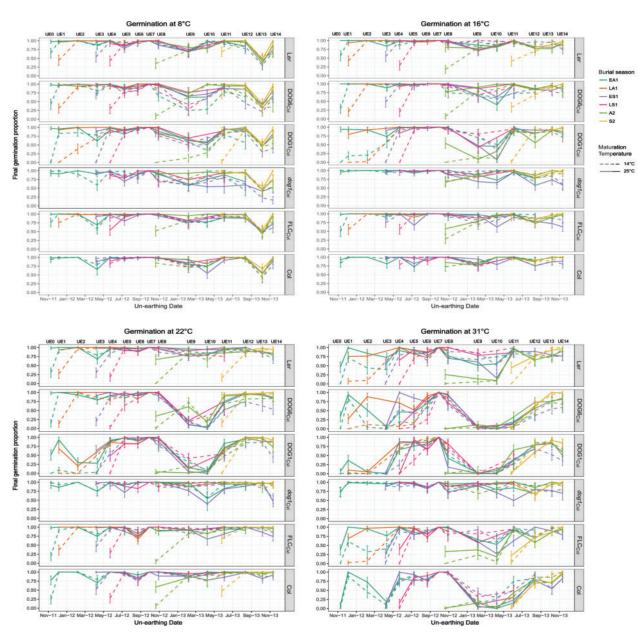
Fig. 2 Mean germination proportions (± SE), for each seed-imbibition temperature in lab germination assays (8 °C, 16 °C, 22 °C, 31 °C), shown in separate panels. Results for each genotype are shown in separate rows within each larger panel. Un-earthing time points (UE0–UE14) are shown across *x*-axis, with UE0 as the seed germination proportion just before the first burial. Each Burial cohort (EA1–S2) is indicated by different colored/greyscale lines (see key), with solid lines representing germination of seeds matured at 25 °C temperature and dotted lines representing germination of seeds matured at 14 °C temperature.

regained dormancy over winter (losing the ability to germinate at high temperature), and lost secondary dormancy over the subsequent summer.

Genotypic differences in dormancy cycling

The genotypes used in this experiment were chosen because they are known to differ in primary dormancy. As expected, Col, FLC$_{Cvi}$, and *dog1*$_{Cvi}$ had higher germination proportions than L*er* in several initial assessments of germination before seed burial,

and DOG6$_{Cvi}$ and DOG1$_{Cvi}$ had much less germination than L*er* (Fig. 2 and Supplementary Fig. S1; Supplementary Tables S1 and S2). These differences among genotypes were manifest as differences in the ability to germinate at high temperature, and they were much more pronounced in seeds matured under cool temperatures than in those matured at warm temperature, because most warm-matured seeds of all genotypes had very low dormancy. Thus, differences in primary dormancy among genotypes tended to be most pronounced under

conditions that induced greater dormancy (cool seed-maturation) and permitted less germination (31 °C imbibition).

Genotypes differed in the intensity of the maternal temperature effect (Supplementary Table S1). For most genotypes, induction of dormancy by cool seed-maturation temperature was most apparent when seeds were subsequently imbibed at 31 °C, but for seeds of DOG1$_{Cvi}$, which were more dormant, maternal temperature effects were also pronounced for seeds imbibed at 22 °C. Seeds of the $dog1_{Cvi}$ mutant had low dormancy and did not exhibit strong maternal effects on germination at any temperature.

Differences in germination behavior between Ler and the more dormant genotypes DOG6$_{Cvi}$ or DOG1$_{Cvi}$ increased over burial time initially (Supplementary Fig. S1; Supplementary Tables S1, S2), as Ler lost primary dormancy more quickly and became indistinguishable from the less dormant genotypes FLC$_{Cvi}$ and $dog1_{Cvi}$. All differences among genotypes disappeared completely by October, when all seeds lost dormancy. Genotypic differences re-emerged as seeds entered secondary dormancy. Therefore, genotypic differences in germination propensity are most likely to be expressed in late autumn through early spring, but not over summer and early autumn.

Genotypes differed not only in primary dormancy, but also in the depth of secondary dormancy induction. Col, DOG1$_{Cvi}$, and DOG6$_{Cvi}$ in particular were induced into strong secondary dormancy during the winter months. Although most genotypes germinated to low percentages at 31 °C after secondary dormancy induction, DOG1$_{Cvi}$ and DOG6$_{Cvi}$ also had low germination percentages at 22 °C. Seeds of Col, DOG1$_{Cvi}$, and DOG6$_{Cvi}$ lost secondary dormancy more slowly than Ler, acquiring high germination percentages at the highest temperature only in the autumn (compared to late spring for Ler).

The $dog1_{Cvi}$ genotype exhibited high germination percentages under almost all conditions. It rarely exhibited significant maternal temperature effects (Tables 1–3), and exhibited little dormancy cycling, although its germination propensity did vary over time in a non-systematic manner. This genotype did exhibit somewhat less germination at the lowest temperature of 8 °C, as did several genotypes that were dispersed in the second year, suggesting that the reduction of germination at low temperature does not depend on the gene DOG1.

In summary, considering the entire seasonal cycle, germination at high temperature was restricted primarily during winter and spring, with Ler, FLC$_{Cvi}$,

and Col exhibiting reduced germination at 31 °C, DOG6$_{Cvi}$ exhibiting reduced germination down to 22 °C, and DOG1$_{Cvi}$ down to 16 °C. The $dog1_{Cvi}$ mutant did not exhibit dormancy cycling and germinated to high percentages over all temperatures across most of the year.

Canalization of germination behavior across dispersal cohorts

We first examined the persistence of effects of burial time within a season. For seeds buried early versus late in autumn (EA1 vs. LA1), differences in the germination behavior between Burial cohorts changed over time (indicated by significant Burial × UE interactions in Supplementary Table S3) and disappeared after a single un-earthing interval (indicated by non-significant effects of Burial treatment by UE2 in Table 1; Fig. 2). For seeds buried in early versus late spring (ES1 vs. LS1), seeds likewise attained similar germination behavior after a single un-earthing interval (Table 1, S3). Thus, differences in germination imposed by variation in burial time within a season were quickly ameliorated.

Considering effects of burial time across seasons within a year, namely between autumn versus spring (EA1, LA1, ES1, LS1), differences among dispersal cohorts disappeared after a single un-earthing interval after spring burial, by July (Table 2, Supplementary Tables S1, S4; Fig. 2). All seeds had identical and complete germination at all temperatures when un-earthed in October. Therefore, seeds dispersed in spring attained germination behavior that was indistinguishable from those dispersed in autumn within a few weeks.

Comparing seeds buried in different years (Fig. 2), seeds buried in autumn in 2011 (EA1) were less dormant when assayed in autumn 2012 than were seeds buried that same autumn (A2). Seeds buried in autumn of 2012 did not lose primary dormancy until the summer of 2013, thereby attaining similar germination ability as seeds buried a year earlier (Table 3, Supplementary Table S5). Therefore, autumn-dispersed seeds buried in different years required months of burial to acquire similar germination behavior, after periods of cold followed by warm temperature. For seeds buried in spring, seeds buried 1 year later acquired similar germination behavior after a single un-earthing interval (Table 3, S5). However, slight differences in germination reappeared in subsequent months, apparent as a slightly reduced ability to germinate at the higher temperatures in seeds buried a year earlier (those entering secondary dormancy).

Table 1 Results of logistic regression analysis of germination to test for the persistence of effects of burial time within a season (Autumn and Spring) for each genotype

A. Autumn burial season

Predictor	DF	UE1	UE2	UE4	UE5	UE6	UE8
L*er*							
Burial	1	30.50***	0.52	2.71	1.89	0.32	0.07
Mat	1	17.25***	1.64	3.46	10.37**	0.15	5.01*
Temp	3	9.67*	90.15***	1.53	26.72***	23.01***	10.06*
Burial × Mat	1	0.84	0.80	0.01	0.14	1.41	0.27
Burial × Temp	3	1.04	0.41	2.36	3.06	1.88	3.77
Mat × Temp	3	2.55	4.09	3.94	1.11	1.01	1.66
Burial × Mat × Temp	3	1.57	0.33	1.05	0.28	2.51	1.65
FLC$_{Cvi}$							
Burial	1	10.71**	0.56	0.20	0.21	0.00	0.16
Mat	1	18.89***	6.11*	5.03*	0.90	0.01	1.01
Temp	3	16.29***	156.89***	4.19	2.33	11.28*	2.72
Burial × Mat	1	2.76	0.35	0.18	0.49	0.02	1.18
Burial × Temp	3	2.58	0.76	3.96	2.51	0.30	4.12
Mat × Temp	3	1.90	39.84***	2.86	7.32	0.30	1.57
Burial × Mat × Temp	3	2.72	2.68	0.78	1.99	1.01	5.13
DOG6$_{Cvi}$							
Burial	1	27.59***	0.00	0.04	0.03	0.56	0.23
Mat	1	21.95***	1.10	5.22*	2.07	3.09	11.53***
Temp	3	32.56***	80.58***	70.00***	139.02***	21.07***	4.87
Burial × Mat	1	14.28***	0.83	3.28	0.57	0.31	0.78
Burial × Temp	3	5.73	6.72	1.11	1.40	3.05	0.57
Mat × Temp	3	0.81	10.64*	0.96	8.60*	3.38	2.77
Burial × Mat × Temp	3	1.51	0.96	0.47	6.70	0.72	0.80
DOG1$_{Cvi}$							
Burial	1	12.17***	0.98	0.26	0.02	0.05	0.31
Mat	1	42.31***	30.61***	2.41	2.68	0.46	5.21*
Temp	3	9.35*	45.28***	17.00***	17.83***	13.08**	36.22***
Burial × Mat	1	4.01*	0.14	0.57	0.60	0.00	0.00
Burial × Temp	3	0.69	0.36	1.09	1.83	1.19	0.82
Mat × Temp	3	1.64	1.82	0.80	1.25	1.04	8.58*
Burial × Mat × Temp	3	2.83	0.40	2.76	3.67	0.09	1.71

B. Spring burial season

Predictor	DF	UE4	UE5	UE6	UE8	UE9	UE11
L*er*							
Burial	1	22.87***	0.92	0.00	8.76**	1.23	1.37
Mat	1	43.57***	0.02	0.03	1.14	1.44	4.80*
Temp	3	16.38***	15.98***	20.97***	0.14	4.19	5.16
Burial × Mat	1	9.77**	0.86	0.00	0.91	0.06	0.30
Burial × Temp	3	7.69	3.58	7.51	1.86	1.97	1.34
Mat × Temp	3	4.20	10.27*	0.78	1.13	2.84	16.24***
Burial × Mat × Temp	3	1.79	1.44	0.58	0.48	1.26	4.45

(continued)

Table 1 Continued

B. Spring burial season Predictor	DF	UE4	UE5	UE6	UE8	UE9	UE11
FLC_{Cvi}							
Burial	1	21.45***	3.34	0.09	1.06	0.01	3.94*
Mat	1	5.41*	4.50*	0.21	1.06	0.03	0.34
Temp	3	13.79**	7.16	9.42*	4.79	8.00*	3.29
Burial × Mat	1	1.38	2.90	0.00	0.07	0.37	19.05***
Burial × Temp	3	4.10	4.33	0.39	0.92	1.10	1.26
Mat × Temp	3	8.76*	5.55	2.61	6.76	8.51*	5.97
Burial × Mat × Temp	3	0.31	1.13	1.37	0.73	1.63	0.29
$DOG6_{Cvi}$							
Burial	1	33.67***	0.08	0.01	0.86	0.01	1.19
Mat	1	18.03***	16.47***	1.15	5.33*	4.02*	5.64*
Temp	3	12.14**	83.59***	37.70***	15.06**	49.05***	115.38***
Burial × Mat	1	0.67	2.57	0.02	0.90	0.00	0.01
Burial × Temp	3	1.77	18.68***	1.18	2.47	1.02	1.43
Mat × Temp	3	3.00	5.69	1.96	1.50	5.74	7.08
Burial × Mat × Temp	3	1.23	6.94	0.17	1.28	1.57	0.36
$DOG1_{Cvi}$							
Burial	1	76.15***	0.71	0.13	2.95	0.02	0.26
Mat	1	30.84***	1.59	0.02	4.17*	1.52	0.26
Temp	3	20.96***	13.84***	28.83***	34.94***	32.64***	79.45***
Burial × Mat	1	8.28**	0.12	0.03	1.54	0.00	0.18
Burial × Temp	3	4.34	1.27	1.24	2.51	0.68	1.54
Mat × Temp	3	5.56	9.68*	2.67	9.32*	13.57**	3.40
Burial × Mat × Temp	3	12.04**	14.13**	3.72	1.37	0.62	0.49
$dog1_{Cvi}$							
Burial	1	0.57	0.45	0.02	0.95	1.93	11.57***
Mat	1	1.82	3.35	0.36	1.34	1.37	3.40
Temp	3	6.90	28.88***	6.48	2.96	16.14***	21.13***
Burial × Mat	1	1.63	0.53	0.09	1.07	0.00	4.25
Burial × Temp	3	4.21	0.39	3.19	0.82	3.96	2.26
Mat × Temp	3	1.97	2.55	2.38	7.82*	6.75	7.43
Burial × Mat × Temp	3	1.36	1.52	0.83	1.67	1.95	0.54
Col							
Burial	1	10.56**	2.09	0.08	1.75	0.00	0.25
Mat	1	4.54*	0.04	0.02	2.30	0.01	1.02
Temp	3	22.28***	26.65***	8.39*	1.18	38.11***	67.91***
Burial × Mat	1	2.92	0.16	0.06	0.91	0.00	3.37
Burial × Temp	3	5.95	9.56*	0.15	4.18	2.61	0.56
Mat × Temp	3	5.54	2.79	1.13	1.20	7.26	0.05
Burial × Mat × Temp	3	1.95	0.66	0.92	1.73	1.53	3.92

Notes: A) For the autumn burial season, EA1 and LA1 were compared through a full year (through the 8th un-earthing cohort). Col and $dog1_{Cvi}$ were not able to be compared because they were not included in LA1. B) For the spring burial season, ES1 and LS1 were compared from a full year (from the 4th through the 11th un-earthing cohort). UE3 and UE7 had inadequate variance and were omitted from the analysis. "UE" indicates un-earthing cohort. "Mat" indicates maternal temperature treatment, and "Temp" indicates seed-imbibition temperature during germination assays. Wald Chi-square values are given for joint tests of significant effects.
*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Table 2 Results of logistic regression analysis of germination to test for the persistence of effects of burial time across autumn versus spring burial seasons for each genotype

Predictor	DF	UE4	UE5	UE6	UE8
L*er*					
Burial	3	50.43***	4.61	0.29	12.04**
Mat	1	28.98***	4.62*	0.02	5.35*
Temp	3	9.42*	32.64***	43.35***	2.18
Burial × Mat	3	18.80***	7.70	1.41	3.79
Burial × Temp	9	16.18	8.81	10.89	8.99
Mat × Temp	3	2.62	5.23	1.73	2.03
Burial × Mat × Temp	9	9.78	7.92	3.17	2.39
FLC$_{Cvi}$					
Burial	3	100.64***	6.13	0.14	1.42
Mat	1	6.63**	5.00*	0.15	2.05
Temp	3	10.22*	7.97*	18.37***	6.08
Burial × Mat	3	4.65	4.78	0.10	1.40
Burial × Temp	9	14.17	11.64	1.50	8.76
Mat × Temp	3	7.58	4.37	2.14	2.07
Burial × Mat × Temp	9	7.77	11.30	2.62	11.10
DOG6$_{Cvi}$					
Burial	3	52.25***	0.11	2.13	1.30
Mat	1	20.94***	14.68***	3.65	16.89***
Temp	3	53.36***	217.29***	49.88***	17.08***
Burial × Mat	3	6.25	5.31	0.43	2.39
Burial × Temp	9	6.65	28.77***	5.63	3.89
Mat × Temp	3	2.87	13.85**	0.88	4.04
Burial × Mat × Temp	9	3.19	14.82	4.64	2.48
DOG1$_{Cvi}$					
Burial	3	64.97***	3.78	0.51	4.47
Mat	1	21.10***	0.24	0.13	9.40**
Temp	3	33.47***	30.25***	24.83***	62.68***
Burial × Mat	3	8.43*	5.19	0.34	2.29
Burial × Temp	9	7.30	3.82	16.68	3.84
Mat × Temp	3	4.68	8.35*	3.70	17.21***
Burial × Mat × Temp	9	12.88	19.70*	5.33	4.75
dog1$_{Cvi}$					
Burial	3	1.42	0.56	0.03	2.49
Mat	1	2.60	7.86**	0.87	2.42
Temp	3	5.90	20.49***	12.13**	6.33
Burial × Mat	3	2.09	1.44	0.16	1.39
Burial × Temp	9	7.42	4.73	5.90	1.81
Mat × Temp	3	2.57	0.86	3.07	3.87
Burial × Mat × Temp	9	2.19	9.86	2.82	4.15
Col					
Burial	3	17.53***	2.52	0.17	2.73
Mat	1	2.85	1.58	0.00	0.65
Temp	3	36.84***	38.25***	14.21**	1.81

(continued)

Table 2 Continued

Predictor	DF	UE4	UE5	UE6	UE8
Burial × Mat	3	5.48	1.69	0.14	4.38
Burial × Temp	9	7.21	12.53	1.24	7.05
Mat × Temp	3	2.68	4.49	1.11	2.64
Burial × Mat × Temp	9	2.84	1.71	1.54	2.29

Note: The analysis uses factorial Burial and Un-earthing (UE) treatments for one full year: specifically, EA1-LS1 for un-earthing cohorts UE4-UE8. "Mat" indicates maternal temperature treatment and "Temp" indicates seed-imbibition temperature during germination assays. Wald Chi-square values are given for joint tests of significant effects. The seventh un-earthing cohort had no variance because of 100% germination in most treatments, so analyses could not be conducted; only $DOG6_{Cvi}$ showed incomplete germination and a significant Mat × Temp interaction for that un-earthing cohort (Wald Chi-square = 11.90, $P < 0.01$). Col and $dog1_{Cvi}$ were not included in LA1, so analysis of these genotypes does not include LA1.

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

Table 3 Results of logistic regression analysis of germination proportions to test for effects of burial time across years within each season (Autumn and Spring) for each genotype

A. Autumn burial season

Predictor	DF	UE8	UE9	UE10	UE11	UE12
Ler						
Burial	1	45.9***	9.33**	2.45	0.31	0.61
Mat	1	23.79***	0.00	0.34	3.26	0.06
Temp	3	27.60***	46.21***	31.75***	6.20	13.20**
Burial × Mat	1	3.67	0.07	0.79	0.78	1.48
Burial × Temp	3	4.33	10.61*	9.11*	2.18	2.84
Mat × Temp	3	2.06	1.26	2.39	6.43	1.10
Burial × Mat × Temp	3	0.73	2.31	2.27	1.18	1.52
FLC_{Cvi}						
Burial	1	30.12***	3.47	0.13	3.26	0.02
Mat	1	6.68**	2.06	0.00	0.54	0.08
Temp	3	6.38	38.62***	56.39***	5.11	14.98**
Burial × Mat	1	7.06**	0.00	0.10	0.92	1.06
Burial × Temp	3	5.12	6.32	11.10*	3.40	2.95
Mat × Temp	3	1.41	0.24	1.49	6.70	1.75
Burial × Mat × Temp	3	8.03*	1.00	1.18	1.55	0.17
$DOG6_{Cvi}$						
Burial	1	64.02***	11.23***	12.47***	5.55*	0.03
Mat	1	27.06***	0.99	3.02	7.36**	6.70**
Temp	3	16.17***	38.91***	67.73***	130.93***	18.40***
Burial × Mat	1	0.02	0.28	0.00	1.35	0.30
Burial × Temp	3	13.31**	3.29	7.89*	2.92	1.25
Mat × Temp	3	9.34*	6.50	0.14	1.51	3.90
Burial × Mat × Temp	3	2.85	1.20	3.54	5.91	0.86
$DOG1_{Cvi}$						
Burial	1	70.17***	4.37*	1.20	0.06	3.80
Mat	1	16.83***	0.60	2.48	13.97***	1.58
Temp	3	15.50**	25.36***	21.74***	64.78***	8.09*
Burial × Mat	1	2.91	0.15	0.04	0.60	0.02

(continued)

Table 3 Continued

A. Autumn burial season

Predictor	DF	UE8	UE9	UE10	UE11	UE12
Burial × Temp	3	3.66	5.4	2.35	2.73	6.81
Mat × Temp	3	10.76*	11.39**	0.30	1.13	5.93
Burial × Mat × Temp	3	5.45	5.22	1.64	3.37	1.24
dog1$_{Cvi}$						
Burial	1	0.80	0.54	8.56**	10.77**	5.57*
Mat	1	0.47	4.31*	0.06	5.57*	0.03
Temp	3	5.67	6.49	1.27	11.80**	5.41
Burial × Mat	1	1.37	0.37	1.12	0.06	0.56
Burial × Temp	3	6.10	6.50	2.93	0.62	4.07
Mat × Temp	3	1.24	2.19	1.63	2.29	3.21
Burial × Mat × Temp	3	1.47	0.51	2.70	2.95	2.06
Col						
Burial	1	61.15***	0.17	0.49	0.02	0.80
Mat	1	4.77*	0.62	0.04	0.13	0.00
Temp	3	19.95***	70.84***	22.88***	105.93***	17.06***
Burial × Mat	1	1.41	0.09	0.10	1.31	0.39
Burial × Temp	3	22.54***	1.55	2.27	0.10	1.00
Mat × Temp	3	0.62	2.42	1.64	4.66	0.18
Burial × Mat × Temp	3	1.01	1.02	0.43	0.82	2.16

B. Spring dispersal season

Predictor	DF	UE11	UE12	UE13	UE14
L*er*					
Burial	1	20.37***	0.14	6.92**	12.68***
Mat	1	40.00***	0.77	0.30	2.10
Temp	3	24.43***	5.49	55.37***	0.90
Burial × Mat	1	20.98***	0.28	0.08	0.23
Burial × Temp	3	13.31**	1.09	2.51	1.60
Mat × Temp	3	0.86	2.29	3.01	2.31
Burial × Mat × Temp	3	3.10	1.98	2.79	2.16
FLC$_{Cvi}$					
Burial	1	6.49*	0.81	4.46*	22.73***
Mat	1	2.15	0.00	3.20	0.35
Temp	3	45.36***	8.53*	26.76***	0.69
Burial × Mat	1	28.70***	0.08	0.17	0.42
Burial × Temp	3	34.59***	1.78	3.46	0.09
Mat × Temp	3	7.12	2.63	2.85	1.46
Burial × Mat × Temp	3	6.28	2.13	0.51	0.35
DOG6$_{Cvi}$					
Burial	1	3.60	3.05	10.71***	19.78***
Mat	1	33.31***	4.13*	3.55	4.56*
Temp	3	93.26***	28.86***	61.12***	4.85
Burial × Mat	1	7.65**	0.35	0.00	0.04
Burial × Temp	3	4.53	5.90	6.87	3.32

(continued)

Table 3 Continued

| B. Spring dispersal season | | | | | |
Predictor	DF	UE11	UE12	UE13	UE14
Mat × Temp	3	7.39	2.35	1.29	1.05
Burial × Mat × Temp	3	7.09	1.19	0.59	0.57
$DOG1_{Cvi}$					
Burial	1	50.40***	1.92	5.22*	25.67***
Mat	1	26.76***	0.12	1.60	8.27**
Temp	3	30.33***	20.63***	40.60***	10.86*
Burial × Mat	1	20.82***	0.82	0.03	0.78
Burial × Temp	3	0.96	3.29	3.90	0.86
Mat × Temp	3	3.41	1.30	0.56	3.21
Burial × Mat × Temp	3	1.02	1.14	2.20	1.50
$dog1_{Cvi}$					
Burial	1	19.77***	2.57	7.18**	36.04***
Mat	1	8.71**	0.00	0.44	5.21*
Temp	3	8.43*	6.73	43.98***	4.02
Burial × Mat	1	3.57	0.54	4.06*	5.14*
Burial × Temp	3	2.58	8.23*	1.98	1.85
Mat × Temp	3	0.83	2.78	2.15	1.94
Burial × Mat × Temp	3	5.18	0.49	0.55	0.15
Col					
Burial	1	4.36*	0.00	5.75*	21.86***
Mat	1	0.56	0.24	0.01	0.71
Temp	3	90.50***	12.71**	13.63**	1.12
Burial × Mat	1	9.55**	0.07	0.01	0.47
Burial × Temp	3	7.51	5.77	3.52	0.47
Mat × Temp	3	3.24	0.77	3.41	1.24
Burial × Mat × Temp	3	6.34	1.72	1.94	1.03

Notes: A) For the Autumn burial season, EA1 and A2 were compared from November of the second year through September of the third year (the 8th through the 12th un-earthing time points). B) For the Spring burial season, ES1 and S2 were compared from late spring through autumn of the second year (the 11th through the 14th un-earthing time points). "UE" indicates un-earthing time point. "Mat" indicates maternal temperature treatment, and "Temp" indicates seed-imbibition temperature during germination assays. Wald Chi-square values are given for joint tests of significant effects.
*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

One genotype in particular, DOG6$_{Cvi}$, retained differences across years for longer than Ler; this effect was apparent as a reduced ability to germinate at 22 °C in seeds with secondary, but not primary, dormancy (Supplementary Fig. S2; Table 3; and Supplementary Table S2). That is, DOG6$_{Cvi}$ exhibited deeper secondary dormancy than primary dormancy but Ler did not, such that inter-annual differences were manifest in DOG6$_{Cvi}$ but not in Ler. Therefore, differences between primary and secondary dormancy behavior can be manifest as inter-annual differences in germination ability.

In summary, seeds were able to canalize their germination behavior in response to variation in burial time within and between seasons, and they did so within weeks of being buried. All cohorts exhibited identical germination behavior after complete loss of primary dormancy, and the cohorts cycled into and out of secondary dormancy in a consistent manner thereafter. Nonetheless, differences were apparent between seeds buried in similar seasons but in different years, reflecting differences between primary and secondary dormancy.

Dissipation of maternal temperature effects over time

Maternal temperature effects were pronounced in freshly shed seeds, such that seeds matured under

cool temperature germinated less than seeds matured under warm temperature, especially when seeds were imbibed at high temperatures (22 °C and 31 °C; Fig. 2). These maternal temperature effects dissipated over time (indicated by significant Mat x UE interactions in Tables S3–S5). For seeds buried in autumn, maternal effects persisted until spring in most genotypes. For seeds buried in spring, maternal effects persisted during the summer in some genotypes, but dissipated by autumn. Maternal effects reappeared in seeds induced into secondary dormancy (after UE8) in some genotypes, but they were smaller in magnitude than those expressed in seed with primary dormancy.

Effects of field environmental variables on germination

A greater accumulation of hydrothermal units at optimal temperatures, but less hydrothermal accumulation at low (<6 °C) and high (>27 °C) temperature, was associated with higher germination. This relationship was found for environmental conditions experienced both 2 weeks before un-earthing and earlier (Table 4, Supplementary Table S6), revealing long-term effects of environmental conditions during burial. The direction of environmental effects was similar across burial times and seed-maturation temperature, although differences in magnitude were detected, indicating that experimental reproductive timing altered the sensitivity of seeds to ambient conditions. Specifically, germination of seeds buried in autumn was more strongly impeded by low temperature (especially that experienced shortly before un-earthing), and germination of seeds buried in spring were more impeded by hot temperature. See Supplemental Text 2 for more details of treatment- and genotype-specific responses to ambient environmental conditions.

Discussion

The appropriate seasonal timing of developmental transitions is necessary for expressing adaptive life cycles in seasonal environments. However, variation in the developmental timing in one life stage may influence developmental transitions of subsequent life stages within and across generations. We investigated potential processes whereby the seed life stage may counter effects of variation in the reproductive life stage in the previous generation, to regulate the critical developmental transition of seed germination.

We found that effects of seed-maturation temperature and timing of burial had strong effects on germination soon after seeds were buried. However,

seeds canalized their germination behavior in response to variation in burial time, both within and across seasons, and they did so within weeks of being buried. Thus, ambient conditions appear to quickly over-ride effects of primary dormancy imposed at the time of seed burial. After the loss of primary dormancy, all cohorts cycled into and out of secondary dormancy in a consistent manner. Therefore, secondary dormancy and responses of buried seeds to ambient conditions may canalize germination behavior across cohorts after the loss of primary dormancy.

Seed responses to ambient conditions can canalize germination behavior

Maternal reproductive timing influenced seed germination via effects of the temperature during seed-maturation and via effects of the environmental conditions experienced by seeds immediately after burial, determined by the timing of burial. Cool seed-maturation temperature imposed stronger primary dormancy, manifest as a decreased ability to germinate at warm temperature, as has been shown previously (Kendall and Penfield 2012; Burghardt et al. 2015a). However, these maternal effects quickly dissipated as seeds responded to their own environments in the soil.

Seeds were influenced by environmental conditions experienced soon (2 weeks) before un-earthing, but also by conditions experienced before that. In particular, the hydrothermal accumulation within the temperature range that is promotive of germination in *A. thaliana* (Burghardt et al. 2015) most strongly predicted germination propensity. The observation that hydrothermal accumulation, as opposed to thermal or hydro-accumulation independently (see Supplemental Text 2), was a better predictor of germination suggests that dry after-ripening alone does not determine germination ability, and that hydrothermal accumulation is a key determinant of germination ability even in seeds with some degree of primary dormancy (Meyer et al. 2000; Bradford 2002; Allen 2003; Alvarado and Bradford 2005). Surprisingly, increased exposure to cool temperatures, considered to be a dormancy-breaking treatment in *A. thaliana* (Nordborg and Bergelson 1999; Coughlan et al. 2016), did not increase germination of seeds in this experiment, but instead was associated with decreased germination, whether through lack of opportunity to accumulate hydrothermal progress in the permissive range, or through inhibition of germination by low temperature. Increased exposure to supra-optimal

Table 4 Results of logistic regression of germination to test for effects on seed dormancy of ambient environmental variables experienced during burial

Effect	Full	Autumn burial only			Spring burial only		
	x Burial	Envir	x Mat	x Geno	Envir	x Mat	x Geno
Duration	0.55	−0.356***	0.13	8.24	−0.366***	7.35**	9.00
2-week HT_{opt}	0.45	1.90E-4	3.05	20.27**	8.214E-4***	3.33	22.70***
2-week HT_{cold}	15.35***	−0.224***	0.29	54.95***	−0.165***	5.22*	60.33***
2-week HT_{supra}	2.30	−0.001***	2.70	8.55	−0.001***	11.97***	14.59*
2-week Range	2.82	−1.11	1.67	15.43**	−1.85*	1.74	7.61
Prior HT_{opt}	0.57	1.3E-4***	0.00	8.76	2.3E-4***	6.35*	5.69
Prior HT_{cold}	3.79	−0.026***	0.04	32.12***	−0.022***	11.20***	8.08
Prior HT_{supra}	3.92*	2.17E-5	0.05	2.83	−3.14E-4**	4.88*	7.56
Prior range	1.46	5.00***	24.12***	45.34***	5.32***	2.54	12.78*

Notes: The two longest time series were analyzed: EA1 and ES1. We tested for effects of hydro-thermal accumulation during the 2 weeks immediately before the germination assessments (2 weeks) and before that, during the time interval between burial and 2 weeks before unearthing (prior). "HTopt" refers to the hydrothermal accumulation within the permissive temperature range (between 6 °C and 27 °C); "HTcold" refers to hydrothermal accumulation below 6 °C; "HTsupra" refers to hydrothermal accumulation above 27 °C; "Range" refers to the temperature range within the indicated time interval. In addition to these ambient environmental predictors, the following fixed cofactors were included in the model: Burial cohort, seed-maturation temperature ("Mat"), Genotype ("Geno"), and temperature of imbibition during the germination assay ("Temp"). The duration of burial ("Dur") was used as a continuous covariate. The following interactions were also included: Mat x Temp; Geno x Mat; Geno x Temp; Geno x Mat x Temp; Burial x Mat; Burial x Temp; Burial x Geno; Mat x Duration of burial; Geno x Duration of burial. The "x Burial" column presents the Wald Chi-square to test whether effects of environmental factors differed between burial seasons, based on a model that included both Burial cohorts, using timepoints shared between Burial cohorts. Separate analyses of each Burial cohort ("Autumn" and "Spring") included all time points for each burial season. For each Burial cohort separately, "Envir" reports the parameter estimate (x100) for the main effect of each environmental variable. The "x Mat" column reports the Wald Chi-square that tests for an interaction between environmental factors and maternal temperature treatment. The "x Geno" column reports the Wald Chi-square that tests for interactions between environmental variables and genotype. The effect of maternal temperature was significant (Wald Chi-square = 7.96, P < 0.01) in the full model in Burial cohort EA1 (Wald Chi-square = 16.93, P < 0.01), but not in Burial cohort ES1 (Wald Chi-square = 2.18, P > 0.05).
*P < 0.05, **P < 0.01, ***P < 0.001.

temperatures was also associated with reduced germination, reflecting a lack of hydrothermal accumulation within the permissive temperature range, or potentially an inhibitory effect of high temperature, which has been shown to impose dormancy in this and other species (Khan and Karssen 1980; Corbineau et al. 1988; Auge et al. 2015). Thus, the major predictor of germination propensity of seeds under natural conditions was the accumulated exposure to permissive germination temperatures under moist soil conditions.

Ambient conditions sometimes influenced seeds buried in autumn versus spring differently, with germination of autumn-buried seeds more strongly impeded by cold temperature and germination of spring-buried seed more strongly impeded by supra-optimal temperature. This contrast may be caused by differences in the sensitivity of the seeds or by differences in exposure to cold versus hot temperature in the different seasons. The stronger inhibitory effect of cold temperatures in autumn-buried seeds reduced differences in germination across seasonal Burial cohorts, although this compensatory

effect was manifest only in the ability to germinate at the highest temperature.

In addition, seeds matured under different temperatures sometimes had different magnitudes of response to ambient conditions. Spring-buried seeds of some genotypes that had matured under warm temperature were more strongly impeded in their germination by cold and supra-optimal temperatures. This increased sensitivity of warm-matured seeds to inhibitory factors counter-acted the greater induction of primary dormancy in cold-matured seeds, and potentially facilitated the dissipation of that maternal temperature effect. Therefore, differences in the sensitivity of seeds of different cohorts to ambient environmental conditions appear to be able to mitigate effects of variation both in burial timing and in seed-maturation temperature.

The dissipation of maternal effects with increased time since dispersal (Hermann and Sultan 2011) is consistent with theory that predicts that the relative contribution of parental versus progeny cues to progeny phenotypes should decrease as the predictability of parental cues declines (Ezard et al. 2014;

English et al. 2015), which can occur as the time between parental cues and progeny selection increases. If so, then although parental cues may contribute useful information to progeny at a time when progeny may lack cues, progeny may be selected to respond to cues that they perceive themselves rather than to cues perceived by their parents.

In summary, although germination behavior was strongly influenced by variation in reproductive timing (burial time and seed-maturation temperature) when seeds were first buried, those effects diminished over time. The increasingly more uniform germination behavior of different cohorts over time appears to be achieved by similar responses of seeds to their own environment, and sensitivities that differed somewhat in magnitude across cohorts.

Maternal temperature effects may compensate for difference in dispersal season

Seeds dispersed in autumn are likely to be matured under cool temperature, whereas seeds dispersed in spring may be more likely to be matured under warmer temperature. Cool seed-maturation temperature imposes stronger primary dormancy than warm temperature in *A. thaliana* (Donohue et al. 2007; Kendall and Penfield 2012; Murphey et al. 2015), and Burghardt et al. (2015) hypothesized that such maternal temperature effects could compensate for differences in dispersal timing. Results of this study show that cool-matured seeds (as in late-autumn seed maturation) do have higher dormancy even when exposed to natural seasonal cycles in the field, and this higher dormancy was manifest primarily as an inability to germinate at the highest temperatures (22 °C and 31 °C). Therefore, cool-matured seeds may be less likely to germinate in warm spring conditions than warm-matured seeds, potentially postponing germination until autumn. However, the observed maternal effect would postpone the germination of such autumn-matured seeds only if temperatures remained high throughout spring, since those seeds can germinate at cool temperature. Therefore, maternal effects may contribute to the synchronization of germination time across autumn and spring seasonal cohorts, but the conditions for that are narrow.

Alternatively, maturation at cool temperatures within either autumn or spring could postpone the germination of autumn-matured seeds until spring, and postpone the germination of spring-matured seeds until autumn. If so, then maternal temperature effects may enhance seasonal variation in germination time. How these maternal effects determine germination time in natural populations requires further field studies of seeds not only buried in the seed bank but of seeds on the soil surface.

Maternal effects have been shown to adaptively alter progeny phenotypes and phenology in diverse taxa (Mousseau and Fox 1998). For instance, in *Campanulastrum americana*, maternal timing of seed dispersal, mediated by maternal light environment, determines progeny germination time, flowering time, and life history in an adaptive manner (Galloway and Etterson 2007). In winter months, maternal parents compensated for their own late emergence by inducing earlier emergence in progeny in a manner that better matched hatch-out to the phenology of their food source (van Asch et al. 2008). Thus, maternal effects have the potential to compensate for variation in the maternal generation by inducing adaptive progeny phenotypes. However, our study shows that they may be able to do so only under a restricted set of conditions.

Germination behavior was canalized during secondary dormancy cycling

Cohorts differed in germination only as long as they maintained primary dormancy. Once they lost primary dormancy, secondary dormancy induced dormancy cycling similarly in all cohorts. Thus, secondary dormancy led to canalized germination that was much less influenced by reproductive timing. However, seeds with secondary dormancy did not behave the same as fresh seeds with primary dormancy even under identical ambient environmental conditions.

These findings have implications for the germination behavior of first-year cohorts compared to cohorts maintained in the seed bank. When seeds are freshly shed, differences in primary dormancy across seed cohorts are expected to cause differences in germination time, if those seed do not become buried. The weeks required for the canalization of germination behavior across seed cohorts, even if few, could enable the germination of less dormant seeds on the soil surface under cooler temperatures, while seeds that are more strongly induced into primary dormancy, or that have had less time to lose dormancy, may not yet germinate. Therefore, first-year seeds on the soil surface may exhibit effects of variation in reproductive timing. However, if seeds get buried, they apparently canalize their germination behavior. Once those seeds are disturbed, they may exhibit a highly uniform germination behavior. Therefore, first-year seeds may vary in germination time more than seeds from the seed bank. If most seeds end up

in the seed bank, germination could be highly synchronous.

Genotypic differences in dormancy cycling

Genotypes differed not only in primary dormancy at the time of seed dispersal, but in how quickly they lost that primary dormancy and, as a consequence, how quickly seed cohorts were canalized across seed-maturation temperature and Burial cohorts. In particular, DOG1$_{Cvi}$ and DOG6$_{Cvi}$ exhibited greater initial primary dormancy and a slower release of that dormancy, as expected (Alonso-Blanco et al. 2003; Bentsink et al. 2006; Burghardt et al. 2015). As a consequence, although Ler canalized its germination behavior across cohorts by mid-summer, DOG1$_{Cvi}$ and DOG6$_{Cvi}$ did not achieve uniform germination behavior until the autumn.

DOG1$_{Cvi}$ and DOG6$_{Cvi}$ also exhibited greater secondary dormancy induction. While Ler lost secondary dormancy by spring, these genotypes did not lose it until autumn. The genetic differences in both primary and secondary dormancy suggests that genotypes are likely to differ in germination time not only when first-year seeds remain on the soil surface, but also when seeds are disturbed from the seed bank.

The mutant genotype $dog1_{Cvi}$ germinated to high proportions at all times and at most temperatures, regardless of seed-maturation temperature and burial time. Although the germination behavior itself is quite homogeneous over time, such high germinability under a broad range of conditions likely leads to highly variable germination time, since seeds can germinate immediately after being dispersed. Thus, some dormancy is required to ensure that reproductive timing alone does not determine germination time, and dormancy is required to allow seeds to express sensitivity to ambient temperatures. In short, functional DOG1 is required to regulate germination time independently of reproductive time, even though DOG1 appears to delay the canalization of germination behavior across cohorts.

It should be noted that the magnitude of the differences among the genotypes varied across the season. Genetic differences were most pronounced soon after dispersal in all seed cohorts (Supplementary Fig. S1), but differences among genotypes diminished dramatically during summer and early autumn. Genetic differences re-appeared with secondary dormancy induction. Therefore, all genotypes are capable of germinating during the autumn, when *A. thaliana* typically germinates in temperate climates (Ratcliffe 1965; Montesinos-Navarro et al. 2012; Postma and Ågren 2016). If germination in the autumn is optimal (Donohue et al. 2005; Postma and Ågren 2016), genotypes differ most during times of year that may be sub-optimal. If, however, optimal germination time varies geographically (Kronholm et al. 2012), then variation in these loci could contribute to local adaptation because genetic differences in germination would be expressed at other times of year.

Summary and conclusion

Reproductive timing, via seed-maturation temperature and the timing of dispersal, influenced germination behavior under natural field conditions, but seeds exhibited processes whereby their germination behavior can be canalized. Primary seed dormancy imposed variation in germination across Burial cohorts, seed-maturation temperature, and genotypes. After primary dormancy was lost, however, secondary dormancy cycling did not strongly reflect variation in reproductive phenology, suggesting secondary dormancy after the loss of primary dormancy re-sets dormancy cycling. Maternal effects themselves may contribute to the canalization of germination under some conditions; first, by delaying the germination of autumn-dispersed seeds matured under cool temperature, and second by modifying the responses of seeds to their ambient environment. Finally, DOG1 appears to be necessary for dormancy and consequently for any canalization of germination behavior.

The observation that seed dormancy is necessary for the canalization of germination behavior suggests that other mechanisms of developmental arrest in different taxa, such as diapause, may be required to regulate progeny phenology in the presence of variation in phenology at prior life stages. Dormancy provides an essential mechanism that enables progeny to respond to their own ambient conditions; without dormancy, development (germination) proceeds indiscriminately.

In conclusion, a combination of genetically imposed seed dormancy, maternally induced seed dormancy, and secondary dormancy can offset variation in germination behavior imposed by variation in reproductive phenology, potentially contributing to the maintenance of adaptive germination phenology. Such autoregulatory mechanisms whereby organisms can maintain adaptive phenology despite developmental variation in prior life stages are likely important components of adaptation to seasonality.

Acknowledgments

Supplementary data

Supplementary data are available at *ICB* online.

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