CANALIZATION OF SEASONAL PHENOLOGY IN THE PRESENCE OF DEVELOPMENTAL VARIATION: SEED DORMANCY CYCLING IN AN ANNUAL WEED

Brianne Edwards[1,2], Liana T. Burghardt[1,3], Katherine Kovach[1], Kathleen Donohue[1,4]

[1]Biology Department, Duke University, Box 90338, Durham, NC 27708 USA

[2]Current address: Department of Plant and Microbial Biology, North Carolina State University, 851 Main Campus Drive, Raleigh, NC 27606

[3]Current address: Department of Plant and Microbial Biology, University of Minnesota, 150 Gortner Ave, St. Paul, MN 55108

[4]Author for correspondence: k.donohue@duke.edu; Tel: 919-613-7467

ABSTRACT

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 un-earthed 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.

Key words: *Arabidopsis thaliana*, canalization, dormancy, germination time, hydrothermal, life history, maternal effects, phenology

Total words (excluding abstract, acknowledgments, references, tables, and figure legends): 5543

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 and c). 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 1985, 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 2012, Montesinos et al. 2012, Akiyama and Agren 2014, Postma et al. 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, *Arabidopsis 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 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.* 2016). 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*$_{Cvi}$ knockout, 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°C and 14°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 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). Fig. 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 2-way, 3-way, and 4-way interactions, but the 4-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 (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 one-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°C and 27°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°C) that are known to break or induce dormancy depending on the duration of time spent at those temperatures (Coughland *et al.* 2016), and supra-optimal temperatures ("supraT" = > 27°C) that are known to induce secondary dormancy in the lab (Coughland *et al*. 2016, Auge *et al*. 2015). We also investigated the effects of environmental conditions during two time periods: two weeks before un-earthing ("2-weeks"), and the time from burial up to two 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 x 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 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°C). Temperature-dependent germination and the effect of seed-maturation temperature also varied among genotypes, as described below.

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°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), 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, 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* (Figs. 2 and S1, Table 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 (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 L*er* and the more dormant genotypes $DOG6_{Cvi}$ or $DOG1_{Cvi}$ increased over burial time initially (Fig. S1, Table S1, S2), as L*er* 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 L*er*, acquiring high germination percentages at the highest temperature only in the autumn (compared to late spring for L*er*).

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 L*er*, 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 x UE interactions in 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, 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 (Tables 3, 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 one year later acquired similar germination behavior after a single un-earthing interval (Table 3, S5). However, slight differences in germination re-appeared 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).

One genotype in particular, $DOG6_{Cvi}$, retained differences across years for longer than L*er*; this effect was apparent as a reduced ability to germinate at 22°C in seeds with secondary, but not primary, dormancy (Fig. S2, Table 3 and S2). That is, $DOG6_{Cvi}$ exhibited deeper secondary dormancy than primary dormancy but L*er* did not, such that inter-annual differences were manifest in $DOG6_{Cvi}$ but not in L*er*. 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 re-appeared 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 (Tables 4 and 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 (two weeks) before un-earthing, but also by conditions experienced prior to 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 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 Carsen 1980, Corbineau et al. 1988, Auge et al. 2016). 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 (Mouseau 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 moths, 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 Arch 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 L*er* 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 L*er* 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 (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 (Ratcliff 1965, Montesinos et al. 2012, Postma and Agren.

2016). If germination in the autumn is optimal (Donohue et al 2005, Postma and Agren 2016), genotypes differ most during times of year that may be sub-optimal. If, however, optimal germination time varies geographically (Kronholm 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.

ACKNOWLEDGEMENTS

The authors thank the staff of the Duke Phytotron for excellent care of the plants. We also thank Eden Ashebir for assistance with data management and lab assays. Zoe Hill, Chunhui Zhang, Jennifer Zou, Tarek Elnacash, Mercedes Zapata-Garcia, and Joseph Provenzano provided technical assistance with the field work and lab assays. We thank Gabriela Auge, Logan Blair, Lindsay Leverett, and Michelle D'Aguillo for useful discussions and comments that improved this manuscript and the reviewers for providing useful suggestions. Funding was provided by grant NSF-DEB-1020963 and NSF-IOS-11-46383 to K.D.

LITERATURE CITED

Akiyama R, Ågren J. 2014. Conflicting selection on the timing of germination in a natural population of *Arabidopsis thaliana*. *Journal of Evolutionary Biology* **27**: 193-199.

Allen PS. 2003. When and how many? Hydrothermal models and the prediction of seed germination. *New Phytologist* **158**: 1-3.

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

Alvarado V, Bradford K. 2005. Hydrothermal time analysis of seed dormancy in true (botanical) potato seeds. *Seed Science Research* **15**: 77-88.

Andrewartha HG. 1952. Diapause in relation to the ecolog of insects. *Biological Reviews* **27**: 50–107.

Auge G, Blair L, Donohue K. 2017. Maternal vernalization and vernalization-pathway genes influence progeny seed germination. *New Phytologist* **In press**.

Auge G, Edwards B, Blair L, Burghardt L, Coughlan J, Leverett L, Donohue K. 2015. Secondary dormancy induction depends on primary dormancy status. *Seed Science Research* doi:10.1017/S0960258514000440.

Auge G, Leverett L, Edwards B, Donohue K. 2017. Adjusting phenotypes via within- and across-generational plasticity. *New Phytologist*.

Baskin CC, Baskin JM. 2014. *Seeds: Ecology, biogeography and evolution of dormancy and germination*. Academic Press, San Diego.

Baskin JM, Baskin CC. 1972. Ecological life cycle and physiological ecology of seed germination of *Arabidopsis thaliana*. *Canadian Journal of Botany* **50**: 353-360.

-. 1983. Seasonal changes in the germination responses of buried seeds of *Arabidopsis thaliana* and ecological intepretation. *Botanical Gazette* **144**: 540-543.

Bentsink L, Hanson J, Hanhart CJ, Blankestijn-de Vries H, Coltrane C, Keizer P, El-Lithy M, Alonso-Blanco C, Teresa de Andres M, Reymond M et al. 2010. Natural variation for seed dormancy in *Arabidopsis* is regulated by additive genetic and molecular pathways. *Proceedings of the National Academy of Sciences of the United States of America* **107**: 4264-4269.

Bentsink L, Jowett J, Hanhart CJ, Koornneef M. 2006. Cloning of *DOG1*, a quantitative trait locus controlling seed dormancy in *Arabidopsis*. *Proceedings of the National Academy of Sciences* **105**: 17042-17047.

Bewley JD. 1997. Seed germination and dormancy. *Plant Cell* **9**: 1055-1066.

Blair L, Auge G, Donohue K. 2017. Effect of FLOWERING LOCUS C on seed germination depends on dormancy. *Functional Plant Biology*: In press.

Bradford K. 2002. Applications of hydrothermal time to quantifying and modeling seed germination and dormancy. *Weed Science* **50**: 248-260.

Burghardt L, Edwards B, Kovach K, Donohue K. 2015. Multiple paths to similar germination behavior in Arabidopsis thaliana. *New Phytologist*: doi: 10.1111/nph.13685.

Burghardt L, Metcalf CJE, Wilczek A, Schmitt J, Donohue K. 2015. Modeling the influence of genetic and environmental variation on the expression of plant life cycles across landscapes. American Naturalist 185: 212-227.

Burghardt L, Metcalf J, Donohue K. 2015. Seed dormancy cline conserves reproductive environment of Arabidopsis thaliana across the range. American Journal of Botany 103: 47-59.

Carter, A. W., R. M. Bowden, and R. T. Paitz. 2017. Seasonal shifts in sex ratios are mediated by maternal effects and fluctuating incubation temperatures. *Functional Ecology*, DOI: 10.1111/1365-2435.12801

Chiang GCK, Barua D, Amasino R, Donohue K. 2009. A major flowering-time gene, FLOWERING LOCUS C, controls temperature-dependent germination in *Arabidopsis thaliana*. *Proceedings of the National Academy of Sciences* **106**: 11661-11666.

Corbineau F, Rudnicki RM, Come D. 1988. Induction of secondary dormancy in sunflower seeds by high temperature. Possible involvement of ethylene biosynthesis. *Physiologial Plantarum* **73**: 368–373.

Coughlan J, Saha A, Donohue K. 2016. Effects of pre- and post-dispersal temperature on primary and secondary dormancy dynamics in contrasting genotypes of Arabidopsis thaliana (Brassicaceae). *Plant Species Biology*: Doi: 10.1111/1442-1984.12145.

Denlinger DL. 1986. Dormancy in tropical insects. *Annual Review of Entomology* **31**: 239-264.

Denlinger DL. 2002. Regulation of diapause. *Annual Review of Entomology* **47**: 93–122.

Donohue K. 2014. Why ontogeny matters during adaptation: Developmental niche construction and pleiotropy across the life cycle in *Arabidopsis thaliana*. *Evolution* **68**: 32-47.

Donohue K, Dorn LA, Griffith C, Schmitt J, Kim E-S, Aguilera A. 2005. The evolutionary ecology of seed germination of *Arabidopsis thaliana*: Variable natural selection on germination timing. *Evolution* **59**: 758-770.

Donohue K, Heschel MS, Chiang GCK, Butler CM, Barua D. 2007. Phytochrome mediates germination responses to multiple seasonal cues. *Plant, Cell and Environment* **30**: 202-212.

Donohue K, Rubio de Casas R, Burghardt L, Kovach K, Willis C. 2010. Germination, post-germination adaptation, and species ecological ranges. *Annual Review of Evolution, Ecology and Systematics* **41**: 293–319.

Edwards B, Zappata M, Li R, Burghardt LT, Donohue K. 2016. Dormancy-mediated germination responses to low water potential in Arabidopsis thaliana. *Environmental and Experimental Botany* **126**: 55-67.

English S, Pen I, Shea N, Uller T. 2015. The information value of non-genetic inheritance in plants and animals. *Plos One* **10**.

Ezard THG, Prizak R, Hoyle RB. 2014. The fitness costs of adaptation via phenotypic plasticity and maternal effects. *Functional Ecology* **28**: 693-701.

Footitt S, Clay HA, Dent K, Finch-Savage WE. 2014. Environment sensing in spring-dispersed seeds of a winter annual Arabidopsis influences the regulation of dormancy to align germination potential with seasonal changes. *New Phytologist* **202**: 929-939.

Footitt S, Douterelo-Soler I, Clay H, Finch-Savage WE. 2011. Dormancy cycling in Arabidopsis seeds is controlled by seasonally distinct hormone-signaling pathways. *Proceedings of the National Academy of Sciences USA* **108**: 20236–20241.

Footitt S, Huang Z, Clay HA, Mead A, Finch-Savage WE. 2013. Temperature, light and nitrate sensing coordinate *Arabidopsis* seed dormancy cycling, resulting in winter and summer annual phenotypes. *Plant Journal* **76**: 1003 - 1015.

Galloway LF. 2001. Parental environmental effects on life history in the herbaceous plant *Campanula americana*. *Ecology* **2001**: 2781-2789.

Galloway, L. F. and J. R. Etterson. 2007. Transgenerational plasticity is adaptive in the wild. *Science* **318**:1134-1136.

Hermann JJ, Sultan SE. 2011. Adaptive transgenerational plasticity in plants: case studies, mechanisms, and implications for natural populations. *Fronteirs in Plant Science* **2**: doi:10.3389/fpls.2011.00102.

Huang X, Schmitt J, Dorn L, Griffith C, Effgen S, Takao S, Koornneef M, Donohue K. 2010. The earliest stages of adaptation in an experimental plant population: strong selection on QTLS for seed dormancy. *Molecular Ecology* **19**: 1335-1351.

Kendall S, Penfield S. 2012. Maternal and zygotic temperature signalling in the control of seed dormancy and germination. *Seed Science Research* **22**: S23-S29.

Khan AA, Karssen CM. 1980. Induction of secondary dormancy in *Chenopodium bonus-henricus L*. seeds by osmotic and high temperature treatments and its prevention by light and growth regulators. *Plant Physiology* **66**: 175–181.

Kronholm I, Xavier Pico F, Alonso-Blanco C, Goudet J, de Meaux J. 2012. Genetic basis of adaptation in *Arabidopsis thaliana*: Local adaptation at the seed dormancy QTL *DOG1*. *Evolution* **66**: 2287-2302

Lacey EP, Pace R. 1983. Effect of parental flowering and dispersal times on offspring fate in *Daucus carota* (Apiaceae). *Oecologia* **60**: 274-278.

Marcer, A., D. S. D. S. Vidigal, P. M. A. James, M.-J. Fortin, B. Mendez-Vigo, H. W. M. Hilhorst, L. Bentsink, C. Alonso-Blanco, and F. X. Pico. 2017. Temperature fine-tunes

Mediterranean Arabidopsis thaliana life-cycle phenology geographically. Plant Biology doi:10.1111/plb.12558.

Meyer SE, Debaene-Gill SB, Allen PS. 2000. Using hydrothermal time concepts to model seed germination response to temperature, dormancy loss, and priming effects in *Elymus elymoides*. *Seed Science Research* **10**: 213–223.

Miller, C. W. 2008. Seasonal effects on offspring reproductive traits through maternal oviposition behavior. *Behavioral Ecology* **19**:1297-1304.

Montesinos-Navarro A, Xavier Picó F, Tonsor SJ. 2012. Clinal variation in seed traits influencing life cycle timing in *Arabidopsis thaliana*. *Evolution* **66**: 3417–3431.

Mousseau TA, Fox CW, eds. 1998. *Maternal Effects As Adaptations*. Oxford University Press, New York.

Murphey M, Kovach K, Elnacash T, He H, Bentskink L, Donohue K. 2015. DOG1-imposed dormancy mediates germination responses to temperature cues. *Environmental and Experimental Botany* **112**: 33-43.

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

Postma FM, Agren J. 2016. Early life stages contribute strongly to local adaptation in Arabidopsis thaliana. *Proceedings of the National Academy of Sciences USA* **113**: 7590-7595.

Ratcliffe D. 1965. Germination characteristics and their inter- and intra-population variability in *Arabidopsis*. *Arabidopsis Information Service* **13**.

Roach DA, Wulff RD. 1987. Maternal effects in plants. *Annual Review of Ecology and Systematics* **18**: 209-235.

Sinclair BJ, Addo-Bediako A, Chown SL. 2003. Climatic variability and the evolution of insect freeze tolerance. *Biological Reviews* **78**: 181–195.

Sultan SE. 1995. Phenotypic plasticity and plant adaptation. *Acta Bot Neerl* **44**: 363-383.

Valladares F, Gianoli E, Gomez JM. 2007. Ecological limits to plant phenotypic plasticity. *New Phytologist* **176**: 749-763.

van Asch, M., R. Julkunen-Tiito, and M. E. Visser. 2010. Maternal effects in an insect herbivore as a mechanism to adapt to host plant phenology. *Functional Ecology* **24**:1103-1109.

van Kleunen M, Fischer M. 2005. Constraints on the evolution of adaptive phenotypic plasticity in plants. *New Phytologist* **166**: 49-60.

Vidigal, D. S., A. C. Marques, L. A. Willems, G. Buijs, B. Méndez-Vigo, H. W. Hilhorst, L. Bentsink, F. X. Picó, and C. Alonso-Blanco. 2016. Altitudinal and climatic associations of seed dormancy and flowering traits evidence adaptation of annual life cycle timing in Arabidopsis thaliana. Plant Cell Environment **39**:1737-1748.

Wilchesa DM, Lairdb RA, Floatea KD, Fields PG. 2016. A review of diapause and tolerance to extreme temperatures in dermestids (Coleoptera). *Journal of Stored Products Research* **68**: 50–62.

Wright SD, McConnaughay KDM. 2002. Interpreting phenotypic plasticity: the importance of ontogeny. *Plant Species Biology* **17**: 119-131.

FIGURES

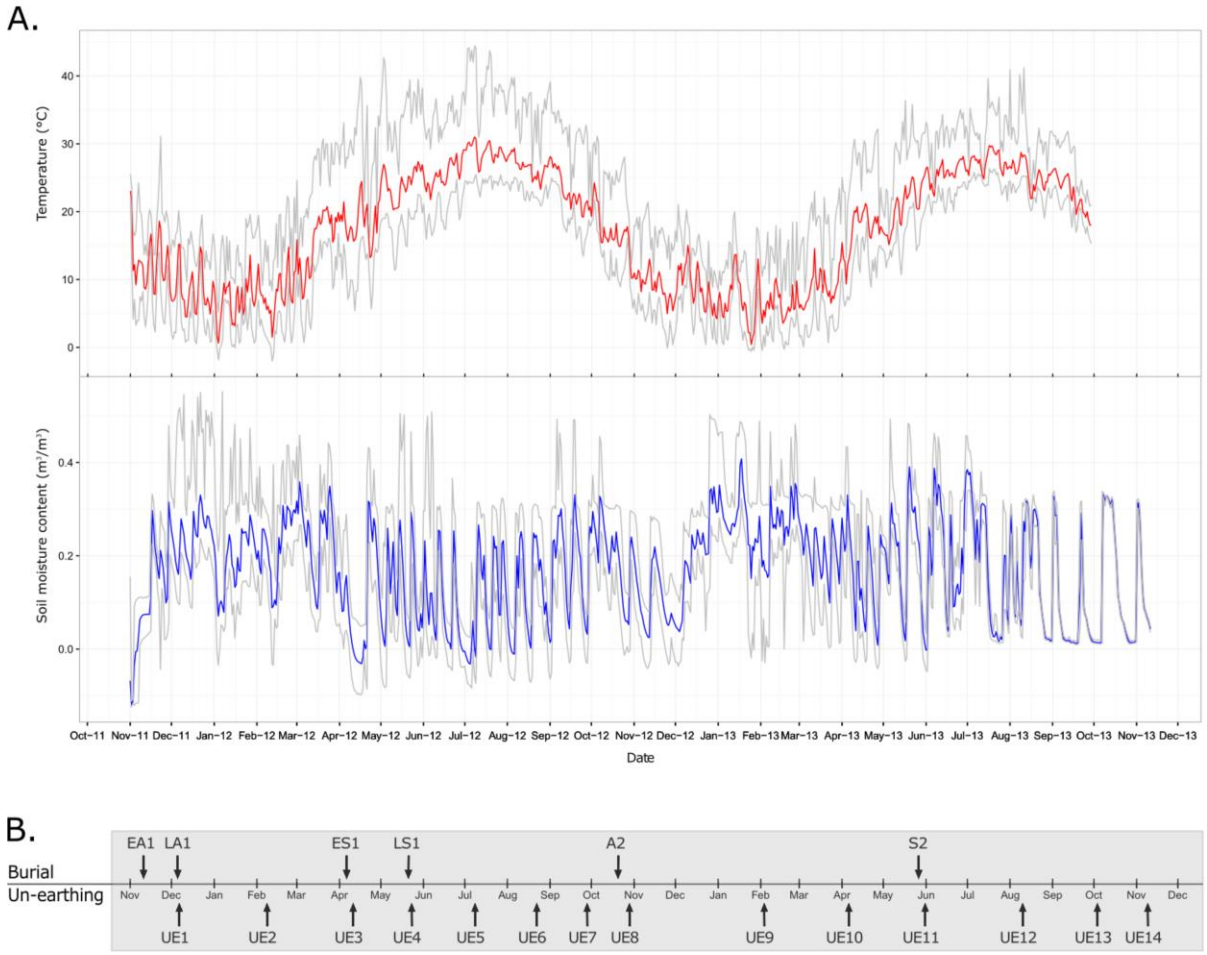


Figure 1. Experimental design and environmental conditions during the experiment. A) Hourly soil temperature (upper) and soil moisture (lower) over the course of the experiment (x-axis) averaged over all blocks. Temperature and moisture were smoothed (solid line). B) Experimental schedule. Downward arrows 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.

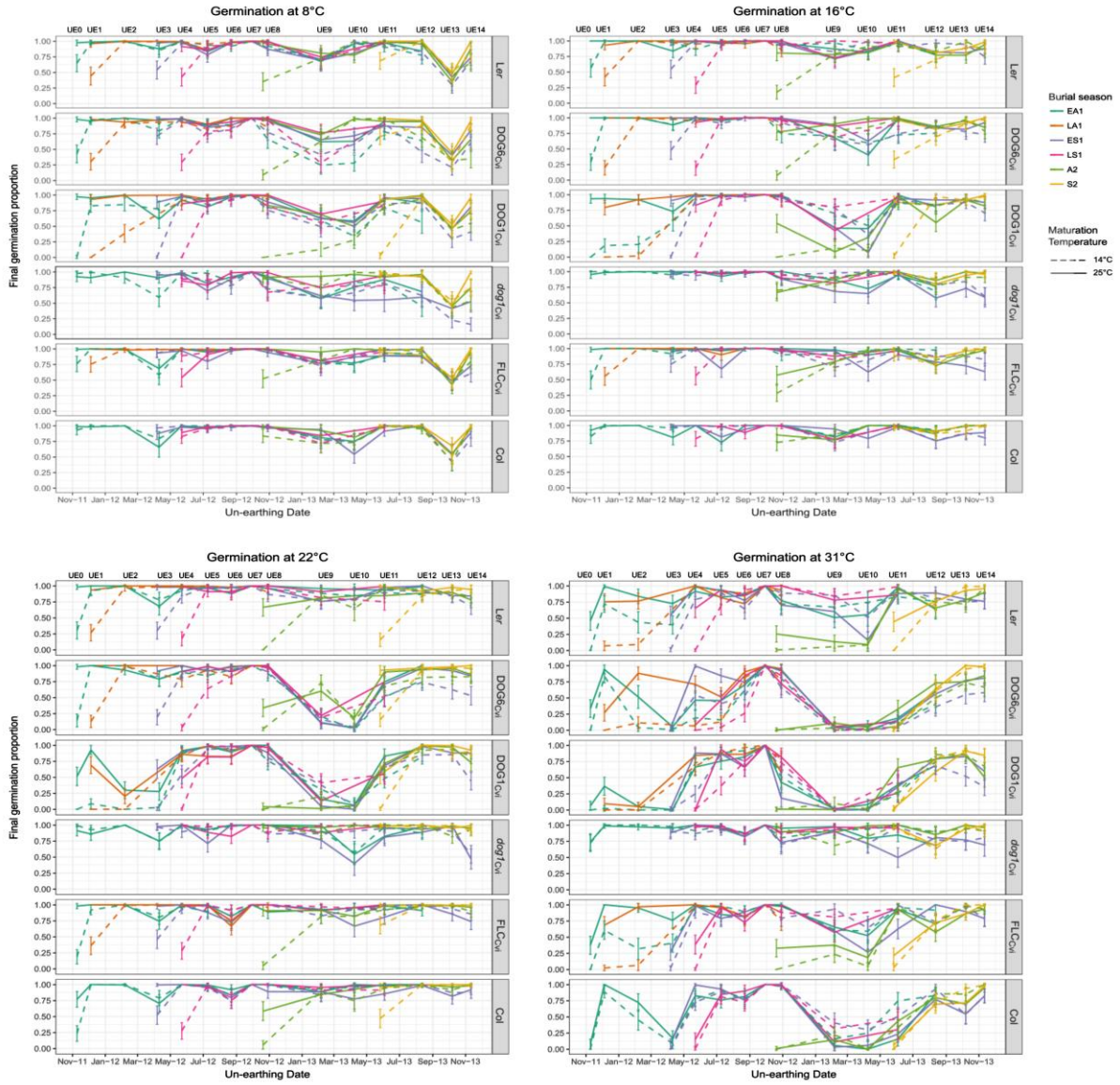
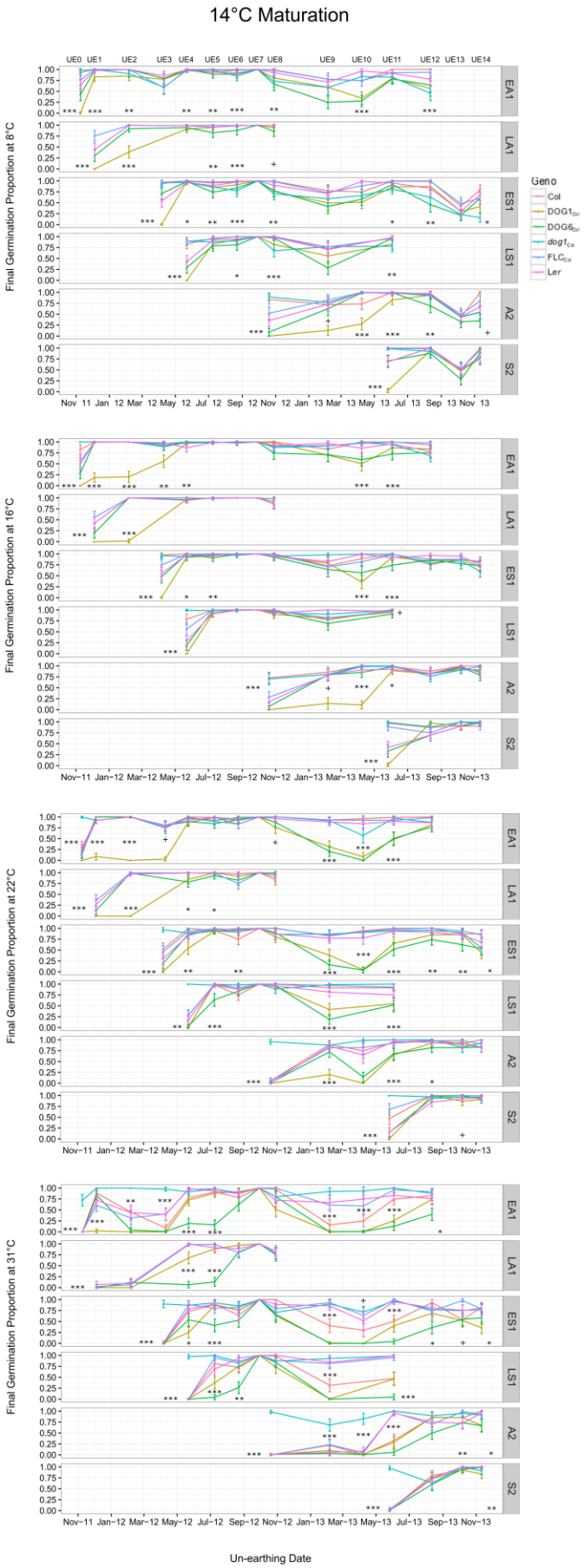


Figure 2. Mean germination proportions (± SE), for each seed-imbibition temperature in lab germination assays (8°C, 16°C, 22°C, 31°C), shown in separate panels. Results for each genotype are shown in separate rows within each larger panel. Un-earthing time points (UE0-UE14) are shown across X-axis, with UE0 as the seed germination proportion just before the first burial. Each Burial cohort (EA1 -S2) is indicated by different colored 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.

A.


14°C Maturation
UE0 UE1 UE2 UE3 UE4 UE5 UE6 UE7 UE8 UE9 UE10 UE11 UE12 UE13 UE14
Final Germination Proportion at 8°C
Final Germination Proportion at 16°C
Final Germination Proportion at 22°C
Final Germination Proportion at 31°C
EA1
LA1
ES1
LS1
A2
S2
Nov 11 Jan 12 Mar 12 May 12 Jul 12 Sep 12 Nov 12 Jan 13 Mar 13 May 13 Jul 13 Sep 13 Nov 13
Geno
Col
DOG1Col
DOG6Col
dog1Col
FLCCol
Ler
Un-earthing Date

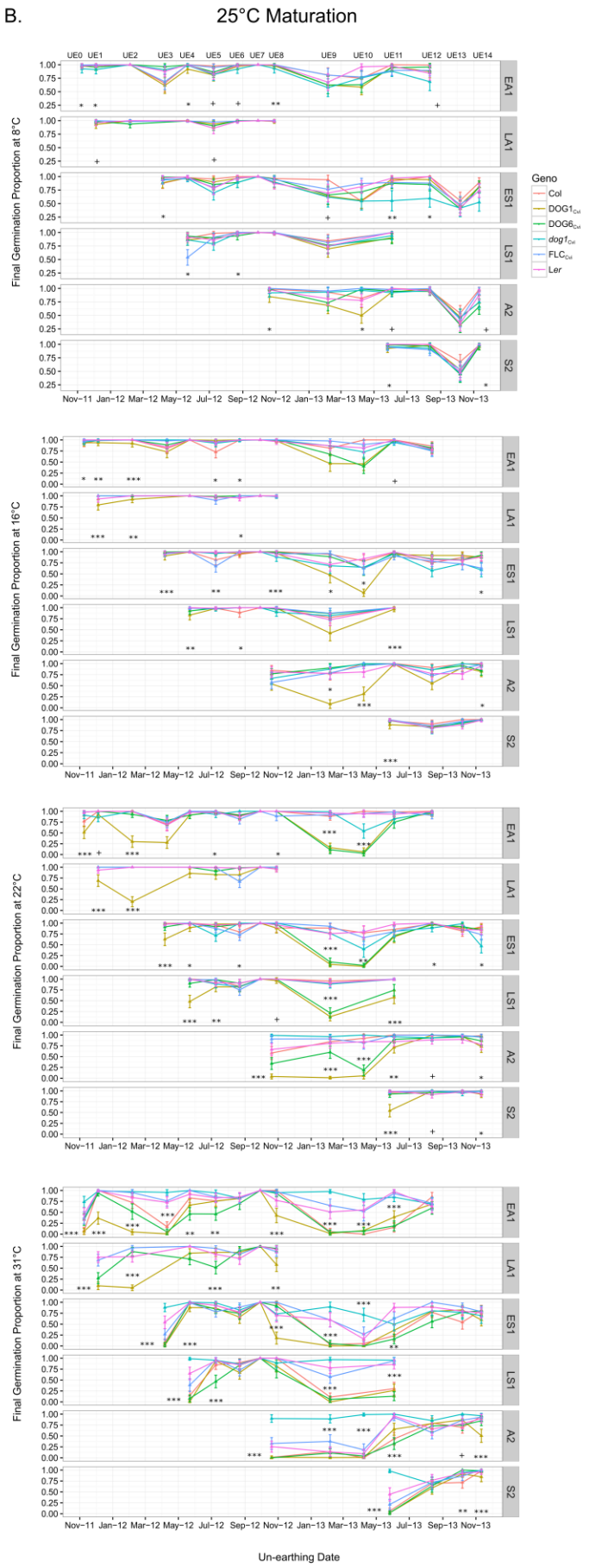

B.
25°C Maturation
UE0 UE1 UE2 UE3 UE4 UE5 UE6 UE7 UE8 UE9 UE10 UE11 UE12 UE13 UE14
EA1
LA1
ES1
LS1
A2
S2
Final Germination Proportion at 8°C
Final Germination Proportion at 16°C
Final Germination Proportion at 22°C
Final Germination Proportion at 31°C
Nov−11 Jan−12 Mar−12 May−12 Jul−12 Sep−12 Nov−12 Jan−13 Mar−13 May−13 Jul−13 Sep−13 Nov−13
Un-earthing Date
Geno
Col
DOG1Col
DOG6Col
dog1Col
FLCCol
Ler

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) 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). "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.

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

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

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
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

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) For the Autumn burial season, EA1 and A2 were compared from Nov. of the second year through Sept. 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. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

A. Autumn burial season

Predictor	**DF**	**UE8**	**UE9**	**UE10**	**UE11**	**UE12**
L*er*						
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
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
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

Table 4: Results of logistic regression of germination to test for effects on seed dormancy of ambient environmental variables experienced during burial. The two longest time series were analyzed: EA1 and ES1. We tested for effects of hydro-thermal accumulation during the two weeks immediately before the germination assessments (two-week) and prior to that, during the time interval between burial and two weeks before un-earthing (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"), 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 < 0.001. * P < 0.05, ** P < 0.01, *** P < 0.001.

	Full	**Autumn Burial only**			**Spring Burial only**		
Effect	**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**	-01.85*	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*	7.558
Prior Range	1.46	5.00***	24.12***	45.34***	5.32***	2.54	12.778*