CLINAL VARIATION IN SEED TRAITS INFLUENCING LIFE CYCLE TIMING IN *ARABIDOPSIS THALIANA*

Alicia Montesinos-Navarro,[1,2,3] F. Xavier Picó,[2] and Stephen J. Tonsor[1]

[1]*Department of Biological Sciences, University of Pittsburgh, 162 Crawford Hall, 4249 Fifth Avenue, Pittsburgh, Pennsylvania 15260*

[3]*E-mail: ali.montesinos@gmail.com*

[2]*Departamento de Ecología Integrativa, Estación Biológica de Doñana (EBD), Consejo Superior de Investigaciones Científicas (CSIC), Av. Américo Vespucio, s/n 41092 Sevilla, Spain*

Early life-history transitions are crucial determinants of lifetime survival and fecundity. Adaptive evolution in early life-history traits involves a complex interplay between the developing plant and its current and future environments. We examined the plant's earliest life-history traits, dissecting an integrated suite of pregermination processes: primary dormancy, thermal induction of secondary dormancy, and seasonal germination response. We examined genetic variation in the three processes, genetic correlations among the processes, and the scaling of germination phenology with the source populations' climates. A spring annual life history was associated with genetic propensities toward both strong primary dormancy and heat-induced secondary dormancy, alone or in combination. Lineages with similar proportions of winter and spring annual life history have both weak primary dormancy and weak thermal dormancy induction. A genetic bias to adopt a spring annual strategy, mediated by rapid loss of primary dormancy and high thermal dormancy induction, is associated with a climatic gradient characterized by increasing temperature in summer and rainfall in winter. This study highlights the importance of considering combinations of multiple genetically based traits along a climatic gradient as adaptive strategies differentiating annual plant life-history strategies. Despite the genetic-climatic cline, there is polymorphism for life-history strategies within populations, classically interpreted as bet hedging in an unpredictable world.

KEY WORDS: **after-ripening, dormancy, germination-timing evolution, life-history, plasticity, structural equation models.**

Adaptive plasticity in the phenology of early life-history stage transitions can be critical in determining the fate of the organism, in that plasticity can synchronize the most vulnerable life-history stages with favorable environmental windows (Biere 1991; Denver et al. 1998; Gómez-Mestre and Tejedo 2002; Donohue et al. 2005a,b; Forrest and Miller-Rushing 2010). This can not only maximize survival early in life-history but can also influence the timing and hence the fitness of later life-history stages (Evans and Cabin 1995; Hilhorst 1995; Vleeshouwers et al. 1995; Bewley 1997; Li and Foley 1997; Morey and Reznick 2000; Donohue 2002; Twombly and Tisch 2002; Altwegg and Reyer 2003; Baskin and Baskin 2004; Fenner and Thompson 2005; Walsh et al. 2008). In plants, germination is the first and most important early stage transition and contrasting life histories can result from polymorphic germination responses to seasonal cues (Baskin and Baskin 1985). For many annuals, this results in a choice of two alternative life cycles. Winter annuals germinate in fall, and overwinter as seedlings or rosettes, while spring annuals germinate in spring, and overwinter as dormant seeds (Baskin and Baskin 1974, 1983, Nordborg and Bergelson 1999; Baskin et al. 2004; Cici and Van

Acker 2009). Winter and spring annual life cycles nevertheless are generally synchronous in maturation and dispersal of seeds in late spring or early summer.

Arabidopsis thaliana is an annual displaying this life-history polymorphism. In this species and many others, primary dormancy is induced during seed maturation and persists in dispersed seeds. Catabolism of germination inhibitors and/or synthesis of germination enhancers often follows dispersal during a period of dry conditions. This process by which primary dormancy is lost is referred to as after-ripening, and when completed results in responsiveness to germination cues. Exposure to cold conditions can also accelerate the breakdown of a known germination inhibitor, abscisic acid, and enhance responsiveness to germination cues (Finch-Savage and Leubner-Metzger 2006; Holdsworth et al. 2008). Further complicating the process of seed readiness for germination, dispersed seeds of temperate annuals often experience a warm summer period during which they may be induced into secondary dormancy (Baskin and Baskin 1998). This is thought to prevent germination during briefly benign periods that are often interspersed within generally inhospitably dry and hot summers (Donohue et al. 2005b). At some point, external stimuli such as cooler fall–winter conditions together with internal biochemical transitions result in both primary and secondary dormancy breakage (Baskin and Baskin 1983, 1998; Schmitt et al. 1992; Platenkamp and Shaw 1993; Donohue et al. 2005a; Penfield et al. 2005). However, even with a knowledge of the gain and loss of both primary and secondary dormancy, the fate of seeds can remain unpredictable. Together, both the known processes of after-ripening, heat-induced secondary dormancy and chilling-based dormancy breakage as well as additional as yet unknown processes result in differential responses to fall and spring germination cues. As a result, both individual seeds and contrasting genotypes can differ in germination responses to seasonal cues (Baskin and Baskin 1983; Donohue et al. 2005a; Tsiantis 2005). We refer to observed differences in response to fall versus spring germination cues as seasonal germination bias.

In this study, we used the model species *A. thaliana* to disentangle the genetically based mechanisms that collectively trigger or suppress germination across the succession of seasonal conditions that follow seed production. We evaluate the genetic variation in and correlations among primary dormancy, secondary dormancy and seasonal germination response, and their potential to collectively shape an adaptive combination of seed trait values along a climatic gradient. We expected that two different genetically based combination of seed traits could result in a spring annual life history (Fig. 1): (1) a genetic association between high primary strength and high seasonal germination bias toward spring (Fig. 1a-e-f[s]) and (2) a genetic association between low primary dormancy strength, high thermal dormancy induction, and high seasonal germination bias toward spring (Fig. 1a-b-d-f[s]). Likewise, the winter annual life-history phenotype would be expressed by a combination of low primary dormancy, low thermal dormancy induction, and low seasonal germination bias toward spring (Fig. 1a-b-c[s]). Genetic variation in any element of these alternative pathways could shift the extent of seasonal germination bias. We sought to describe the extent of potentially adaptive variation in these processes and their influence on seasonal germination bias.

Three main goals of the this study are (1) testing for genetic variation in traits related to multiple sequential processes involved in germination phenology, (2) testing for genetic covariation among these seed traits, potentially shaping spring and winter life histories, and finally (3) testing for adaptive divergence of genetically based seed combinations along a climatic gradient. To our knowledge, no study has approached the combination of these three pregermination processes while considering their potential to collectively shape adaptive strategies along a climatic gradient. Studying the potential of annual plants to adapt to different environments can contribute to understanding how annual plant species expand their geographic and environmental ranges.

Methods

STUDY SYSTEM

We used *A. thaliana* as a study organism. Being highly selfing (Abbott and Gomes 1989) it is highly homozygous (Todokoro et al. 1995; Berge et al. 1998; Bergelson et al. 1998), greatly minimizing the genetic variation among a lineage's seeds. We collected seeds in 17 natural populations (Fig. S1) along an altitudinal gradient (109–1668 m above sea level) in NE Spain. This region shares a history of isolation from the other regions of the Iberian Peninsula (Picó et al. 2008). The populations within this region present strong neutral genetic differentiation without detectable isolation-by-distance among populations (Montesinos et al. 2009). In nine of these natural populations, we censused life-history events in the field. Seed release occurred in late May and early June and germination primarily in September–October and March (Montesinos et al. 2009). Both daily mean temperatures and temperature fluctuation decrease in fall in the natural sites and increase during spring (Tonsor, Picó, and Montesinos-Navarro, unpubl. data).

We georeferenced each population and extracted climatic variables from the digital climatic atlas of the Iberian Peninsula (Ninyerola et al. 2005) using 50-year averages of monthly temperatures and rainfall (see Montesinos-Navarro et al. 2011 for more details). We selected climatic variables that we hypothesized would exert strong selection on seed phenotypes from seed release in June to favorable germination conditions in September and March: maximum monthly average temperature from June to

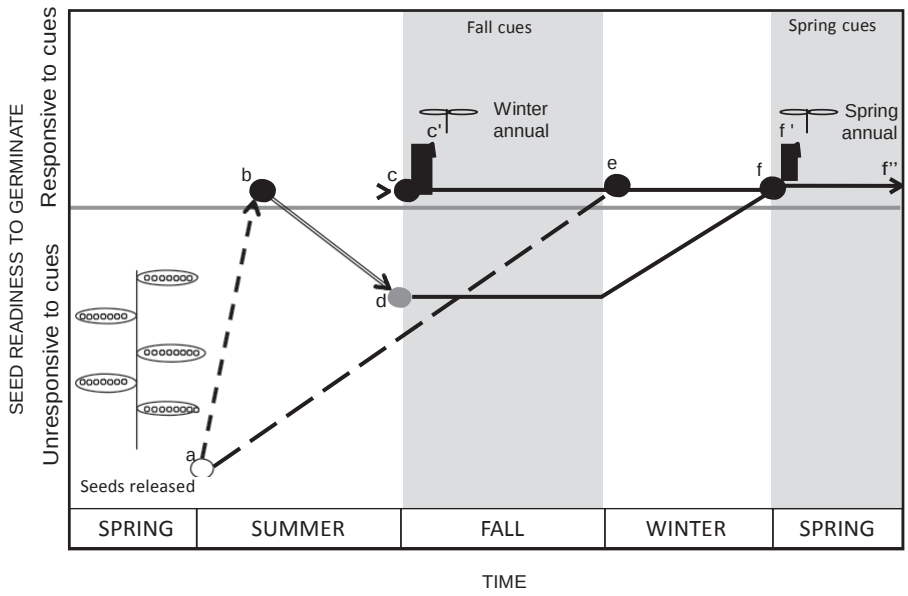

Figure 1. **Schematic decision tree representing the influence of the traits examined in this study on the ability to respond to germination queues: combinations of "primary dormancy strength," thermal dormancy induction, and seasonal germination bias determine entry into winter and spring annual life cycles. The vertical axis represents physiological progress in seed readiness to germinate with a threshold between responsive versus unresponsive to cues triggering germination. The horizontal axis represents seasonal climatic conditions along a temporal axis. Circles represent seed stages (white: newly released, black: postprimary dormancy, gray: heat-induced secondarily dormant). Arrows represent transitions in physiological condition (loss of primary dormancy: dashed, thermal dormancy induction: double solid, loss of secondary dormancy: single solid). Branch points symbolize the two extremes in the spectrum of variability in each trait: primary dormancy strength (*a-b* low, *a-e* high), sensitivity to thermal induction of dormancy (*b-c* low, *b-d* high), and seasonal germination bias (c-c^t low, f-f^t high). Periods shaded are those in which seasonal cues that trigger germination are present.**

August, minimum monthly average temperature from December to February, and mean rainfall in both periods. We used field soil temperature data from Hobo UA-002–08 temperature loggers (www.OnSetcomp.com) as a reference to set conditions in the controlled environment chambers (see Montesinos-Navarro et al. 2011 for more details).

SEED PRODUCTION

We collected seeds from 138 field-collected plants, and bulked them up enforcing self-fertilization in Conviron PGW36 (http://www.conviron.com) controlled environment chambers at the University of Pittsburgh (Montesinos-Navarro et al. 2011). The offspring from these self-fertilized field-collected plants are highly homozygous lineages with very high within-lineage homogeneity. We used these lineages for the experiments reported here. For the purposes of these experiments, the seeds within a lineage are considered genetic replicates of each other. To minimize maternal position effects (Boyd et al. 2007) we only used seeds released within the period between the fifth and 15th days following maturation of the first fruit. We quantified maternal position effects on germination within these 10 days with pilot experiments using a subset of 88 lineages. Germination rates of the same lineages between the two collection dates differed by 2.9% on average (SE = 0.9 Wilcoxon's $n = 88$, $Z = -4.5$, $P < 0.001$). Accordingly, we considered sampling differences smaller than 2.9% to be inseparable from sampling error.

Some experiments subjected seeds to a gradual change of environmental conditions. These experiments therefore require lineages whose seeds are produced in synchrony; our experiments were as a result restricted to the use of 115 of the 138 lineages originally collected. The number of lineages used per population ranged from 2 to 13 (details in Table 1).

GERMINATION ASSAYS

We used three replicate assay units (i.e., sets of 50 seeds) per parent lineage in every treatment. We conducted all assays in moisture chambers consisting of Linbro 24-well flat-bottom tissue culture plates (www.hamptonresearch.com) containing filter paper saturated with 140 µl of distilled water with additional water added during the experiment if necessary, closed with transparent lids, and sealed with parafilm. We randomly place replicates in wells and plates within the growth chamber.

Table 1. **Seed traits of 17 populations of *Arabidopsis thaliana*. Population contains population names, the altitude of each lineages population of origin expressed in meters above sea level (masl), and primary dormancy strength expressed as days to reach 50% of the final germination. Entries in the trait columns are population mean values; standard errors in parentheses. Thermal dormancy induction and seasonal germination bias are expressed as reductions in germination percentage between thermal treatments and seasonal germination cues, respectively. Positive values indicates higher germination percentage at 30°C than at 20°C and higher germination percentage under spring than under fall treatments respectively, while negative values indicates the opposite.**

Population	Altitude (masl)	*N*	% germination at release	After-ripening time, days	Thermal dormancy induction	Seasonal germination bias
PIN	109	4	26 (15)	2 (2)	1(4)	15 (6)
RAB	110	5	29 (16)	7 (3)	9(5)	19 (7)
SAL	332	3	1 (1)	31 (9)	2(9)	73 (14)
BAR	429	9	9 (4)	38 (9)	1(4)	27 (8)
HOR	431	9	55 (14)	6 (3)	−0.2(2)	13 (5)
ARU	440	6	29 (13)	6 (2)	6(3)	10 (3)
COC	515	6	5 (4)	34 (5)	10(5)	46 (7)
POB	656	5	33 (18)	13 (6)	14(10)	34 (18)
BOS	719	5	35 (11)	3 (2)	0.4(1)	5 (3)
MUR	836	3	1 (1)	35 (13)	4(4)	52 (13)
VDM	975	13	4 (3)	29 (3)	10(3)	58 (7)
ALE	1225	10	36 (8)	13 (9)	2(2)	5 (4)
HEC	1238	2	1 (1)	20 (2)	29(5)	80 (4)
PAL	1433	11	33 (11)	27 (7)	−0.3(4)	12 (6)
BIS	1450	13	3 (2)	30 (3)	14(5)	75 (4)
VIE	1600	5	1 (1)	24 (8)	6(4)	8 (5)
PAN	1668	6	4 (4)	27 (6)	21(12)	37 (14)

We scored the total number of viable seeds (i.e., golden in color and fully inflated) and the number of germinating seeds (i.e., emerging radical visible) using a Nikon SMZ2-T stereomicroscope and we calculate the percentage of germinated seeds. Germinated seeds were removed after each count to prevent fungus growth.

EXPERIMENTAL DESIGN

We performed three independent but sequential experiments to genetically characterize primary dormancy, thermal germination response, and seasonal germination response. Primary dormancy was characterized testing for germination percentage after different times of storage, thermal germination response by comparing cumulative germination curves of seeds exposed to low and high temperature, and seasonal germination response by comparing cumulative germination curves of seeds germinating under spring versus fall simulated conditions. First, we partitioned the genetic variance of these traits among populations, lineages, treatments, and their interactions. Second, we tested for genetic covariation among traits using structural equation models. Finally, we tested for adaptive divergence performing canonical correlations between genetically based trait variation and climatic variation along a climatic gradient.

Experiment 1. Primary dormancy

We characterized primary dormancy by storing seeds for increasing lengths of time before recording germination percentage under cool conditions. We collected seeds periodically right after fruit maturation. We stored seeds at 20°C in dark and dry conditions for 0, 10, 60, 90, or 120 days. After 120 days all lineages reached 100% germination. We scored percentage of germination after seeds had been exposed 15 days to moisture and 8 h:16 h light:dark and constant 12°C. This experiment simulates the conditions that seeds experience after release in the field. Seeds released in late spring are buried in the soil from June to August, the driest months of the year (i.e., monthly average temperature across the populations is 19°C). Following dry and warm soil conditions, seeds experience fall moisture and cooling temperatures (i.e., monthly average temperature in September–October across populations, 16–11.8°C, respectively). Exposure to cool temperature (12°C) following dry storage (20°C) contributes to breakage of primary dormancy, both in the field and in our experimental setting. We used pilot assays to determine the appropriate length of the germination assays. By day 15, germination nearly ceased with an increment lower than 4% for every genotype tested.

A total of 103,500 seeds were used in this experiment (138 lineages × 5 time periods of storage × 3 replicates × 50 seeds per replicate).

We characterized each lineage's primary dormancy using (1) the germination percentage of seeds immediately after seed release from the maternal plant, hereafter "germination at release," and (2) the period of dry storage required prior to exposure to cool temperature to reach 50% of maximum germination (Alonso-Blanco et al. 2003), hereafter "primary dormancy strength" (see section on estimation of germination cumulative parameters for further details).

Experiment 2. Secondary dormancy

We characterized secondary dormancy as plasticity for each lineage in the germination curves of seeds stored at low versus high temperature. All the seeds used in experiment 2 were stored until experiment 1 was completed, that is, every lineage had overcome primary dormancy. Therefore, we exposed two sets of seeds previously stored 120 days dry in the dark (Fig. 2) to 30 days at low 20°C or high 30°C temperature followed by moisture and simulated fall conditions (see experiment 3 below). Temperatures simulated mild versus warm summers based on the thermal range present during summer in the natural sites (i.e., the range of monthly mean temperature from June to August among the 17 sites is 21–30°C). We scored the number of germinants in each well every 3 days over a period of 81 days simulating nearly a full 3 months of fall.

A total of 34,500 seeds were used (115 lineages × 2 temperature treatments × 3 replicates × 50 seed per replicate).

We characterized secondary dormancy for each lineage using: (1) the difference in final germination between seeds exposed to 20°C versus 30°C storage, hereafter "thermal dormancy induction," and (2) the difference in days to reach 50% of final germination between seeds exposed to 20°C and 30°C storage, hereafter "thermal germination acceleration" (see section on estimation of cumulative germination parameters for further details).

Experiment 3. Seasonal germination response

We characterized seasonal germination response as plasticity for each lineage in the germination curves of seeds germinated under simulated spring versus fall conditions. All treatments shared a 120 days dry-stored period to overcome primary dormancy and 30 days at 30°C simulating secondary dormancy induced by summer conditions. Afterwards, we exposed two sets of seeds to simulated fall versus spring germination conditions. Fall germination conditions consisted of moistening and a progressive reduction in hours of light, temperature, and their associated daily amplitude. The mean daily temperature was ramped from 17 ± 4°C to 1 ± 0°C decreasing 2 ± 0.25°C weekly the first month, 1 ± 0.25°C the second, and 1 ± 1°C until the end of the experiment, 81 days. Daily hours of light were ramped from 13 to 9 reducing 15 min weekly (this set of seeds was also used in experiment 2, Fig. 2). Spring germination conditions were simulated by keeping seeds dry and dark during simulated fall, followed by 30 days at constant 4°C, then moistened and exposed to increasing temperatures, hours of light and an increment in their daily amplitudes. The mean spring temperature and daily variation ramped from 7 ± 0.2°C to 14C ± 1.6°C increasing 0.5 ± 0.2 weekly the first month and 1 ± 0.2°C until the 81st day. Daily hours of light were ramped from 9 to 13 increasing 15 min weekly (Fig. S2 shows the actual chamber temperatures during fall and spring germination periods). Fall treatment simulated seasonal conditions from seed release (late spring–summer) through summer and fall conditions, when winter annuals germinate. Spring treatment mimicked a spring annual's germination conditions, including summer, fall, winter, and spring conditions. Experimental conditions were based on monthly mean temperature from September to November for fall treatment and March to May for spring treatment across all 17 source populations.

We scored the number of germinants in each well every 3 days over a period of 81 days. A total of 34,500 seeds were used in this experiment (115 lineages × 2 simulated season treatments × 3 replicates × 50 seed per replicate).

We characterized seasonal germination response for each lineage using: (1) the difference in final germination between seed exposed to spring versus fall conditions, hereafter "seasonal germination bias," and (2) the difference in days to reach 50% germination between the same set of seeds, hereafter "seasonal germination acceleration" (see section on estimation of germination cumulative parameters for further details).

Germination scored under simulated spring conditions do not represent the genetic propensity of a lineage to germinate in spring, as many seeds that germinate in the simulated spring might have germinated in the fall if they had been hydrated. Accordingly, we consider the difference between germination under simulated spring versus fall conditions as a genetically based component of germination bias likely to contribute to differences in the tendency to exhibit winter versus spring annual life cycles in the field. Lack of seasonal germination bias is defined herein as an indistinguishable germination percentage when simulated fall and spring germination are compared. A positive sign indicates higher germination under simulated spring conditions, a value close to zero indicates lack of seasonal germination bias and a negative value indicates a higher germination under simulated fall. The magnitude of the difference indicates the degree of seasonal germination bias.

ESTIMATION OF CUMULATIVE GERMINATION CURVE PARAMETERS

Curves of germination percentage as a function of time in each experiment provided the kinetics of cumulative germination curve of each lineage. We estimated germination parameters from the cumulative germination curves fitting the following four-parameter

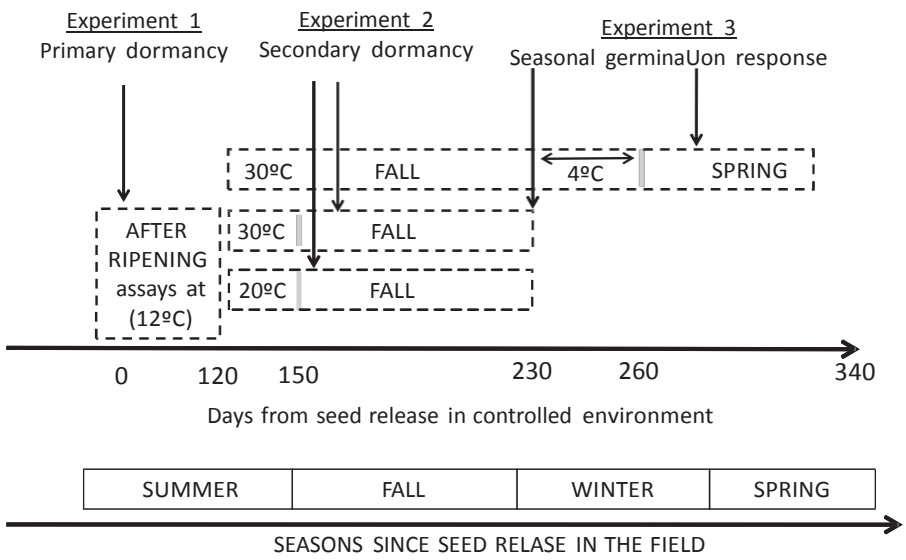

Figure 2. **Schematic representation of the three sequential experiments used to characterize seed traits. The horizontal axis represents our simulation of time, indicating the start and duration of each experiment, from seed release to the following spring, based on the seasonal changes observed in the field. Rectangles with dashed margins represent sets of seeds exposed to the heat and seasonal conditions indicated inside the boxes. Gray lines indicate the start of moisture and light application. Arrows point to the sets of seeds used in each experiment.**

Hill function specifically developed to describe seed germination (El-Kassaby et al. 2008):

$$X_t = Y + (A \times T^b / C^b + T^b),$$

where X is the cumulative germination percentage at time T, Y is initial germination percentage tested immediately after seed release, A is final germination percentage, C is the time required to reach 50% of the final germination percentage, and b is a parameter related with the shape and steepness of the curve (El-Kassaby et al. 2008). Both parameters b and C are related to germination speed. As units in which C is expressed (number of days to reach 50% of final germination) are more intuitive than b, C was selected to characterize germination speed instead of b. For succinctness hereafter we will use the following terms: Y = initial germination, A = final germination, and C = germination speed. In each experimental treatment, experiment and treatment represents first and second subscripts respectively, we designated the cumulative curve parameters as follows (Fig. 3):

Experiment 1: $Y_{1=}$ initial germination (germination at release). A_1 = final germination, which did not have any variation in experiment 1 because our experimental design forced it to be 100% for every lineage in this experiment, C_1 = germination speed (primary dormancy strength). In this experiment only one cumulative germination curve was calculated per individual level using the average of lineage replicates, as lineage replicates cannot be linked across assays performed at different times.

Experiment 2: Y_2 = initial germination, which was 0% in all assays in this experiment because the germination immediately after wells were moistened was 0. $A_{2,20}$ = final germination in seeds exposed to 20°C and $A_{2,30}$ = final germination in seeds exposed to 30°C. $C_{2,20}$ = germination speed in seeds exposed to 20°C and $C_{2,30}$ = germination speed in seeds exposed to 30°C.

Experiment 3: Y_3 = initial germination, which was 0 for the same reason as in experiment 2. $A_{3,\text{fall}}$ = final germination in seeds exposed to fall conditions (note that this is the same as $A_{2,30}$ because the same set of seeds and conditions were used in both experiments) and $A_{3,\text{spring}}$ = final germination in seeds exposed to spring conditions. $C_{3,\text{fall}}$ = germination speed in seeds exposed to fall conditions (note that this is the same as $C_{2,30}$) and $C_{3,\text{spring}}$ = germination speed in seeds exposed to spring conditions.

We calculated four new variables based on the parameters described above and used them in canonical correlation analyses and structural equation models to characterize relationships among seed traits (see Fig. 3 for graphical representation and details in statistical analyses below):

(1) Thermal dormancy induction: $[A_{2,20}–A_{2,30}]$.
(2) Thermal germination acceleration: $[C_{2,20}–C_{2,30}]$.
(3) Seasonal germination bias: $[A_{3,\text{spring}}–A_{3,\text{fall}}]$.
(4) Seasonal germination acceleration: $[C_{3,\text{spring}}–C_{3,\text{fall}}]$.

We fitted nonlinear regressions using SPSS (version 17.0; IBM Company Headquarters, Chicago, IL) to obtain estimates of the parameters.

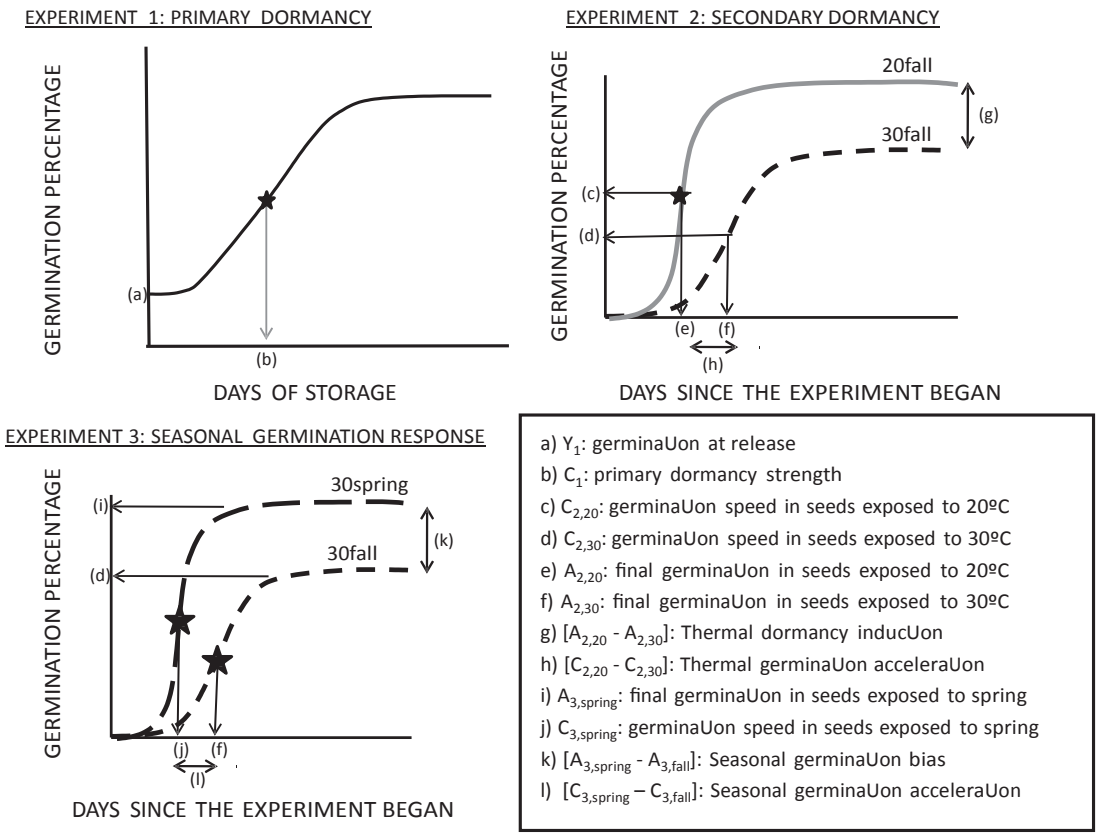

Figure 3. **Schematic representation of the cumulative germination curve parameters used to characterize seed traits. The star indicates 50% of the final germination percentage. In experiments 2 and 3 line types (black, gray, short dashed, and long dashed) indicate different treatments described on top of each line. Each character, in brackets, is followed by the name used for the character throughout the article.**

STATISTICAL ANALYSES

Partitioning of genetic variance

Experiment 1. Primary dormancy. We used two separate mixed models, one each to test for genetic effects of population on germination at release and primary dormancy strength, considering population as a random effect:

$$y_{ij} = \mu + \delta_j + \xi_i\, j.$$

Here y_{ij}, the dependent variable, representing either germination at release or primary dormancy strength for individual i in population j, respectively, μ is the overall mean, δ_j is the population effect with $j = 1,\ldots,17$ populations, and ξ_{ij} is the residual deviation of the ith individual from the grand and population means.

Experiment 2. Thermal germination response. We used mixed models to test for effects of heat treatment, population of origin, lineage nested within population, population by treatment interaction, and lineage by treatment interaction on final germination and germination speed. Heat treatment was considered as a fixed effect and population and lineage as random effects. The model including all the effects did not converge, so population and lineage effect were tested in two separate models. For each response variable, the following two models were used to test for (1) population and population by heat treatment interaction (1) and (2) lineage and lineage by heat treatment interaction (2):

$$y_{ijk} = \mu + \kappa_k + \delta_j + \delta\kappa_{ik} + \xi_{ijk} \quad (1)$$

$$y_{ijk} = \mu + \kappa_k + \imath_i(\delta_j) + \imath_i(\delta_j)\kappa_{ik} + \xi_{ijk}, \quad (2)$$

where y_{ijk} is the dependent variable, either final germination or germination speed for individual i in population j in heat treatment k respectively, μ is the overall mean, κ is the temperature treatment effect with $k = 2$ treatment levels: 20°C and 30°C, δ is the population effect with $j = 17$ populations, $\imath$ is the lineage effect with $i = 115$ lineages but with a variable number (2–13) of lineages nested within each population, $\delta\kappa$ is the population by treatment interaction effect, $\imath\kappa$ is the individual by treatment interaction effect, and ξ is the residual error.

In the analyses involving final germination the residual error did not meet homoscedasticity assumptions of general linear models. GLIMM models were therefore used, adjusted to a beta

distribution with which the residual error meet the assumptions required.

Experiment 3. Seasonal germination response. We used mixed models to test for effects of season treatment, population of origin, lineage nested within population, population by treatment interaction, and lineage by treatment interaction on final germination and germination speed. The full model with both population $\times$ season and lineage $\times$ season failed to converge. We therefore used mixed models (1) and (2) as described above. Season treatment κ was considered a fixed effect with treatment levels: fall versus spring, and population, population by season treatment interaction, lineage and lineage by season treatment interaction as random effects. In the analyses involving final germination the residual error did not meet homoscedasticity assumptions of general linear models. GLIMM models were therefore used, adjusted to a beta distribution with which the residual error meet the assumptions required.

For all three experiments significance of fixed effects was calculated using restricted maximum likelihood. For models using a beta distribution, dual quasi-Newton pseudo-likelihood estimation was used instead because of its greater calculation efficiency. We tested for significance of random effects by comparing nested models. We removed each factor one at a time and compared -2 Log pseudo-likelihood (-2 LogPL) of the models (Burnham and Anderson 2002; Bolker et al. 2009). Each full model was compared to a null model $y_{ijk} = \mu$ and the -2 LogPL used to test for its overall significance.

All analyses were performed with PROC GLIMMIX in SAS (version 9.2; SAS Institute Inc., Cary, NC) using COVTEST option.

Genetic correlations among seed traits

We tested for genetic correlations between primary dormancy and secondary dormancy and their independent effects, on seasonal germination response. We tested the causal pathways illustrated in Figures 1 and 6A using structural equation models as confirmatory analysis (Pugesek et al. 2003). We used seasonal germination bias and seasonal germination acceleration as the response variables and primary dormancy strength, thermal dormancy induction, and thermal germination acceleration as putative causal variables. A nonsignificant χ^2 *P*-value indicates that the model has a high goodness-of-fit and the pattern of covariance predicted by the model is not distinguishable from that observed (Mitchell 1992, 1993; Shipley 1997).

Lineages were clustered within populations. We fitted the same model using both lineage means ($n = 115$) and population means ($n = 17$). In both cases the *P*-value of the goodness-of-fit of our proposed model was above 0.05 (0.76 and 0.92, respectively). Both models had the same significant paths with the same signs, thus potential nonindependence is not conditioning the results. Results are presented for models fitted with lineage means, because they provide higher precision. We removed the six observations furthest from the centroid to examine whether deviation from multivariate normality was affecting the results, and this resulted in outputs that were consistent with the full dataset. Accordingly, we included the full dataset in our analyses without further transformation. Analyses were performed using the AMOS module of PASW statistics (version 18.0; SPSS Inc., Chicago, IL).

Genetic population differentiation associated with an environmental gradient

We tested for clinal variation in genetically based seed traits along the climatic gradient. Canonical correlation analyses were used to establish the linear combination of traits and climatic variables that maximize the Pearson product–moment accounting for nonindependence of the variables (McGarigal et al. 2000), thus accounting for as much colinearity as possible between trait values and climate of origin and providing the greatest predictive value for climate with regard to variation in seed traits.

We estimated the canonical correlation between population mean values of: primary dormancy strength, thermal dormancy induction, and seasonal germination bias versus the climatic variables: maximum temperatures from June to August, minimum temperatures from December to February, and mean rainfall in those same periods. Canonical correlations produces variates (i.e., orthogonal linear combinations) of both traits and environmental variables and these were tested using the likelihood ratios among nested models, sequentially excluding the previous variates (Scheiner and Gurevitch 2001). We present only the significant variates in the results. The significant variates' standardized canonical coefficients were calculated for each trait and environmental variable, and their magnitudes and signs were used to interpret their relative contribution to the overall canonical correlation. The canonical variates and their constituent trait and climate coefficients provide the linear combinations of trait and climate values that best estimate their mutual covariation. The magnitude represents the contribution of that trait or environmental variable to the multivariate correlation. Similar or opposite signs for two variable's coefficients represent respectively either concordant or opposing effects of the two variables on the multivariate correlation.

The statistic most often reported from a canonical correlation analysis is the squared canonical correlation, which estimates the proportion of the multivariate variance in both trait and climate measures explained by the canonical covariance. Although this is an important measure and we report it, for this study the most important measure is the proportion of variance in the seed traits that is explained by variance in climate. We report this as well. The

Table 2. **Results of the generalized mixed linear model tests for population effects on primary dormancy. Significance based on likelihood ratio (−2 Log likelihood, 2LOD) and variance component (SE) of population considered as random are reported. The full model was significant for both germination at release (initial germination percentage) and primary dormancy strength (days to reach 50% germination) (2LOD = 25.53***; df = 1 and 2LOD = 983.39***; df = 1, respectively). (n.s > 0.005, *P < 0.05; **P < 0.01; ***P < 0.001).**

Trait	Effect	2LOD	Variance component (SE)
Initial germination percentage			
	Population	17.4***	0.02 (0.01)
	Residual		0.06 (0.01)
Days to reach 50% germination			
	Population	13***	100.96 (52.90)
	Residual		287.26 (41.10)

analysis was performed using PROC CANCOR in SAS (version 9.2; SAS Institute Inc.).

Results

PARTITION OF GENETIC VARIANCE

Experiment 1. Primary dormancy strength

In our germination assay conditions, all lineages broke primary dormancy before 120 days postrelease. On average, 50% of germination was achieved by day 20 ± 19 (SE). Germination at release was 90% in 5% of the lineages, whereas 20% of the lineages did not germinate at all at release, thus some lineages are able to germinate at 12°C without a period of dry storage. We found significant population differentiation in germination at release (Table 2) and in primary dormancy strength (Table 2; Table 1 for population mean values).

Experiment 2. Secondary dormancy

Exposing seeds to 30°C tended to reduce final germination by 7% and increase the number of days to reach 50% germinants by 1.5 days, on average, compared to seeds exposed to 20°C. Exposure to 30°C thus slowed germination speed by 1.5 ± 0.6 days.

There were significant population, lineage, and lineage-by-heat interaction effects on final germination and germination speed in response to the heat treatment (Table 3, Fig. S3A, B). The population-by-heat treatment interaction effect was not significant for either variable (Table 3), indicating that all populations respond similarly to the heat treatment.

Experiment 3. Seasonal germination response

We found a significant effect of simulated fall versus spring conditions on germination behavior (Table 4). Exposure to fall conditions on average reduced final germination by 33 ± 3% and increased the days to reach 50% of final germination by 1 ± 0.6 days, compared to spring germination conditions. Germination showed increased synchrony across lineages in the spring compared to the fall treatment (germination speed standard deviations: 1.4 days and 7.8 days, respectively; Fig. S3C and D).

There was no significant average effect across seasons of populations or lineages for either final germination or germination speed in experiment 3. However, both populations and lineages were differentiated in their responses to the season treatment for both final germination and germination speed, as indicated by significant interaction terms (Table 4).

GENETIC CORRELATIONS AMONG SEED TRAITS

There were no significant differences between the covariance structures proposed in our model and the observed structure, indicating a good fit of the structural equation model (n = 115, χ^2 = 0.09, df = 1, P = 0.76). The model had a high adjusted goodness-of-fit index (AGF) of 0.99 (index ranges from 0 to 1) and a root mean square error of approximation (RMSA) of 0 with a confidence interval of 0–0.17. Our proposed model fit the empirical data considerably better than the independence model, with a comparative fit index of 1 and normed fit index of 0.99 (Byrne 2009). Holter's critical N focuses on the adequacy of the sample size, values bigger that 200 indicate that the model adequately represents the sample data (Byrne 2009). For a significant difference of 0.05 our model had a Holter's critical N of 4861.

The proposed model explained 43% and 50% of the variation in seasonal germination bias and seasonal germination acceleration, respectively. The variance explained accounts for positive genetic influences of primary dormancy strength (standardized regression coefficient [SRC] = 0.30), thermal dormancy induction (SRC = 0.51), and thermal germination acceleration (SRC = 0.23) on seasonal germination bias (Fig. 4A and B) (all P < 0.001). We also found a significant association between thermal germination acceleration and seasonal germination acceleration (SRC = 0.71, Fig. 4A and B). No overall genetic correlation was detected between primary dormancy strength and thermal germination response.

GENETIC POPULATION DIFFERENTIATION ASSOCIATED WITH THE ENVIRONMENTAL GRADIENT

Clinal variation in seed traits along a climatic gradient is demonstrated by a significant canonical correlation between population mean seed traits values and site-specific environmental variables values (n = 17, Wilk's Lambda 0.1, F = 3.3, Num DF 12, Den

Table 3. **Results of generalized mixed linear model test for genetic variance in thermal germination response. For each dependent variable the effects of (1) population and population by treatment interaction and (2) lineage and lineage by treatment interaction were tested independently. Significance of fixed (heat treatment), random (all the rest) based on likelihood-ratio (−2 Log likelihood, 2LOD) effects and variance component (SE) are reported. Lineage was nested within population for all analyses (n.s: $P > 0.05$, *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$).**

Trait	Source	2LOD	*F*	df	Variance component (SE)
Final germination	Model	529.23***		2	
	Population	10.8***			1.65 (0.65)
	Heat		18.8***	1/123	
	Population×Heat	0n.s			0 (.)
	Residual				2.84 (0.36)
	Lineage	129.47***			4.48 (0.65)
	Lineage×Heat	17.85***			0.37 (0.08)
	Residual				10.77 (0.74)
Germination speed	Model	188.98***		2	
	Population	6.59**			0.46 (0.26)
	Heat		4.28*	1/123	
	Population×Heat	0n.s			0 (.)
	Residual				3.74 (0.35)
	Lineage	34.9***			3.09 (0.62)
	Lineage×Heat	21.21***			1.44 (0.41)
	Residual				4.95 (0.31)

Table 4. **Results of generalized mixed linear model tests for genetic effects on seasonal germination response. For each dependent variable the effects of (1) population and population by treatment interaction and (2) lineage and lineage by treatment interaction were tested independently. Significance of fixed (season treatment), random (all the rest) based on likelihood-ratio (−2 Log likelihood, 2LOD) effects and variance component (SE) are reported. Lineage was nested within population for all analyses (n.s: $P > 0.05$, *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$).**

Trait	Source	2LOD	*F*	df	Variance component (SE)
Final germination	Model	542.61***		2	
	Population	−21.2n.s			1.20 (0.63)
	Season		311.59***	1/123	
	Population×Season	56.18***			0.35 (0.31)
	Residual				4.19 (0.49)
	Lineage	−70.44n.s			3.11 (0.61)
	Lineage×Season	474.84***			1.34 (0.30)
	Residual				15.69 (1.02)
Germination speed	Model	184.42***		2	
	Population	0n.s			0.003(0.14)
	Season		56.27***	1/123	
	Population×Season	3.77*			0.25 (0.20)
	Residual				2.59 (0.25)
	Lineage	0n.s			0.00 (.)
	Lineage×Season	133.28***			3.16 (0.38)
	Residual				2.93 (0.19)

DF 26.7, $P < 0.005$). Only the first variate of the three obtained in the canonical correlation analysis was significant, explaining 81% of the total variation ($R^2 = 0.81$, $P = 0.005$). The other two climate variates were not significant ($P = 0.2$ and 0.77, respectively). The climatic gradient, in order of decreasing contributions to the variate, is one of increasing maximum temperatures in June–August, increasing rainfall December–February, increasing minimum temperatures December–February, and increasing rainfall

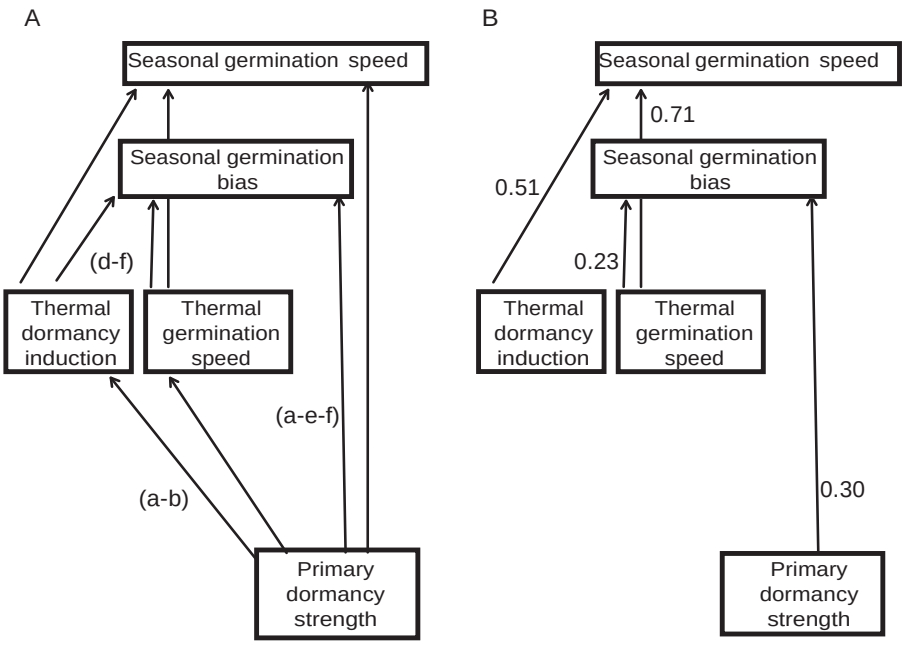

Figure 4. **Results of structural equation model to test for primary dormancy strength and thermal dormancy induction effects on seasonal germination bias. (A) Representation of the model tested. (B) Standardized coefficients only for the significant paths (*P*-value ≤ 0.01) (*N* = 115, χ^2 = 0.09, df = 1, *P*-value = 0.76, R^2 (S_Y) = 0.43, R^2 (S_C) = 0.50). Characters in brackets designate groups of relationships testing for specific paths hypothesized in Fig. 1: (a-e-f^t) after-ripening time and seasonal germination bias, (a-b-d) after-ripening time and thermal dormancy induction and (d-f^t) thermal dormancy induction and seasonal germination bias. High values of seasonal germination bias indicate predominant germination under spring cues compared with fall cues.**

Table 5. **Canonical correlation among seed traits and environmental conditions in the natural sites based on population means. The first variates' standardized canonical correlation coefficients (SC) for seed traits and environmental variables accounted for 81% of the total variance (R^2 = 0.81, *P*-value = 0.005). The other two climate variates produced in this analysis, not presented, were not significant (*P*-values 0.2 and 0.77, respectively).**

Traits	SC
Primary dormancy strength	−0.55
Thermal dormancy induction	0.54
Seasonal germination bias	0.74
Environmental variables	
Maximum temperature (June–August)	1.52
Minimum temperature (December–February)	0.23
Mean rainfall (June–August)	0.97
Mean rainfall (December–February)	1.23

June–August, according to the signs of the standardized canonical correlation coefficient (SCCC; Table 5). The climate gradient described by the SCCC is negatively correlated with the first principal component of the WorldClim (http://www.worldclim.org/) climatic data for the Iberian peninsula ($r = -0.65$, $P = 0.005$), which is in turn highly correlated with the altitude of origin of the study populations ($r = 0.93$, $P < 0.001$; M. D. Wolfe and S. J. Tonsor, unpubl. data).

The first principal component of the Iberian WorldClim data, explained 29% of the gradient in standardized variation in seed traits described by the SCCC. Along this climatic gradient, from lower temperature/dryer conditions to higher temperature/wetter conditions, seeds presented lower primary dormancy strength (SCCC: −0.55, Table 5), had higher thermal dormancy induction (SCCC: 0.54, Table 5), and showed increasing seasonal germination bias, predominantly germinating under spring conditions (SCCC: 0.74, Table 5).

Discussion

Genetic variation in germination timing and its evolutionary consequences in wild populations of annuals has been the subject of considerable study (Kalisz 1986; Baskin and Baskin 1998; Donohue et al. 2005a,b; Penfield et al. 2005; Donohue et al. 2007). The disentanglement of the selective pressures and internal mechanisms leading to a given germination phenology remains a matter of active research (Finch-Savage and Leubner-Metzger 2006; Holdsworth et al. 2008; Chiang et al. 2009). In this study, we show that genetic variation at a regional scale in three seed traits contributes to polymorphism in germination phenology in

the highly selfing species *A. thaliana*. We further show that this variation is clinal, associated with a climate gradient whose most important features are a transition from warmer summers and wetter winters to cooler summers and drier winters. This gradient is also associated with the altitude of origin of the study populations.

We observed genetic variation in primary dormancy, thermally induced secondary dormancy, and seasonal germination responses in 115 lineages from 17 populations of *A. thaliana* from NE Spain. The observed integration of this trait variation suggests that *A. thaliana* has evolved a complex bet-hedging strategy in which the likelihood of germinating in the fall is influenced by two clinally covarying but genetically independent mechanisms. These two mechanisms, the rate of loss of primary dormancy, and the sensitivity to thermal dormancy induction, provide in turn two mechanisms that can either inhibit or enable fall germination.

In theory, if release from primary seed dormancy is slow enough, seeds could remain dormant past the window of fall germination cues, ensuring a spring annual life history. However, the longest population mean primary dormancy half-life (time to 50% germination) observed among our 17 populations was 38 days for the BAR population. This population releases seeds in April and May, one of the first populations in which this occurs, and germination occurs in late September or October (Montesinos et al. 2009). Thus, even in this most extreme population the mean remaining primary dormancy can only weakly inhibit fall germination, approximately three half-lives after seed release. Nevertheless, when variation among lineages within populations is considered, we detected significant genetic path coefficients between primary dormancy strength and the extent to which germination is limited to the spring window of cues, and to the speed of spring versus fall germination. This indicates that either primary dormancy directly influences seasonal germination bias and speed, or that the traits are influenced by a common genetic mechanism. The most extreme 20% of the lineages have half-lives between 40 and 90 days, indicating that for many lineages there may not be sufficient residual primary dormancy to substantially inhibit fall germination but for a small number of lineages, primary dormancy will be remain a factor in fall. We have shown a genetic basis to variation in primary dormancy half-life in the laboratory, but environmental conditions in the field most likely will determine the actual primary dormancy half-lives, and the extent to which primary dormancy inhibits fall germination.

For any genotype that loses primary dormancy during the summer, secondary dormancy may be induced in a temperature-dependent manner, providing a second mechanism inhibiting fall germination. Variation in the sensitivity to thermal dormancy induction appears to be genetically independent of primary dormancy strength under our experimental conditions (but see below) and provides a second pathway inhibiting fall germination. Variation in thermally induced secondary dormancy among populations is substantial, ranging from no effect to reductions in germinability of as much as 29%. Secondary dormancy induced by high summer temperatures therefore appears to be highly evolvable.

Because primary dormancy and thermally induced secondary dormancy are evolvable and show strong influences on seasonal germination properties, they appear to be important mechanisms by which seasonal germination bias has evolved across our study populations.

Across the climate gradient described by our first canonical climate variate the trait values associated with primary and secondary dormancy and the tendency to germinate in spring versus fall exhibit a cline, indicating adaptively differentiated dormancy/germination strategies. Populations in warmer, wetter locales exhibit foreshortened primary dormancy, heightened tendency to secondary dormancy, and a tendency to require exposure to winter cold to germinate, consequently germinating preferentially under spring cues. This could provide a mechanism of avoiding germination after sporadic rain within potentially extremely hot summers, very frequent in the Mediterranean climate, especially in localities with average wet and warmer summers. On the other extreme of the climatic gradient, with cooler and drier environments, plants exhibit strong primary dormancy, low secondary dormancy, and greater plasticity in responsiveness to either fall or spring germination cues. In this environment primary dormancy could be uninhibited triggering seeds germination in fall, but in years when summer conditions have been especially dry seeds could have entered secondary dormancy, delaying germination until spring when conditions would have on average improved. At the same time, being able to take advantage of the rare warmer winters where the increased winter warmth brings precipitation as rain rather than snow, could be a tremendous advantage in good years.

One of the challenges of life-history theory is to explain the maintenance of life-history polymorphisms. Despite the observed genetic differentiation in seed traits, all populations in our study retain polymorphism for germination under both spring and fall conditions in both laboratory (this study) and field (Montesinos et al. 2009). Different potential explanations exist for this variation. One explanation, admixture gene flow across the climate gradient, can be discounted in our study system because all population genetic measures thus far point to very low movement of genes between these populations (Montesinos et al. 2009; see Montesinos-Navarro et al. 2011 for a detailed explanation). However, balancing selection, selection favoring bet hedging in responses to germination cues (Pake and Venable, 1996; Venable and Brown 1998) or stochastic switching in gene expression (Acar et al. 2008) all remain as potential causes of the observed polymorphism. In addition, our observed variation in primary and secondary dormancy traits account for only about half of the variation in seasonal germination bias and seasonal

germination speed (0.43% and 0.51%, respectively), suggesting that additional mechanisms may exist that are genetically independent of the mechanisms explored in this study.

The effects of multiple genetic pathways underlying primary dormancy are only partially disentangled in this study and can play an important role in shaping germination time. Germination time is a highly integrative trait influenced by primary dormancy, thermal dormancy induction, and other unaccounted-for influences.

Huang et al. (2010) showed evidence for a genetic association between the speed of after-ripening and thermal dormancy induction using quantitative trait locus analysis (QTLs) in recombinant inbred lines. QTLs associated with after-ripening colocate with QTLs for induction of dormancy by warm temperatures. However, in our results, where primary dormancy is characterized as a combination of timing of after-ripening (in dry storage) and chilling (following 12°C conditions), we did not find an overall genetic association between primary dormancy and thermal dormancy induction (Fig. 4A, B). This may result from the effects of chilling response obscuring the genetic correlation between after-ripening and thermal dormancy induction.

Prediction of germination behavior and its evolution in the field lies outside the aims of this article. Field seed bank dynamics are likely to be influenced by complex environmental cues affecting primary and secondary dormancy after seed release, and other effects such as maternal effects on dormancy, or microenvironmental effects, that we have tried to minimize in our experimental design. In addition, as has been shown in other traits in *A. thaliana* (Tonsor and Scheiner 2007) genetic correlations among seed traits are likely to change with environmental conditions. However, the underlying genetic mechanisms shaping important life-history traits and strategies under controlled conditions in our study are likely to influence phenotypic differences among genotypes in the field as well; just how that influence plays out remains to be studied in the field.

ACKNOWLEDGMENTS

We are grateful to Y. El-Kassaby for providing useful detailed information regarding the implementation of their model, to M. Grabe and E. Revilla for altruistic help with parameter estimation from germination curves and generalized linear mixed models. R. Jovani helped with some figures. This study and its analyses benefited tremendously from many discussions with I. Gomez-Maestre, P. Rey, J. M. Iriondo and K. Donohue, T. Elnaccash, M. Wolfe, T. Helbig, M. Simon, M. Groner, the PEER discussion group at the University of Pittsburgh, and P. Jordano, and all his group, A. Carrillo, J. L. Espinar, and A. Montero in the Estación Biologica de Doñana (Seville, Spain) read the manuscript and suggested considerable improvements. D. Yarnott, S. Valle, J. Wig, and E. York contributed to plant maintenance. AMN was funded by Fundación Caja Madrid, FXP by Ministerio de Educación y Ciencia of Spain (grant CGL2009–07847/BOS), and SJT by National Science Foundation IOS-0809171.

LITERATURE CITED

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

Acar, M., J.T. Mettatal, and A. Oudenaarden. 2008. Stochastic switching as a survival strategy in fluctuating environments. Nat Genet 40:471–475.

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

Altwegg, R., and H.U. Reyer. 2003. Patterns of natural selection on size at metamorphosis in water frogs. Evolution 57:872–882.

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

Baskin, C. C., P. Milberg, L. Andersson, and J. M. Baskin. 2004. Germination ecology of seeds of the annual weeds *Capsella bursa-pastoris* and *Descurainia Sophia* originating from high northern latitudes. Weed Res. 44:60–68.

Baskin, J. M., and C. C. Baskin. 1974. Germination and survival in a population of the winter annual *Alyssum alyssoides*. Can. J. Bot. 52:2439–2445.

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

———. 1985. The annual dormancy cycle in buried weed seeds: a continuum. Bioscience 35:492–498.

———. 2004. A classification system for seed dormancy. Seed Sci. Res. 14:1–16.

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

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

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

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

Bolker, B. M., M. E. Brooks, C. J. Clark, S. W. Geange, J. R. Poulsen, M. H. H. Stevens, and J. S. White. 2009. Generalized linear mixed models: a practical guide for ecology and evolution. Trends Ecol. Evol. 24: 127–135.

Boyd E. W., L. Dorn, C. Weining, and J. Schmitt. 2007. Maternal effects and germination timing mediate the expression of winter and spring annual life histories in *Arabidopsis thaliana*. Int. J. Plant Sci. 168:205–214.

Byrne, B. M., eds. 2009. Structural equation modeling with AMOS: basic concepts, applications and programming. 2nd ed. Routledge Taylor & Francis group, New York.

Burnham, K. P., and D. R. Anderson, eds. 2002. Model selection and multimodel inference: a practical information-theoretic approach. 2nd ed. Secaucus, NJ.

Chiang, G. C. K., Barua, D., Kramer, E. M., Amasino, R. M., and Donohue, K. 2009. Major flowering time gene, FLOWERING LOCUS C, regulates seed germination in *Arabidopsis thaliana*. Proc. Natl. Acad. Sci. USA 106:11661–11666.

Cici, S. Z. F., and R. C. Van Acker. 2009. A review of the recruitment biology of winter annual weeds in Canada. Can. J. Plant Sci. 89:575–589.

Denver, R. J., N. Mirhadi, and M. Phillips. 1998. Adaptive plasticity in amphibian metamorphosis: response of *Scaphiopus hammondii* tadpoles to habitat desiccation. Ecology 79:1859–1872.

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

Donohue, K., L. Dorn, C. Griffith, E. Kim, A. Aguilera, C. Polisetty, and J. Schmitt. 2005a. The evolutionary ecology of seed germination of *Arabidopsis thaliana*: variable natural selection on germination timing. Evolution 59:758–770.

———. 2005b. Environmental and genetic influences on the germination of *Arabidopsis thaliana* in the field. Evolution 59:740–770

Donohue, K., M. S Heschel, G. C. K. Chiang, C. M. Butler and D. Barua. 2007. Phytochrome mediates germination responses to multiple seasonal cues. Plant Cell Environ. 30:202–212.

El-Kassaby, Y. A., I. Moss, D. Kolotelo, and M. Stoehr. 2008. Seed germination: mathematical representation and parameters extraction. Forest Sci. 54:220–227

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

Fenner, M., and K. Thompson, eds. 2005. The ecology of seeds. Cambridge Univ. Press, Cambridge, U.K.

Finch-Savage, W.E., and G. Leubner-Metzger. 2006. Seed dormancy and the control of germination. New Phytol. 171:501–523.

Forrest, J., and A.J. Miller-Rushing. 2010. Towards a synthetic understanding of the role of phenology in ecology and evolution. Philos. Trans. R. Soc. Lond. B. 1555:3101–3112.

Gómez-Mestre, I., and M. Tejedo. 2002. Geographic variation in asymmetric competition: a case study with two larval anuran species. Ecology 83:2102–2111.

Hilhorst, H. W. M. 1995. A critical update on seed dormancy. I. Primary dormancy. Seed Sci. Res. 5:61–73.

Holdsworth, M. J., L. Bentsink, and W. J. J. Soppe. 2008. Molecular networks regulating *Arabidopsis* seed maturation, after ripening, dormancy and germination. New Phytol. 179:33–54.

Huang, X., Schmitt, J., Dorn, L., Griffith, C., Effgen, S., Takao, S., Koornneef, M. and Donohue, K. 2010. The earliest stages of adaptation in an experimental plant population: strong selection on QTLs for seed dormancy. Mol. Ecol. 19:1335–1351.

Kalisz S. 1986. Variable selection on the timing of germination in *Collinsia verna* (Scrophylariaceae). Evolution 40:479–491.

Li, B. L., and M. E. Foley. 1997. Genetic and molecular control of seed dormancy. Trends Plant Sci. 2:384–389.

McGarigal, K., S. Cushman, and S. Stafford, eds. 2000. Multivariate statistics for wildlife and ecology research. Springer-Verlag, New York.

Mitchell, R. J. 1992. Testing evolutionary and ecological hypotheses using path analysis and structural equation modeling. Funct. Ecol. 6: 123–129.

———. 1993. Path analysis: pollination. Pp. 211–231 *in* S. M. Scheiner and J. Gurevitch, eds. Design and analysis of ecological experiments. Chapman and Hall, New York.

Montesinos, A., S. J. Tonsor, C. Alonso-Blanco, and F. X. Picó. 2009. Demographic and genetic patterns of variation among populations of *Arabidopsis thaliana* from contrasting native environments. PLoS One 4:e7213.

Montesinos-Navarro, A., J. Wig, F. X. Picó, and S. J. Tonsor. 2011. *Arabidopsis thaliana* populations show clinal variation in a climatic gradient associated with altitude. New Phytol. 189:282–294.

Morey, S., and D. Reznick. 2000. A comparative analysis of plasticity in larval development in three species of spadefoot toads. Ecology 81:1736–1749.

Ninyerola, M., X. Pons, and J. M. Roure. 2005. Atlas Climático Digital de la Península Ibérica. Metodología y aplicaciones en bioclimatología y geobotánica. Universidad Autónoma de Barcelona, Bellaterra.

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

Pake, C. E., and D. L. Venable. 1996. Seed banks in desert annuals: implication for persistence and coexistence in variable environments. Ecology 77:1427–1435.

Penfield, S., E. M. Josse, R. Kannangara, A. D. Gilday, K. J. Halliday, and I. A. Graham. 2005. Cold and light control seed germination through the bHLH transcription factor SPATULA. Curr. Biol 15:1998–2006.

Picó, F. X., B. Méndez-Vigo, J. M. Martínez-Zapater, and C. Alonso-Blanco. 2008. Natural genetic variation of *Arabidopsis thaliana* is geographically structured in the Iberian Peninsula. Genetics 180:1009–1021.

Platenkamp, G. A. J., and R. G. Shaw. 1993. Environmental and genetic maternal effects on seed characters in *Nemophila meniessi*. Evolution 47:540–555.

Pugesek, B. H., A. Tomer, and A. Von Eye, eds. 2003. Structural equation modeling: applications in ecological and evolutionary biology. Cambridge Univ. Press, Cambridge, U.K.

Scheiner, S. M., and J. Gurevitch, eds. 2001. Design and analysis of ecological experiments. Oxford Univ. Press, Oxford, U.K.

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

Shipley, B. 1997. Exploratory path analysis with applications in ecology and evolution. Am. Nat. 149:1113–1138.

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

Tonsor, S. J., and S. M. Scheiner. 2007. Plastic trait integration across a CO_2 gradient in *Arabidopsis thaliana*. Am. Nat. 169:E119–E140.

Tsiantis, M. 2005. Plant development: multiple strategies for breaking seed dormancy. Curr. Biol. 16:25–27.

Twombly, S., and N. Tisch. 2002. Fitness consequences of the timing of metamorphosis in a fresh water crustacean. Oikos 97:213–222.

Venable, D. L., and J. S. Brown. 1988. The selective interactions of dispersal, dormancy and seed size as adaptations for reducing risk in variable environments. Am. Nat. 131:360–384.

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

Walsh, P. T., J. R. Downie, and P. Monaghan. 2008. Larval over-wintering: plasticity in the timing of life history events in the common frog. J. Zool. 276:394–401.

Associate Editor: K. Donohue

Supporting Information

The following supporting information is available for this article:

Table S1. Estimates of random effects seed trait variance components related to thermal and seasonal germination response within environments, using restricted maximum likelihood.

Figure S1. Geographic locations of population sites used in the study.

Figure S2. Actual temperature and light conditions recorded in the growth chamber during the experiments simulating fall and spring conditions.

Figure S3. Reaction norms of seed traits related with thermal and seasonal germination response for each lineage.

Supporting Information may be found in the online version of this article.