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RESEARCH ARTICLE

Precipitation timing and soil substrate drive phenology and fitness of *Arabidopsis thaliana* in a Mediterranean environment

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Abstract

1. In Mediterranean climates, the timing of seasonal rains determines germination, flowering phenology and fitness. As climate change alters seasonal precipitation patterns, it is important to ask how these changes will affect the phenology and fitness of plant populations. We addressed this question experimentally with the annual plant species *Arabidopsis thaliana*.
2. In a first experiment, we manipulated the date of rainfall onset and recorded germination phenology on sand and soil substrates. In a second experiment, we manipulated germination date, growing season length and mid-season drought to measure their effects on flowering time and fitness. Within each experiment, we manipulated seed dormancy and flowering time using multilocus near-isogenic lines segregating strong and weak alleles of the seed dormancy gene *DOG1* and the flowering time gene *FRI*. We synthesized germination phenology data from the first experiment with fitness functions from the second experiment to project population fitness under different seasonal rainfall scenarios.
3. Germination phenology tracked rainfall onset but was slower and more variable on sand than on soil. Many seeds dispersed on sand in spring and summer delayed germination until the cooler temperatures of autumn. The high-dormancy *DOG1* allele also prevented immediate germination in spring and summer. Germination timing strongly affected plant fitness. Fecundity was highest in the October germination cohort and declined in spring germinants. The late flowering *FRI* allele had lower fecundity, especially in early fall and spring cohorts. Projections of population fitness revealed that: (1) Later onset of autumn rains will negatively affect population fitness. (2) Slow, variable germination on sand buffers populations against fitness impacts of variable spring and summer rainfall. (3) Seasonal selection favours high dormancy and early flowering genotypes in a Mediterranean climate with hot dry summers. The high-dormancy *DOG1* allele delayed germination of spring-dispersed fresh seeds until more favourable early fall conditions, resulting in higher projected population fitness.

4. These findings suggest that Mediterranean annual plant populations are vulnerable to changes in seasonal precipitation, especially in California where rainfall onset is already occurring later. The fitness advantage of highly dormant, early flowering genotypes helps explain the prevalence of this strategy in Mediterranean populations.

KEYWORDS
climate change, Delay of Germination 1, dormancy flowering, *FRIGIDA*, timegermination, population fitness, rainfall onset

1 | INTRODUCTION

The seasonal timing of germination is crucial to phenology and fitness of natural plant populations. Germination timing determines the environmental conditions under which seedlings emerge and develop (Donohue, 2002; Donohue et al., 2005; Huang et al., 2010; Korves et al., 2007; Miryeganeh et al., 2018; Postma & Agren, 2016, 2022; Wilczek et al., 2009), with cascading effects on flowering, life history expression, and fitness (Burghardt et al., 2015; Chiang et al., 2013; Donohue, 2002, 2005; Galloway, 2001; Gremer et al., 2020; Levine et al., 2011; Olliff-Yang & Ackerly, 2021; Taylor et al., 2017; Wilczek et al., 2009). The seasonal window under which seeds can germinate is mediated by annual cycles of dormancy induction and release in response to environmental cues such as warm after-ripening or chilling exposure (Baskin & Baskin, 2014; Finch-Savage & Leubner-Metzger, 2006; Footitt et al., 2011; Footitt et al., 2015; Martínez-Berdeja et al., 2020; Penfield & Springthorpe, 2012). However, germination in response to these cues also requires adequate soil moisture conditions (Bradford, 2002; Gremer et al., 2020; Huang et al., 2016; Liu et al., 2020). Small annual plants in the upper soil layers are particularly sensitive to rainfall variability (Huxman et al., 2004; Schwinning & Sala, 2004) and the timing and duration of the wet growing season may strongly affect germination and fitness (Gremer et al., 2020; Huang et al., 2016; Kimball et al., 2011). The effects of rainfall variability on germination also depend upon soil water retention properties, which vary with soil texture (Austin et al., 2004; Reynolds et al., 2004). As climate change alters seasonal precipitation regimes (Christensen et al., 2007; Cook et al., 2015; Dong, Leung, Lu, et al., 2019; Dong, Leung, Lu, et al., 2019; Luković et al., 2021; Pausas, 2004; Wang et al., 2017) it is important to ask how variation in rainfall amount and timing will affect the phenology and fitness of annual plant populations (Kimball et al., 2011; Levine et al., 2011).

The timing of seasonal precipitation is particularly critical to plant life cycles in Mediterranean climates, with hot dry summers and cool wet winters (Gremer et al., 2020; Levine et al., 2011). In these environments, annual plants typically germinate with the onset of fall rains and disperse dormant seeds at the end of the growing season in late spring. These seeds gradually lose dormancy with exposure to summer heat (after-ripening), allowing them to germinate under cool wet fall conditions (Chiang et al., 2011; Gremer et al., 2020; Kronholm et al., 2012; Levine et al., 2008, 2011; Montesinos et al., 2009; Olliff-Yang & Ackerly, 2021; Postma & Agren, 2016; Torres-Martínez et al., 2017; Vidigal et al., 2016). The timing of seasonal rains interacts with the release of dormancy by after-ripening to determine germination phenology, which in turn determines flowering phenology and fitness (Gremer et al., 2020; Levine et al., 2011). The amount and timing of precipitation and length of the growing season can vary substantially among years (Lázaro et al., 2001; Luković et al., 2021; Rundel et al., 2016; Swain et al., 2018). Within the rainy season, variation in the timing of rain events commonly results in mid-season droughts of different duration (Mulroy & Rundel, 1977). Moreover, climate change is altering the timing, length, and variability of the wet growing season in Mediterranean climates (Dong, Leung, Lu, et al., 2019; Dong, Leung, Lu, et al., 2019; Gordo & Sanz, 2009; Luković et al., 2021; Peñuelas et al., 2002; Swain et al., 2018), with potential ecological and evolutionary impacts on plant populations. For example, in California the onset of the rainy season is becoming more variable (Swain et al., 2018) and the average onset date has shifted later, from October 1 in 1960–1989 to October 28 over the last 30 years (Luković et al., 2021). This delay in rainfall onset may reduce plant population fitness (Gremer et al., 2020; Levine et al., 2011; Olliff-Yang & Ackerly, 2021). It is therefore important to understand how changes in seasonal rainfall timing will affect plant population fitness in Mediterranean ecosystems and how these effects depend upon variation in seed dormancy and flowering phenology.

The model annual plant *Arabidopsis thaliana* (Brassicaceae) is an excellent system for addressing these questions. This cosmopolitan species spans a broad native climate range across Eurasia and Africa, and is widely naturalized in North America. It is the only *Arabidopsis* species found in Mediterranean climates (Hoffmann, 2005). The species has evolved while expanding and contracting across the landscape with Pleistocene glacial cycles (Durvasula et al., 2017; Hsu et al., 2019; Lee et al., 2017; Toledo et al., 2020), and Mediterranean glacial refugia in the Iberian Peninsula and North Africa are hot spots for ancient "relict" lineages (Durvasula et al., 2017; Toledo et al., 2020). Adaptation to climate in space and time likely involved adaptive evolution of seed dormancy and flowering timing, resulting in geographic clines in these traits across the species range (Debieu et al., 2013; Montesinos-Navarro et al., 2012; Picó, 2012; Stinchcombe et al., 2004; Vidigal et al., 2016). Mediterranean

genotypes flower relatively early, allowing them to complete their life cycle before the onset of harsh summer conditions (Exposito-Alonso, 2020; Exposito-Alonso et al., 2018; Montesinos et al., 2009; Vidigal et al., 2016). They also typically produce seeds with high levels of primary dormancy, which delays germination until the onset of the autumn growing season (Manzano-Piedras et al., 2014; Méndez-Vigo et al., 2011; Montesinos et al., 2009; Montesinos-Navarro et al., 2012; Postma & Agren, 2016; Vidigal et al., 2016; Zacchello et al., 2020). Field experiments suggest that early flowering and high primary dormancy may be adaptive in Mediterranean climates (Ågren & Schemske, 2012; Postma & Agren, 2016).

The genetic basis of variation in seed dormancy and flowering time has been well studied in *A. thaliana* and several major candidate genes have been identified (Bloomer & Dean, 2017; Chiang et al., 2011; Méndez-Vigo et al., 2011; Postma & Ågren, 2015; Postma & Agren, 2016; Vidigal et al., 2016; Zacchello et al., 2020). Here, we focus on effects of well-known functional variants at two important candidate genes: *Delay of Germination 1* (*DOG1*) and *FRIGIDA* (*FRI*). *DOG1* plays an important role in controlling dormancy levels (Bentsink et al., 2010; Footitt et al., 2015; Martínez-Berdeja et al., 2020) and has a strong influence on the seasonal timing of germination in the field (Chiang et al., 2011; Postma & Ågren, 2015; Postma & Agren, 2016; Taylor et al., 2017); it may directly or indirectly influence flowering time (Chiang et al., 2013; Huo et al., 2016). *FRI* confers a vernalization requirement for flowering, and loss-of-function mutations at this locus are strongly associated with early flowering (Bloomer & Dean, 2017; Caicedo et al., 2004; Fournier-Level et al., 2022; Lempe et al., 2005; Méndez-Vigo et al., 2011; Michaels et al., 2003; Scarcelli et al., 2007; Shindo et al., 2005). However, in natural environments, the phenotypic effects of *FRI* depend strongly on germination timing (Burghardt et al., 2015; Wilczek et al., 2009). Near isogenic or recombinant inbred lines (NILs or RILs) contrasting allelic variants of *DOG1* and *FRI* have proved to be a useful tool for experimentally manipulating life history phenotypes to test ecological and evolutionary hypotheses (Chiang et al., 2013; Taylor et al., 2017, 2019; Wilczek et al., 2009). In particular, multilocus RILs or NILs segregating functional variants at both loci provide factorial combinations of dormancy strength and flowering time for elucidation of their interacting effects in different seasonal environments (Taylor et al., 2017).

To investigate how changes in seasonal rainfall will affect phenology and fitness of *A. thaliana* in Mediterranean climates, we performed two manipulative experiments and synthesized the results to project population phenology and fitness in different climate scenarios. First, we experimentally manipulated the date of rainfall onset and soil texture to determine their effects on germination phenology. Second, we experimentally manipulated germination date and growing season length to measure their cascading effects on flowering time and fitness. Within each experiment, we also manipulated seed dormancy and flowering time using multilocus NILs segregating factorial combinations of *DOG1* and *FRI* alleles. We then synthesized germination phenology data from the first experiment with fitness functions from the second experiment to project population fitness of different genotypes under different seasonal rainfall regimes. We addressed the following questions: (1) How does germination phenology respond to variation in seasonal rainfall timing, and how does this response depend upon soil substrate and genotype? (2) How does seasonal germination timing affect flowering phenology and fitness of different genotypes? Specifically, do highly dormant and/or early flowering genotypes have an advantage in a Mediterranean climate? (3) How will changing seasonal precipitation patterns affect population fitness for different genotypes in a Mediterranean climate?

2 | MATERIALS AND METHODS

2.1 | Experiments

We performed the two experiments in a screen house in Davis, California, with a clear plastic roof (Dynaglas SolarSoft 90) that excluded natural precipitation (Figure S1 in Supporting Information). Thus, seasonal water inputs could be controlled while plants experienced natural ambient temperature and light. In the first experiment we simulated different rainfall onset dates by sowing seeds of four NILs on watered sand and soil substrates at monthly intervals, and recorded germination phenology of fresh and after-ripened seeds. In the second experiment, we manipulated germination date by exposing seeds of four NILs to inductive conditions at monthly intervals, planted the seedlings into different season length treatments, and recorded flowering time, survival, and fruit production for each combination of genotype, germination date, and season length. From this experiment, we estimated fitness as a function of germination date for each genotype and season length. We then used these fitness functions to predict fitness of individual seeds in the first experiment based on their observed germination dates and used these predictions to project population fitness for each genotype under different seasonal rainfall scenarios (Figure 1).

2.2 | Genotypes and seed sources

We used four different *A. thaliana* genotypes: (1) Columbia (Col-0), henceforth "Col", an early flowering ecotype with relatively low seed dormancy. (2) *ColFRI-Sf2*, henceforth "*FRI*", a NIL with a functional *FRI* allele from the late flowering *Arabidopsis* ecotype San Feliu-2 (Sf-2) introgressed into Col-0 (Lee & Amasino, 1995). The functional *FRI* allele confers a vernalization requirement for flowering in this NIL, resulting in delayed flowering without vernalization in standard growth chamber conditions (Lee & Amasino, 1995). However, the strength of this allelic effect is reduced by fluctuating temperatures (Burghardt et al., 2016) and differs across seasons and environments in the field (Taylor et al., 2019; Wilczek et al., 2009). (3) *ColDOG1-Cvi*, henceforth "*DOG*", a NIL with a *DOG1* allele from the high dormancy *Arabidopsis* ecotype Cape Verde Islands (Cvi) introgressed into Col-0 for five backcross generations. The *DOG1-Cvi* allele confers high primary dormancy and a long after-ripening requirement

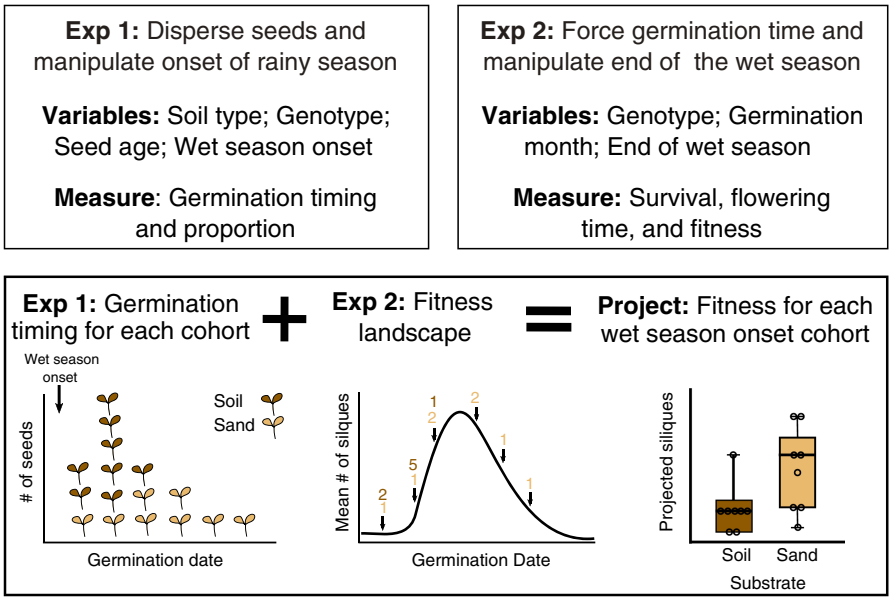

FIGURE 1 Schematic diagram showing how germination phenology data from Experiment 1 was integrated with fitness functions from Experiment 2 to estimate projected fitness.

for germination (Bentsink et al., 2010). (4) a double NIL, henceforth "*FRIDOG*", derived by crossing the *FRI* and *DOG1* NILs and screening F2s for double homozygotes of the introgressed *FRI* and *DOG1* alleles, followed by selfing for two generations.

Three maternal plants of each genotype were planted for seed bulking in the screen house in April 2014 and again in November 2015. Experimental seeds were harvested from these parents in June 2014, and mid March and late May 2015, respectively. Harvested seeds were stored dry in plastic tubes with a mesh top held up in soil in pots in the screen house to after-ripen under ambient seasonal temperatures until they were sown into the two experiments.

2.3 | Experiment 1: Manipulating rain onset timing to assess germination phenology

We conducted monthly germination trials from Nov. 2014 to Oct. 2015 with seeds of *Col*, *FRI*, *DOG*, and *FRIDOG* on soil and sand substrates (Figure S2). From November 2014 to March 2015 we planted naturally after-ripened seeds produced in spring 2014. Starting in April 2015, we planted fresh seeds produced in spring 2015 as well as after-ripened seeds from 2014 in separate replicated pots within each genotype × soil × planting date combination. This allowed us to contrast how freshly dispersed seeds and seeds from the previous year's seed bank responded to spring and summer rains as they after-ripened over time. Rainfall onset treatments from September to January simulated the wide interannual range of rainfall onset dates observed in California since 1960 (Luković et al., 2021).

In all, there were 19 planting date × seed age combinations ("cohorts") on both soil and sand. Each genotype × soil × cohort combination was replicated in at least 10 pots, with additional replicates in December 2014 (18 per genotype-treatment combination) and September–November 2015 (10–12 per combination). On planting dates from April 2015 on, the 10 pots included at least 5 replicates each of fresh and after-ripened seeds. In all there were 1816 experimental pots, of which 1689 were used in the analysis after excluding pots that suffered mishaps or irretrievable errors in data collection.

At each monthly simulated rain onset we scattered 20 seeds of each genotype into 6.4 cm diameter by 17.8 cm depth containers (Stuewe & Sons, Inc., Deepot Cell) filled with either (1) saturated potting soil (1:1:1 sand:compost:peat moss with 1.49 kg/m^3 of Dolomite) or (2) a thin layer (~5 mm) of sand on top of potting soil, such that the seeds were surrounded by sand. Replicate pots were randomly assigned to positions in adjacent 5 × 5 racks on one of two benches in the screen house and randomly moved across benches throughout the experiment. Watering for each cohort started at the beginning of the month and continued until each pot reached 100% germination. Twice a week pot racks were top watered twice a day with a water mister, and bottom watered in plastic tubs to saturate the soil with 1/3 strength fertilizer (GrowMore 4-18-38 modified to N:P:K at 60:29:60 ppm). Germination was recorded daily for 2 weeks and subsequently every 2 days until 100% germination was scored in a pot or until the experiment ended in March 2016. Germinated seedlings were removed from pots, so their growth did not disrupt the germination timing of other seeds. For each pot, we calculated the median and range of days to germination (DTG) and the proportion of planted seeds that germinated.

2.4 | Experiment 2: Manipulating germination timing and season length to assess effects on flowering phenology and fitness

To assess the effects of seasonal germination timing on flowering phenology and fitness, we induced germination by exposing seeds of the four NILs to inductive cold stratification at monthly intervals from November 2014 to May 2015, and in September 2015

and October 2015, creating different seasonal germination cohorts (Figure S3). To examine the effects of growing season length on fitness, we also manipulated the length of the growing season with watering treatments ending in March, April and May for each planting in the 2014–2015 growing season, and in January, February, March and April for the 2015–2016 growing season. This gave a total of 24 watering treatments. We also report methods and results for six additional treatments simulating mid-season droughts in the Appendix.

For each planting, seeds of each genotype were stratified in agar at 4°C for 3 days to break dormancy before sowing in well-watered potting soil in 6.4 cm diameter by 17.8 cm depth pots in 5 × 5 container racks in the screen house. These pots were hand misted twice a day for the first week after sowing to promote germination. After seedlings emerged, the pots were moved to preassigned random positions in 5 × 5 treatment racks on each bench. From the first planting cohort start date to the assigned end date, plants were bottom watered up to twice a week as needed to saturate the soil with 1/3 strength fertilizer (GrowMore 4-18-38, Grow More Inc, Gardena, CA; modified to N:P:K at 60:29:60 ppm). Racks were placed in plastic tubs for bottom watering.

In the 2014–2015 season, each end date treatment was applied to an array of four adjacent racks on each bench. Three end date treatment arrays were arranged in random order on each bench, and genotypes and watering onsets were randomized within each array. In the 2015–2016 season, genotypes, watering onsets, and end dates were randomized across three racks on each bench, and on their assigned rainfall end date plants were moved to random positions on an adjacent unwatered rack. In all plantings, there were three replicates of each genotype per watering treatment per bench for a total of 1152 experimental plants (4 genotypes × 24 watering treatments × 4 benches × 3 replicates).

We recorded days to flowering (DTF), when the first flower appeared, every other day and harvested plants at senescence. After harvest, we counted siliques as a proxy for fitness.

2.5 | Inferring projected fitness

We combined results from the germination and fitness experiments described above to estimate the projected fitness of seeds of the four NILs under different rainfall scenarios in two substrates (sand and soil). We estimated fitness as a function of germination date for each genotype and season length by linearly interpolating from observed fruit counts in each experimental cohort. We interpolated fitness functions using *approxfun* in R. This generates a piecewise linear function that predicts fruit counts for any date (Figure S4). For biological realism and simplicity this estimate did not include fitness data from the three watering treatments ending in January and February. Because plants in the September and October cohorts were completely senesced by March and April, respectively, we assumed that their fitness at senescence would be the same if rainfall ended at later dates in April or May. We assumed that seeds germinating during summer drought from June through August would not survive and would have zero fitness. Fitness functions were then used to project fitness from observed germination dates for individual seeds in different environments in the germination phenology trials. To simulate 1 year of an annual plant life-cycle, we used seeds with dormancy levels similar to those of annual plants in the field. We assumed that the year started in April with the dispersal of fresh seeds (represented by seeds collected in spring 2015), which after-ripened over the summer (April–October 2015 planting cohorts). To project fitness of germination cohorts from November–March we used germination data from fully after-ripened seeds (collected in spring 2014 and planted in November 2014–March 2015). These projections allowed us to predict the fitness of each genotype on each soil type in different rainfall timing scenarios following seed dispersal in spring.

2.6 | Measurement of environmental variables during experiments

To assess the accumulation of photothermal time and exposure to vernalizing temperatures for plants in the different seasonal cohorts in Experiment 2, we measured temperature at 10 minute intervals from October 2014 to July 2015 and from September 2015 to July 2016 with an Onset HOBO Pendant temperature logger (Figure S5). From these readings we extracted hourly average temperatures and used them to calculate hourly accumulation of photothermal units (PTUs) from germination to bolting and flowering, with a base temperature of 3°C (Granier et al., 2002; Wilczek et al., 2009) (Figure S6). This metric allowed us to compare time to phenological events in common developmental units across cohorts in different seasonal environments (Fournier-Level et al., 2013, 2022; Wilczek et al., 2009). Hourly temperature data were also used to calculate accumulation of vernalization units using the vernalization effectiveness function of Wilczek et al. (2009; Figure S7).

2.7 | Statistical analyses

The central goal of this study was to investigate how the timing of rainfall onset affected phenology and fitness of different genotypes, and how those effects depended on other ecological factors (soil, rainy season length). To approach these questions, we first conducted a full factorial ANOVA (lm function in R) with all relevant treatment factors for each variable of interest. When higher order interactions from the full models were significant, we then ran additional ANOVA models within treatment factors to examine relevant contrasts within these interactions, using Bonferroni corrections for multiple comparisons as appropriate. For all analyses we checked for violations of statistical assumptions, including normality and homoscedasticity of residuals. We compared natural and log-transformed values with a constant offset and for each variable we used the transformation that gave the best distribution of model residuals.

2.7.1 | Germination timing and proportion

We examined the effects of rainfall onset date, genotype, seed age and soil type on median and range of days to germination in two ways. First, for the 7 months where we planted seeds from both 2014 and 2015, we conducted a full factorial ANOVA with 5 factors: substrate (with two levels: sand and soil), genotypes at *FRI* and *DOG1* (two levels each: functional and null), wet-season onset (with 7 levels), and seed age (with two levels: 2014 and 2015). After detecting significant higher order interactions with rainfall onset, we tested for effects of substrate, *FRI*, *DOG1*, seed age, and their interactions with ANOVA within each rainfall onset treatment. Second, to analyse the full set of 19 plantings we combined wet-season onset and seed age into cohorts (with 19 levels) and used ANOVA to test for effects and interactions of substrate, *FRI*, *DOG1*, cohort, and their interactions. Again, significant higher order interactions involving cohort led us to test for effects of substrate, *FRI* and *DOG1*, and their interactions within each cohort. For all these analyses, median and range of days to germination were both square-root transformed.

Germination proportions within cohort were analysed using a GLM model with binomial family and logit link (glm function in R) to explore the effect of substrate (with two levels: soil and sand), *FRI* and *DOG1* (two levels each plus their interaction). We assessed the importance of each term by quantifying the change in scaled deviance (twice the difference in log-likehood from a saturated model divided by the residual degrees of freedom) and comparing to a χ^2 distribution with degrees of freedom equal to the change in number of parameters.

2.7.2 | Flowering phenology from germination timing manipulation experiment

To test for effects of germination timing and genotype on flowering phenology, we used factorial ANOVA models with log transformed days to flowering (DTF) and PTUs from germination to flowering as dependent variables. The full models included planting cohort, *FRI* genotype, and *DOG1* genotype as main effects, with all their interactions and bench as a block effect.

2.7.3 | Fitness from germination time manipulation experiment

We examined the effects of germination timing, rainfall end date, and genotype on fitness in an ANOVA model with germination cohort, rainfall end date, *FRI*, and *DOG1* as main effects, all interactions, and bench as a block effect. Note that the experiment was not fully factorial because not all planting cohorts were planted early enough or lived long enough for all end dates to be relevant. We included zeros in the fitness data for non-survivors. Generalized linear models for count data (Poisson, quasipoisson or Negative-binomial glms fit with the glm or glm.nb function in R, respectively) did not converge because of highly variable overdispersion across treatments. Therefore, to test the joint effects of all factors across the whole experiment we used a Gaussian linear model (lm function in R) on log-transformed count data after adding a constant of 10 to standardize the variance as well as possible. In order to dissect the effects of germination date and growing season length on fitness, we also ran an ANOVA with planting cohort, number of months of growth, *FRI*, and *DOG1* as main effects, all interactions, and bench as a block effect.

3 | RESULTS

3.1 | Effects of rainfall timing, genotype and soil substrate on seasonal germination phenology

Our experimental manipulation of rainfall timing revealed strong effects of substrate and seasonal cohort on germination phenology, as well as changing seasonal effects of substrate, seed age, and genotype across the year (higher order interactions in Table 1, Tables S1 and S2; Figure 2). We explored these seasonal changes with factorial ANOVA within rainfall onset dates (Table S3) and cohorts (Table 1). Seed germination timing was consistently later and frequently more variable on sand than on soil throughout the year (Figure 1, Table 1). Notably, many seeds in the spring and summer cohorts on sand delayed germination until the typical onset of the fall rainy season (Figure 1). However, effects of seed age and genotype on germination timing depended strongly on rainfall onset date. Fresh seeds collected in spring 2015 germinated later than after-ripened seeds from 2014 in rainfall onset treatments from April through September 2015, but this effect disappeared by October 2015, indicating that all seeds were fully after-ripened by fall (Figure 1, Table S3). The strong *DOG1* allele significantly delayed germination in fresh 2015 seeds immediately exposed to rainfall in April and June. This effect was more pronounced on soil, and in a *FRI* null background. However, *DOG1* effects on germination timing disappeared in subsequent rainfall onset treatments as seeds after-ripened (Figure 1, Table 1). Although *DOG1* has been reported to contribute to variability of *A. thaliana* germination time (Abley et al., 2021), we did not detect an effect on the range of days to germination in this experiment.

The cumulative proportion of seeds germinating in each cohort followed similar patterns (Figure S8, Table S4). Overall germination was generally lower on sand than on soil in most cohorts, except for the cool months of November, December and January. Germination was also lower in fresh seeds harbouring a strong *DOG1* allele in the April rain onset treatment (Figure S8, Table S4). In aged 2014 seeds, we also observed low germination of functional *FRI* genotypes in the September and October 2015 rain onset treatments, especially in a strong *DOG1* background (Figure S8, Table S4).

TABLE 1 ANOVA table for the effect of substrate, *FRI*, *DOG1*, *FRI*×*DOG1*, wet-season cohort, and all their interactions for squared root transformed median (a) and range (b) days to germination per pot. Table cells contain *F*-values, each term of the model has $df = 1$, and bold font indicates significant values. We used Bonferroni correction for multiple comparisons among 19 cohorts tested at 0.05 significance (*p*-values <0.0026).

Cohort	*FRI*	*DOG1*	*FRI*×*DOG1*	Substrate	*FRI*×substrate	*DOG1*×substrate	*FRI*×*DOG1*×substrate	Residuals
(a)								
2014 seeds								
November	0.93	0.04	0.33	**191.51**	0.31	0.02	0.006	72
December	0.08	5.13	1.05	3.14	0.03	0.27	1.25	136
January	0.11	1.64	0.08	**29.29**	0.74	0.44	0.30	72
February	0.11	0.58	4.24	**26.79**	0.76	0.22	5.95	72
March	0.05	0.03	1.31	**680.95**	0.21	0.005	1.69	72
April	0.63	1.26	3.64	**126.78**	1.37	0.62	2.58	72
May	1.72	0.31	0.37	**501.15**	1.48	0.37	1.17	72
June	1.86	0.40	0.45	**7.64**	0.007	0.34	0.39	72
July	3.74	0.05	2.12	**177.76**	0.01	0.70	1.35	72
August	3.56	0.74	0.17	**410.90**	2.61	0.06	3.08	65
September	1.83	0.08	0.19	**529.73**	2.33	0.08	0.74	72
October	0.0004	2.07	1.04	**199.05**	0.48	1.09	0.009	76
2015 seeds								
April	4.10	**95.74**	**75.47**	**147.24**	0.48	**43.59**	**44.51**	72
May	0.03	4.67	5.29	**765.49**	0.16	0.12	0.17	72
June	0.002	**17.04**	**15.99**	**29.17**	3.84	0.44	4.92	71
July	1.07	0.06	4.30	**858.88**	2.86	0.01	0.68	72
August	0.51	0.0005	1.51	**431.19**	2.43	0.04	0.91	70
September	1.82	2.48	8.53	**547.78**	0.06	0.03	8.38	102
October	0.38	0.28	0.58	**250.34**	0.04	0.08	0.23	88
(b)								
2014 seeds								
November	1.07	0.12	0.10	**29.43**	0.0003	0.0001	1.15	72
December	1.12	0.92	0.42	1.83	0.57	0.41	0.02	136
January	0.22	0.76	1.58	1.89	0.24	3.37	0.03	72
February	0.77	0.09	6.16	**46.66**	0.05	0.008	7.62	72
March	1.79	0.008	0.59	**276.13**	0.02	0.44	0.57	72
April	0.05	3.35	3.40	**77.58**	0.38	0.36	4.28	72
May	5.21	0.15	2.75	**88.85**	3.03	0.41	0.26	72
June	0.28	2.91	0.03	**12.56**	1.17	0.60	1.49	72
July	3.74	1.47	0.29	**146.55**	0.31	0.03	1.34	72
August	0.43	2.23	0.77	**46.49**	0.11	3.79	0.46	65
September	5.12	0.02	0.75	**67.22**	6.84	0.60	1.18	72
October	2.45	0.12	0.04	**68.80**	0.09	0.03	5.77	76
2015 seeds								
April	0.89	0.06	0.82	5.20	1.78	8.44	**11.58**	72
May	2.56	0.47	0.23	8.26	0.39	1.33	0.08	72
June	0.03	1.25	5.33	1.10	2.24	0.006	1.75	71
July	0.07	0.03	0.27	**46.27**	0.45	0.06	0.49	72
August	0.02	0.01	6.72	**28.96**	0.92	3.00	1.20	70
September	0.44	0.34	0.0001	**105.68**	0.90	0.01	0.07	102
October	0.04	0.06	2.67	**174.73**	1.27	2.13	0.32	88

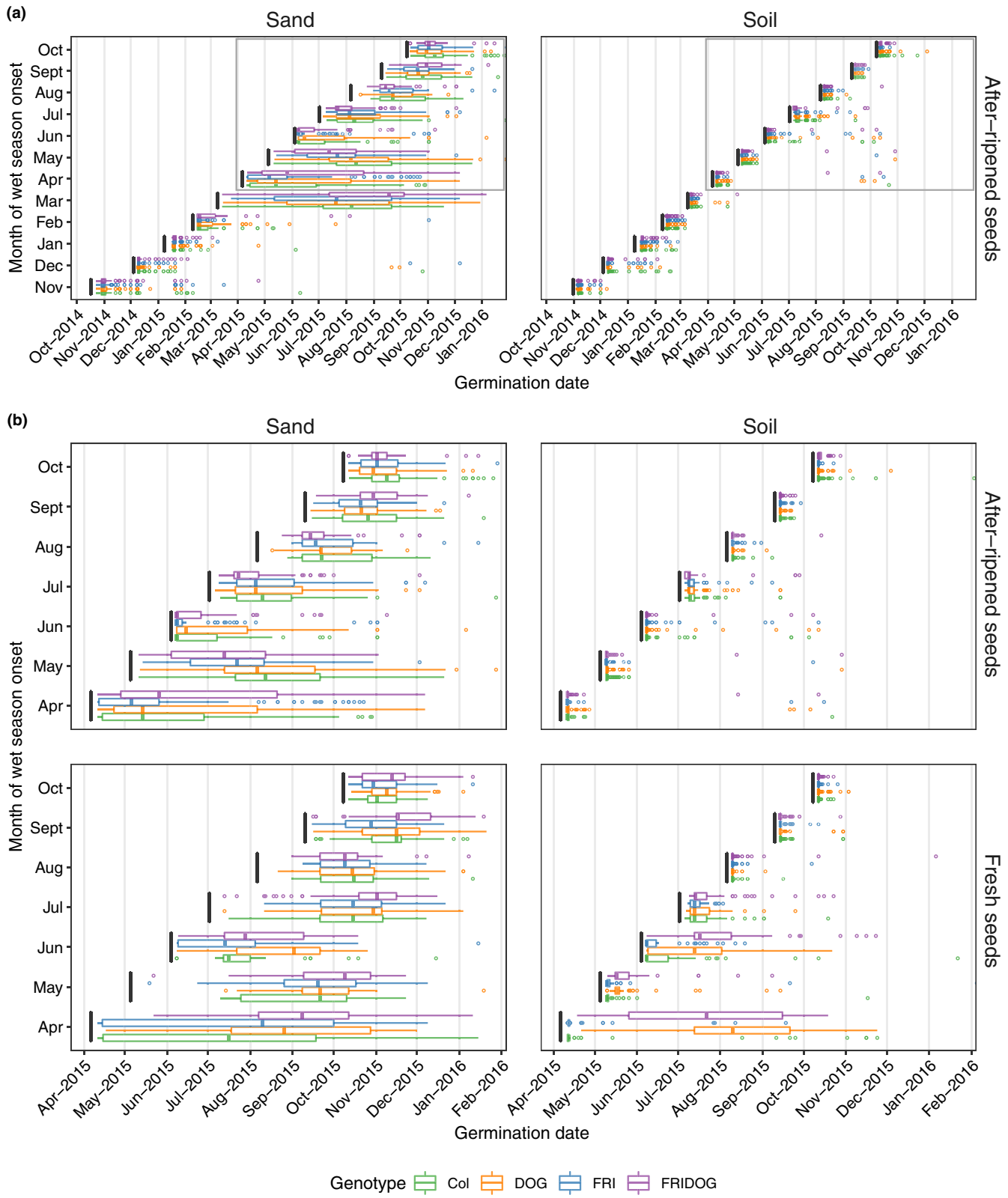

FIGURE 2 Boxplots showing the effects of rainfall onset timing on seed germination phenology for (a) all 2014 after-ripened seeds in sand and soil and (b) cohorts with both 2015 fresh and 2014 after-ripened seeds in sand and soil. Note that the after-ripened seed cohorts enclosed by a grey square on the upper part of (a) are enlarged for comparison with fresh seeds in the bottom part of (b). Vertical lines in the boxplot extend 1.5× the interquartile range, and values outside this range are denoted with circles.

3.2 | Effects of germination timing and genotype on flowering phenology

Seasonal flowering phenology depended strongly on germination timing and *FRI* genotype (Figure 2, Figure S9), consistent with prior model predictions (Burghardt et al., 2015; Wilczek et al., 2009, 2010). Successively later germination cohorts generally flowered successively later in the season, despite a concurrent decrease in the number of days to flowering with exposure to increasing day lengths in spring (Figure 2, Figure S9). Functional *FRI* alleles significantly delayed flowering, but the magnitude of the effect depended on the planting cohort and *DOG1* background (significant *FRI*×*DOG1*, and *FRI*×planting cohort interactions, Figure 3, Figure S9, Table 2). Functional *FRI* genotypes were most delayed in early fall (September and October) and spring (March, April and May) cohorts, when plants received less exposure to vernalizing temperatures (Figure 2, Figures S7 and S9). This effect was somewhat stronger in a weak *DOG1* background (Figure 2, Figure S9). The effect of *FRI* was most dramatic in the September cohort, where plants with null alleles flowered rapidly without vernalization in fall but *FRI* genotypes exhibited a winter annual life history.

3.3 | Effect of precipitation timing and genotype on individual fitness

The onset and duration of precipitation strongly affected fitness (silique number). Fitness peaked in the October and November fall germination cohorts, and decreased monotonically with later germination (Figure 4, Figure S4). Silique number also increased significantly with later end of the rainy season (Figure 4, Table 3). *FRI* functionality had a significant overall negative effect on fitness, but the magnitude varied among planting cohorts (Figure 4, Table 3, Figure S10). Plants with functional *FRI* alleles had dramatically lower fitness than those with nonfunctional alleles in the September planting cohort, possibly because *FRI* functional genotypes delayed flowering until stressful winter conditions had passed whereas nonfunctional genotypes flowered rapidly in response to inductive daylength in early fall (Figure 4, Figures S7 and S10). Functional *FRI* alleles were also disadvantageous for plants in the spring planting, possibly because early flowering genotypes had more time to produce seeds before the onset of summer drought (Figure 4, Figure S10). However, *FRI* had little effect on fitness in late autumn and winter cohorts, in which exposure to vernalizing temperatures overrode the delaying effect of the functional allele (Figures 3 and 4 and Figure S10). Fitness declined dramatically with decreasing number of months in the growing season, but the effect of germination timing was still significant after accounting for season length (Table S5, Figure S10).

3.4 | Population fitness projections

We projected population fitness of each genotype on soil and sand under different rainfall scenarios by combining individual

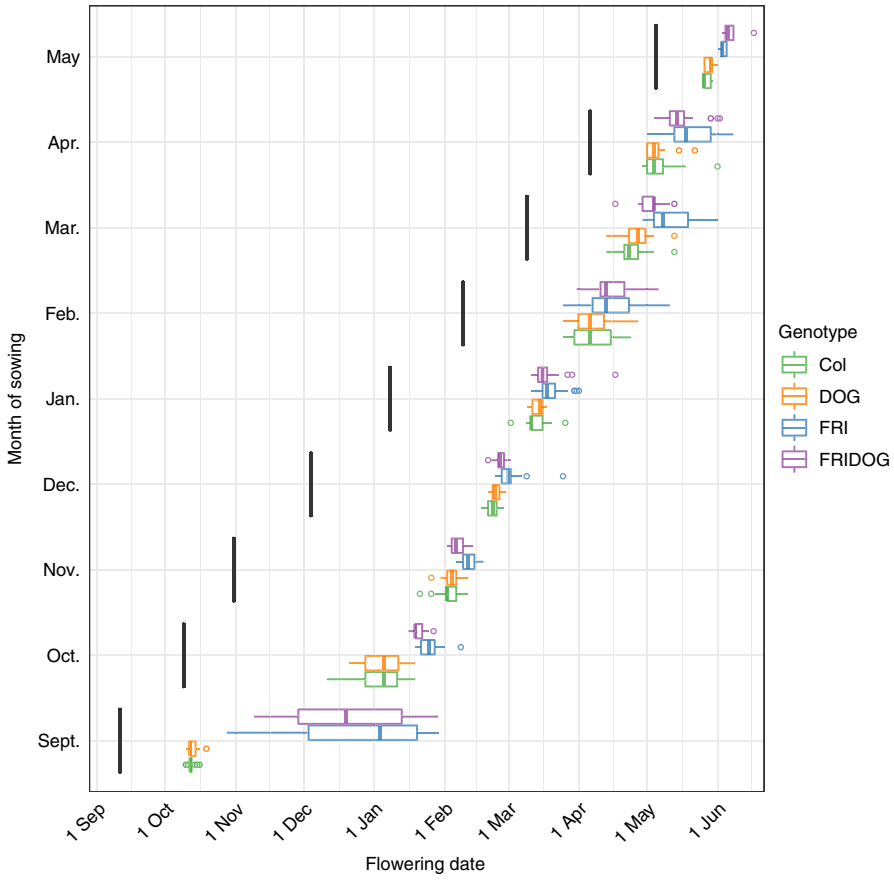

FIGURE 3 Boxplots for flowering time for each planting cohort from germination to flowering date. Genotype is indicated by different colours. Black vertical lines indicate the germination time for each planting. Horizontal lines extend 1.5× the interquartile range, and values outside this range are denoted with circles.

Source	df	DTF		PTUs	
		F	*p*	*F*	*p*
FRI	1	1653.44	**<0.001**	1000.13	**<0.001**
DOG1	1	3.28	0.07	4.56	0.033
FRI×*DOG1*	1	11.55	**0.0007**	15.84	**<0. 001**
Planting cohort	8	1066.98	**<0.001**	197.18	**<0.001**
Bench	3	2.78	0.04	5.50	**0.0009**
FRI×planting cohort	8	258.13	**<0.001**	96.72	**<0.001**
DOG1×planting cohort	8	1.26	0.26	1.78	0.077
FRI×*DOG1*×planting cohort	8	1.44	0.18	2.12	0.032
Error	1021				

TABLE 2 ANOVA table for the effect of *FRI*, *DOG1*, *FRI*×*DOG1*, planting cohort and their interaction, and the effect of bench, on log transformed days to flowering (DTF) and PTUs from germination to flowering. We used Bonferroni correction for multiple comparisons among significant *p*-values tested at 0.05 significance (*p–values* < 0.00833) (bold).

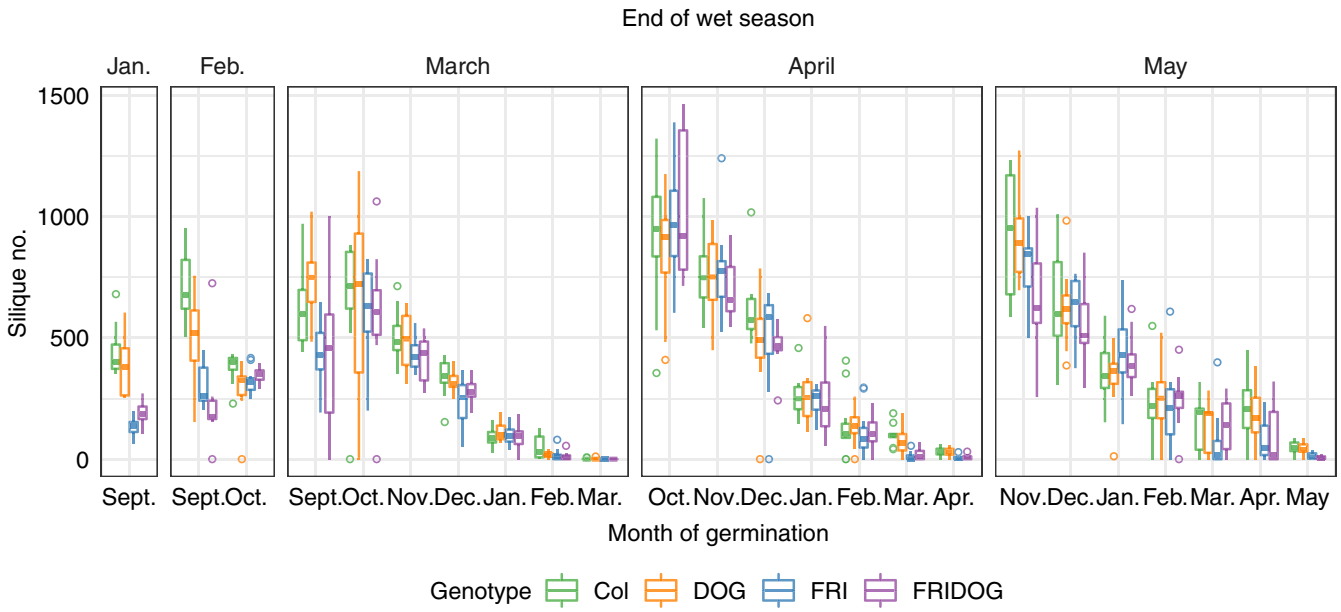

FIGURE 4 Boxplots for total silique number in each planting cohort. Facets indicate the end of the growing season for each planting cohort; genotypes are indicated by different colours. November to May planting cohort end in March and April 2015, and September and October planting cohort end in March and April 2016.

observations of seed germination timing in different rainfall onset treatments (Experiment 1) with empirical fitness functions relating individual silique production to germination time and season length (Experiment 2, Figure 1). This analysis revealed strong effects of rainfall onset on population fitness, which differed strikingly between soil and sand substrates (Figure 5). On soil, where germination timing was earlier and more synchronous for most rainfall onset cohorts (Figure 2, Table 1), our simulations predicted high reproductive success only if precipitation onset occurred during a relatively narrow window from September to November (Figure 5). Rainfall onsets outside of this fall window resulted in low projected fitness due to germination in unfavourable seasonal conditions. On sand, however, slow and variable germination timing buffered the fitness impact of rainfall onset in spring or summer. In these conditions, many seeds delayed germination until favourable fall conditions, resulting in higher population fitness for these cohorts on sand. Even though maximal fitness under favourable autumn rainfall onset conditions tended to be lower on sand than on soil, delayed germination on sand allowed seeds from spring and summer planting cohorts to attain relatively high reproductive success across a broader range of precipitation onset dates. On both sand and soil the width of the favourable precipitation onset window was narrower if the wet season ended in March and wider if the wet season lasted until May. Rainfall onset in winter resulted in low fitness on both substrates.

The projected population fitness effects of *DOG* and *FRI* genotypes were subtler and differed among seasonal cohorts and soil substrates (Figure 5, Figures S11 and S12). Highly dormant *DOG1* alleles conferred higher projected population fitness in late spring and summer cohorts, especially in soil where they prevented lethal immediate germination, but were not under strong selection in other

TABLE 3 ANOVA table for the effect of *FRI*, *DOG1*, *FRI*×*DOG1*, planting cohort, wet-season end and all the interactions, and the effect of bench, on log transformed silique number + 10 data in the non-drought treatments. We used Bonferroni correction for multiple comparisons among significant *p*-values tested at 0.05 significance (*p-values*< 0.00833) (bold).

Source	*df*	*F*	*p*
FRI	1	88.07	**<0.001**
DOG1	1	3.15	0.077
FRI×*DOG1*	1	0.05	0.820
Planting cohort	8	344.30	**<0.001**
Wet-season end	4	124.65	**<0.001**
Bench	3	2.09	0.10
FRI×planting cohort	8	10.28	**0.001**
DOG1×planting cohort	8	0.24	0.982
FRI×*DOG1*×planting cohort	8	1.31	0.236
FRI×wet-season end	4	0.48	0.748
DOG1×wet-season end	4	0.84	0.500
FRI×*DOG1*×wet-season end	4	1.55	0.185
Planting cohort×wet-season end	11	10.35	**<0.001**
FRI×planting cohort×wet-season end	11	2.21	0.012
DOG1×planting cohort×wet-season end	11	0.64	0.791
FRI×*DOG1*×planting cohort×wet-season end	11	0.86	0.576
Error	1048		

cohorts where seeds after-ripened before the onset of precipitation (Figure 5). *FRI* null alleles tended to confer higher projected fitness in summer and early fall cohorts, particularly on sand with precipitation ending in May (Figure 5).

4 | DISCUSSION

In Mediterranean climates, the growing season depends on the timing of seasonal precipitation, which is highly variable among years (Lázaro et al., 2001; Luković et al., 2021; Rundel et al., 2016; Swain et al., 2018) and already changing in response to ongoing climate change (Gordo & Sanz, 2009; Luković et al., 2021; Peñuelas et al., 2002; Swain et al., 2018). It is therefore important to ask how changing rainfall patterns will affect the phenology and fitness of Mediterranean annual plant populations. Our experiments revealed strong effects of rainfall onset timing and soil substrate on germination phenology of *A. thaliana*, which in turn influenced flowering phenology and fitness. Precipitation timing also determined the phenotypic expression of allelic variation in seed dormancy and flowering time genes. By integrating our experimental germination phenology data with empirical fitness functions, we could project the consequences of changing precipitation timing for population fitness of different genotypes. The results suggest that ongoing changes in seasonal rainfall patterns may have significant impacts on *A. thaliana* population fitness in Mediterranean climates but these impacts will also depend on soil water holding capacity and genotype.

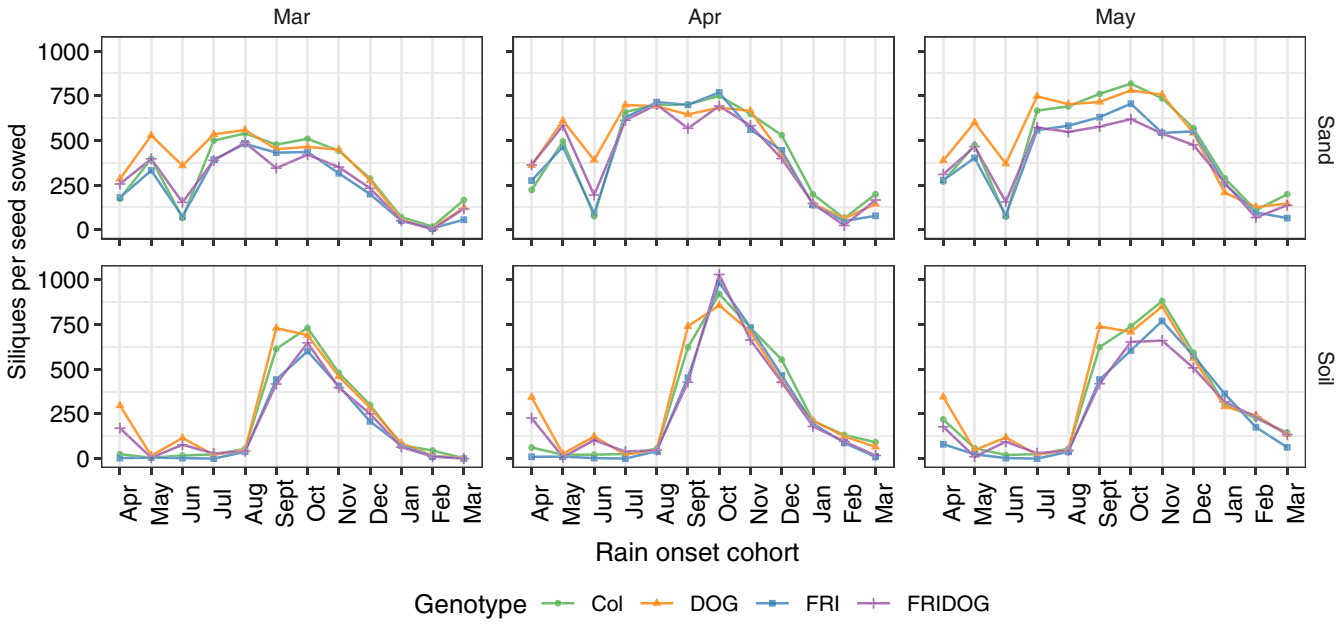

FIGURE 5 Median projected siliques per seed sowed for two substrates (sand–top panels, soil–bottom panels) and four genotypes (indicated in different colours).

4.1 | Drivers of seasonal germination phenology

Mediterranean annual seeds are typically dispersed in spring and remain dormant in the soil during the summer, thus avoiding seedling emergence during the hot summer months (Manzano-Piedras et al., 2014; Méndez-Vigo et al., 2011; Montesinos et al., 2009; Montesinos-Navarro et al., 2012; Postma & Agren, 2016; Vidigal et al., 2016; Zacchello et al., 2020). This dormancy is often lifted by warm after-ripening over the summer and/or brief chilling exposure, allowing germination in moist soils with the onset of cool autumn rains (Chiang et al., 2011; Kendall et al., 2011; Gremer et al., 2019, 2020). Thus, the expression of a winter-annual life cycle greatly depends on the seasonal timing of germination (Galloway, 2001; Gremer et al., 2019; Miryeganeh, 2020; Miryeganeh et al., 2018; Wilczek et al., 2009, 2010). Not surprisingly, our experimental manipulation of seasonal rainfall onset strongly influenced germination phenology and the proportion of seeds that germinated. As expected, the germination of after-ripened seeds closely tracked the timing of first rainfall in autumn and winter; later rainfall onset resulted in later germination. However, germination responses to seasonal rainfall timing depended strongly on soil texture. The observation of lower, slower, more variable germination on sand than on soil is likely due to the lower water holding capacity of sandy soil (Austin et al., 2004; Reynolds et al., 2004) which slowed the accumulation of sufficient hydrothermal time to germinate (Bradford & Somasco, 1994; Hu et al., 2015; Huang et al., 2016). Consequently, many seeds dispersed on sand in spring and summer delayed germination until the cooler temperatures of autumn, sampling a broader range of seasonal environments for seedling establishment.

The season-specific effects of *DOG1* suggest that this gene has a specific ecological function in Mediterranean climates with hot dry summers: preventing immediate germination of freshly dispersed seeds in response to spring and summer rainfall. Consequently, spring-dispersed seeds carrying a strong *DOG1* allele could express a winter annual life cycle. In contrast, seeds with the weak *DOG1* allele are more likely to germinate immediately and experience lethal summer conditions. Delaying germination until favourable autumn conditions would thus be advantageous in Mediterranean climates where spring germinating seedlings experience high mortality due to summer heat and drought. In fact, southern *A. thaliana* accessions exhibit higher *DOG1* expression and higher primary dormancy compared with northern accessions (Chiang et al., 2011; Kronholm et al., 2012; Vidigal et al., 2016), suggesting a role in climate adaptation. Moreover, strong *DOG1* alleles from an Italian population were associated with delayed germination in field experiments, resulting in higher fitness in the Mediterranean climate of Italy and lower fitness in Sweden (Postma & Agren, 2016, 2022; Zacchello et al., 2020).

4.2 | Effects of germination timing and drought on flowering phenology and fitness

Germination timing may have cascading effects on later life cycle events such as flowering (e.g. Burghardt et al., 2015; Donohue, 2002; Gremer et al., 2020; Miryeganeh et al., 2018), and can determine lifetime fitness (Burghardt et al., 2015; Donohue, 2002; Gremer et al., 2020; Olliff-Yang & Ackerly, 2021). In our manipulative experiments, later germination resulted in later flowering in spring, as reported for other California species (Gremer et al., 2020; Olliff-Yang & Ackerly, 2021). Germination timing also strongly affected plant fitness. Fecundity reached a maximum in the October germination cohort and declined in successive cohorts to extremely low levels in spring germinants, evidence of stabilizing and directional selection on germination time. This decline in fitness with later germination involves two factors: seasonal changes in the favorability of conditions at the time of emergence, and declining length of time for growth and reproduction before the end of the rainy season. Negative effects of later rainfall onset or germination timing on fitness have been reported for several other California species (Gremer et al., 2020; Levine et al., 2011; Olliff-Yang & Ackerly, 2021), with some exceptions (Levine et al., 2011). Zacchello et al. (2020) observed strong stabilizing selection on germination timing in an Italian population of *A. thaliana*, with maximum survival and fecundity in November germinants. All of these observations suggest that changes in the timing of rainfall onset in Mediterranean regions will have major impacts on fitness of annual plant populations.

Germination timing may also influence the expression of genetic variation in flowering time (Chiang et al., 2013; Taylor et al., 2017; Wilczek et al., 2009, 2010). We therefore asked how variation in seasonal rainfall in a Mediterranean climate would influence flowering phenology of genotypes carrying different combinations of weak and strong *FRI* and *DOG1* alleles. *FRI* in particular has been implicated in Mediterranean climate adaptation because at least 11 loss-of-function *FRI* alleles have been identified in Iberian accessions and *FRI* polymorphisms are significantly associated with winter temperatures in the site of origin (Méndez-Vigo et al., 2011, 2013; Sanchez-Bermejo et al., 2012). Our experiment revealed that the phenological effects of this functional polymorphism depend dramatically on the timing of germination, which mediates exposure to vernalization and inductive day length cues (Burghardt et al., 2015; Wilczek et al., 2009). The effects of *FRI* functionality were greatest in early autumn and spring cohorts, where non-functional genotypes flowered rapidly without vernalization exposure under inductive photoperiods. Notably, in the September cohort *FRI* functionality made the difference between rapid-cycling and winter annual life histories, consistent with the observations and model of Wilczek et al. (2009). The fecundity effects of *FRI* also depended upon germination timing; *FRI* functional alleles had low relative fecundity overall, but especially in the early autumn and late spring cohorts where phenology effects were strongest. Thus, selection favoured early flowering genotypes in our experiment, as we might expect if the frequent occurrence of early flowering in southern Mediterranean populations reflects climate adaptation (Exposito-Alonso, 2020; Exposito-Alonso et al., 2018; Montesinos et al., 2009; Vidigal et al., 2016) but the strength of this selection depended upon the timing of rainfall onset.

4.3 | How will changing seasonal precipitation patterns affect population fitness for different genotypes in a Mediterranean climate?

Mediterranean regions are already experiencing higher temperatures and ongoing changes in the amount and distribution of rainfall. These changes include a decrease in spring, summer and autumn rainfall (Christensen et al., 2007; Cook et al., 2015; Pausas, 2004), and more high-intensity rain events in the winter with longer drought periods between them (Easterling et al., 2000; Miranda et al., 2009; Wang et al., 2017). In California, the rainy season is coming later (Luković et al., 2021) and becoming narrower, with precipitation concentrated in the winter months (Dong, Leung, Lu, et al., 2019; Dong, Leung, Lu, et al., 2019; Luković et al., 2021). Seasonal precipitation is also becoming more variable among years in California (Swain et al., 2018; Wang et al., 2017). These changes are likely to affect plant population phenology (Llorens & Peñuelas, 2005, Lloret et al., 2004, Miranda et al., 2009; Peñuelas et al., 2004) and fitness (Gremer et al., 2020, Levine et al., 2011, Olliff-Yang & Ackerly, 2021; Torres-Martínez et al., 2017). In fact, our experiments revealed that changing precipitation patterns may have a substantial impact on the phenology and individual fitness of *A. thaliana* in Mediterranean climates with hot, dry summers.

Integrating our germination phenology observations with empirical fitness functions from our manipulation of germination timing to project population fitness under different rainfall scenarios led to several important insights:

4.3.1 | Later onset of autumn rains and shorter duration of the rainy season may have major negative impacts on annual plant population fitness in Mediterranean climates

This result is especially relevant for annual plant populations in California, where the average date of rainfall onset is currently 27.1 days later than in the 1960s (Luković et al., 2021), a delay that caused significant decreases in fecundity and population fitness in our study. Reports of fitness declines with later fall germination in other California species (Gremer et al., 2020; Levine et al., 2011; Olliff-Yang & Ackerly, 2021) suggest that later rainfall onset may have general negative effects on the fitness and viability of California plant populations. Our experiments also revealed significant negative fitness impacts from an earlier end to the rainy season, which has been observed recently in California (Luković et al., 2021). Thus, ongoing changes in seasonal rainfall timing are likely to reduce the viability of California annual plant populations.

4.3.2 | Slow, variable germination on sand buffers fitness impacts of variation in rainfall timing

Our projections revealed a striking role for soil texture in mediating population fitness responses to variation in seasonal rainfall. The manipulation of seasonal germination timing detected major fecundity costs for spring germinants. Non-dormant seeds dispersed on wet soil in spring often germinated immediately and incurred those fitness costs. Our projections therefore showed that rainfall events in late spring and summer would result in very low population fitness on soil, especially for low-dormancy genotypes, whereas rainfall onset in early fall would allow germination under optimal conditions and maximize population fitness. In contrast, germination on sand was much slower and highly variable, especially in the spring and summer rainfall treatments. Consequently, many seeds dispersed on sand in spring did not germinate until the favourable conditions of fall, thereby realizing high fecundity. On the other hand, some seeds in the optimal early fall rainfall treatment delayed germination until less favourable conditions in later fall and winter, incurring a fecundity cost. The net result was that projected population fitness was much higher on sand than on soil in spring and summer rainfall treatments, with a broader, slightly lower peak in the early autumn months. Paradoxically, the slow accumulation of hydrothermal time due to low water potential on sand turned out to buffer population fitness against the negative impacts of spring and summer rainfall. For *A. thaliana* populations on sandy soils, such buffering may serve as a form of bet hedging in the face of unpredictable rainfall timing.

4.3.3 | Seasonal selection favours high dormancy and early flowering genotypes in a Mediterranean climate

DOG1 also played an important role in buffering population fitness against spring and summer rainfall. Although *DOG1* had no direct effect on individual fecundity within any germination cohort, the strong *DOG1* allele delayed germination of fresh seeds, especially on soil, allowing some of them to germinate under more favourable early fall conditions and achieve higher fecundity. Consequently, strong *DOG1* alleles conferred higher projected population fitness than weak alleles when fresh seeds were exposed to spring rainfall. The projections of population fitness also reflected the observed seasonal variation in fecundity advantage for early flowering *FRI* null genotypes. Early flowering loss-of-function *FRI* alleles had higher projected fitness, particularly in spring, summer, and early autumn rainfall treatments. These results indicate that high-dormancy, early flowering genotypes are generally favoured by selection in a Mediterranean climate with hot, dry summers, although the intensity of selection may depend upon rainfall onset timing, wet season length and soil substrate.

These findings are consistent with our initial predictions, based on the geographic distribution of these phenotypes (Exposito-Alonso, 2020; Manzano-Piedras et al., 2014; Martínez-Berdeja et al., 2020; Méndez-Vigo et al., 2011; Montesinos et al., 2009; Montesinos-Navarro et al., 2012; Postma & Agren, 2016; Vidigal et al., 2016; Zacchello et al., 2020). The high-dormancy, early flowering genotypes in our experiment correspond to a "Mediterranean rapid cycler" strategy, concentrated in southern coastal Mediterranean

areas with hot dry summers (Exposito-Alonso, 2020), a climate similar to that in our experimental site in California. Although selection favouring early flowering genotypes has also been observed in agricultural environments in central Europe (Fournier-Level et al., 2013, 2022; Taylor et al., 2019), in these regions early flowering combines with low seed dormancy to construct a "weedy summer/spring cycler" strategy (Exposito-Alonso, 2020), taking advantage of abundant summer rainfall to produce multiple generations per year (Burghardt et al., 2015). Late flowering, low-dormancy genotypes are favoured by selection in Nordic environments (Ågren & Schemske, 2012; Dittmar et al., 2014; Fournier-Level et al., 2022; Postma & Agren, 2016, 2022). Thus, adaptation to climate requires coordinated evolution of germination and flowering phenology (Exposito-Alonso, 2020; Martínez-Berdeja et al., 2020).

Our results highlight the importance of conducting studies that enable integration across the entire life cycle for understanding how plant populations will respond to climate change. We found that seasonal germination timing is critical for individual fitness. Population fitness therefore depends upon seeds germinating during favourable seasonal conditions, which depends upon the timing of seasonal rainfall and soil water potential—as well as regulation of seed dormancy to prevent germination at unfavourable times of the year. By integrating germination phenology data with fitness functions from experimentally manipulated rainfall treatments, we could project population fitness of different genotypes under different rainfall timing scenarios. These simulations show that later onset of autumn rains and shorter growing seasons will have negative consequences for Mediterranean annual plant populations. Moreover, the simulations reveal a fitness advantage for high-dormancy genotypes, which can avoid germinating in response to spring rains and wait until more favourable conditions in fall. Such temporal niche construction through seed dormancy (Donohue, 2005) may be a critical component of adaptation to Mediterranean climates.

AUTHOR CONTRIBUTIONS

Johanna Schmitt, Miki Okada and Alejandra Martínez-Berdeja designed research; Miki Okada, Alejandra Martínez-Berdeja and Martha D. Cooper performed research; Alejandra Martínez-Berdeja, Liana T. Burghardt, Johanna Schmitt and Daniel E. Runcie analysed data; Alejandra Martínez-Berdeja, Liana T. Burghardt and Johanna Schmitt wrote the paper. All authors contributed critically to the drafts and gave final approval for publication.

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CONFLICT OF INTEREST STATEMENT

None.

DATA AVAILABILITY STATEMENT

Data deposited in the Dryad Digital Repository: https://doi.org/10.25338/B8MH07 (Martínez-Berdeja et al., 2023).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Appendix. Methods and results for six additional treatments simulating mid-season droughts.

Figure S1. Screen house photographs.

Figure S2. Germination experiment design schematic (Experiment 1).

Figure S3. Rain simulations for the repeated plantings experiment schematic (Experiment 2).

Figure S4. Fitness functions from April to March for monthly plantings with three end dates (March, April and May) for four genotypes (*Col*, *FRI*, *DOG*, and *FRIDOG*).

Figure S5. Screenhouse mean, maximum and minimum daily temperature values and photoperiod for October 21, 2014 to July 1, 2015 and September 4, 2015 to July 25, 2016 data.

Figure S6. Hourly cumulative photothermal units for each monthly planting during the period of November 2014–May 2015 and September–October 2015.

Figure S7. Hourly cumulative vernalization units for each monthly planting during the period of November 2014–May 2015 and September– October 2015.

Figure S8. Genotype germination proportion for each rain onset cohort for two different substrates sand and soil, for the 2014 and 2015 seeds. Genotype is indicated by different colours.

Figure S9. Boxplots for DTF and PTUs to flowering from germination to flowering date for each planting cohort, genotype is indicated by different colours.

Figure S10. Boxplots for total silique number for each planting cohort—end date, facets indicate the length of the growing season, and different colours indicate genotypes.

Figure S11. Boxplots for projected siliques per seed for each rain onset cohort for two substrates, and four genotypes ending in March, April and May.

Figure S12. Proportion of seeds that survived to projected reproduction for each rain onset cohort for two substrates, and four genotypes ending in March, April and May.

Table S1. ANOVA table for the effect of substrate *FRI*, *DOG1*, *FRI*×*DOG1*, planting, seed age and all their interactions for median and range days to germination for both 2014 and 2015 seeds.

Table S2. ANOVA table for the effect of substrate, *FRI*, *DOG1*, *FRI*×*DOG1*, cohort and all their interactions for median and range days to germination.

Table S3. ANOVA table for the effect of *FRI*, *DOG1*, *FRI*×*DOG1*, substrate, seed age and all their interactions for median and range days to germination for each wet-season onset for both 2014 and 2015 seeds.

Table S4. ANDEVA table for GLM model for the effect of *FRI*, *DOG1*, *FRI*×*DOG1*, substrate, and all their interactions for the proportion of germinated seeds for each cohort.

Table S5. ANOVA table for the effect of *FRI*, *DOG1*, *FRI*×*DOG1*, planting cohort, and growth season length and all the interactions, and the effect of bench, on number of siliques.

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