New Phytologist

Maternal vernalization and vernalization-pathway genes influence progeny seed germination

Gabriela A. Auge[1,2], Logan K. Blair[1], Hannah Neville[1] and Kathleen Donohue[1]

[1]Department of Biology, Duke University, Box 90338, Durham, NC 27708, USA; [2]Present address: Fundación Instituto Leloir, IIBBA-CONICET, Av. Patricias Argentinas 435, C1405BWE, Buenos Aires, Argentina

Author for correspondence:
Gabriela A. Auge
Tel: +54 11 5238 7500
Email: gauge@leloir.org.ar

Received: *16 January 2017*
Accepted: *5 February 2017*

New Phytologist (2017) **216:** 388–400
doi: 10.1111/nph.14520

Key words: *Arabidopsis thaliana*, flowering pathway, maternal effects, pleiotropy, seed germination, transgenerational plasticity, vernalization.

Summary

- Different life stages frequently respond to the same environmental cue to regulate development so that each life stage is matched to its appropriate season. We investigated how independently each life stage can respond to shared environmental cues, focusing on vernalization, in *Arabidopsis thaliana* plants.
- We first tested whether effects of rosette vernalization persisted to influence seed germination. To test whether genes in the vernalization flowering pathway also influence germination, we assessed germination of functional and nonfunctional alleles of these genes and measured their level of expression at different life stages in response to rosette vernalization.
- Rosette vernalization increased seed germination in diverse ecotypes. Genes in the vernalization flowering pathway also influenced seed germination. In the Columbia accession, functional alleles of most of these genes opposed the germination response observed in the ecotypes. Some genes influenced germination in a manner consistent with their known effects on *FLOWERING LOCUS C* gene regulation during the transition to flowering. Others did not, suggesting functional divergence across life stages.
- Despite persistent effects of environmental conditions across life stages, and despite pleiotropy of genes that affect both flowering and germination, the function of these genes can differ across life stages, potentially mitigating pleiotropic constraints and enabling independent environmental regulation of different life stages.

Introduction

Because different life stages have different environmental tolerances, plants must regulate the timing of developmental transitions such that each life stage is exposed only to environmental conditions that permit its survival and growth. In short, each life stage must be matched to its appropriate season. The appropriate timing of developmental transitions requires accurate sensing of seasonal cues. To effectively coordinate developmental transitions across the life cycle, however, each separate life stage must respond optimally to each cue, although frequently different life stages sense and respond to the same environmental cue. It is of interest to know if environmental cues experienced by one life stage carry over to influence following life stages, and if different life stages use the same genetic pathways of environmental sensing and response to regulate distinct developmental transitions across the life cycle.

In annual plants, two key developmental transitions are the transition from vegetative to reproductive status (flowering) and the transition from seed to seedling (seed germination). The timing of both transitions can be under intense natural selection (reviewed in Donohue *et al.*, 2010; Munguía-Rosas *et al.*, 2011; Ehrlén, 2015) and exhibit strong environmental regulation, with both responding to temperature, light quality, photoperiod and other environmental factors (reviewed in Simpson & Dean, 2002; Amasino, 2004; Michaels *et al.*, 2005; Holdsworth *et al.*, 2008; Baskin & Baskin, 2001).

To understand how independently these two life stages are regulated by shared seasonal environmental factors, it is first necessary to evaluate the degree to which environmental conditions experienced at one life stage influence subsequent life stages. It is commonly observed that environmental conditions experienced by maternal parents influence the behavior of progeny (Marshall & Uller, 2007; English *et al.*, 2015). Such 'maternal environmental effects' are well documented in plants (Gutterman, 1982; Donohue & Schmitt, 1998; Donohue, 2009). For instance, in *Arabidopsis thaliana*, low temperatures experienced before or during seed maturation reduce germination propensity, alter seed responses to chilling and water availability during imbibition, and reduce seed longevity, demonstrating that environmental conditions experienced at one life stage can influence subsequent developmental transitions (Kendall & Penfield, 2012; Penfield & Springthorpe, 2012; Chen *et al.*, 2014; He *et al.*, 2014; Auge *et al.*, 2015; Burghardt *et al.*, 2015; MacGregor *et al.*, 2015; Coughlan *et al.*, 2016; Edwards *et al.*, 2016). These effects of temperature also persist as seeds after-ripen (lose primary

dormancy over time) and undergo dormancy cycling (Auge *et al.*, 2015; Burghardt *et al.*, 2015; Edwards *et al.*, 2016).

In addition to such persistent effects of environmental conditions experienced by prior life stages or generations, individual life stages also should respond appropriately to environmental factors that they experience directly for adaptive phenology to be expressed. It is therefore important to know how genetically independent environmental sensing and response pathways are across the life cycle. Pleiotropy occurs when one gene regulates more than one trait, potentially facilitating the evolution of coordinated responses of complex integrated phenotypes, or alternatively, imposing evolutionary constraints on each trait attaining its own adaptive optimum (Fisher, 1958; Atchley, 1984; Wagner, 1988; Barton, 1990; Crespi, 2000; Orr, 2000; Griswold & Whitlock, 2003; Brakefield, 2006; Wagner *et al.*, 2008; Walsh & Blows, 2009). For traits that are regulated by the environment and determined by pleiotropic genetic pathways, environmental responses of one trait or life stage may be associated with environmental responses of other traits or life stages. In this manner, pleiotropy may constrain the expression of adaptive life cycles by preventing independent environmental responses of different life stages. Pleiotropy is expected to be strongest when entire genetic pathways are shared among traits. However, constraints of pleiotropy may be mitigated if genes in the pathway are regulated independently at different life stages. Pleiotropy can also be mitigated if genetic pathways diverge, such that different downstream genes, each with their own environmental responses, regulate different traits. It is consequently of interest to know whether pleiotropy exists in the regulation of different developmental transitions, and if so, whether pleiotropic genes operate in the same manner and in the same genetic pathways at multiple life stages.

Here we investigate the independence of the environmental regulation of flowering and seed germination, focusing on an important environmental cue for flowering: chilling at the vegetative stage (rosette vernalization). Temperatures experienced by maternal parents are known to influence seed germination, as mentioned earlier (Kendall & Penfield, 2012; Burghardt *et al.*, 2016). Regarding pleiotropy, the major flowering regulator, *FLOWERING LOCUS C* (*FLC*), has been previously shown to be pleiotropic, regulating both flowering (Michaels & Amasino, 1999) and germination (Chiang *et al.*, 2009; see later Fig. 5a). *FLC* is a major repressor of flowering in the family *Brassicaceae*, and it is induced by *FRIGIDA* (*FRI*), the *FRI*-complex and other genes such us *VERNALIZATION INDEPENDENCE3* (*VIP3*) (Zhang *et al.*, 2003; Michaels *et al.*, 2004; Choi *et al.*, 2009; reviewed in Ream *et al.*, 2012). *FLC* represses the floral integrators *FT*, *SOC1* and *FD*, thereby delaying flowering and setting the requirement for vernalization. When plants are vernalized by winter chilling, *FLC* is epigenetically silenced via histone modification mediated by genes such us *VERNALIZATION INSENSITIVE3* (*VIN3*) and *VERNALIZATION2* (*VRN2*) (Gendall *et al.*, 2001; Sung & Amasino, 2004; reviewed by Ream *et al.*, 2012). Consequently, *FT*, *SOC1* and *FD* expression is derepressed, and flowering is induced. *FLC* silencing is reset during fertilization, re-imposing the vernalization requirement in the next generation (Sheldon *et al.*, 2000; Michaels *et al.*, 2003). *FLC* and some genes downstream of it also influence germination. Seeds of *A. thaliana* with high-expressing *FLC* alleles show higher germination propensity than seeds with weak or null alleles, and *FLC* appears to regulate germination through other downstream genes of the flowering pathway, such as *FT*, *SOC1* and *AP1* (Chiang *et al.*, 2009). It has also been shown that *FT* is involved in seed germination responses to maternal temperature by affecting phenylpropanoid deposition in, and consequently permeability of, the seed coats (Chen *et al.*, 2014). Thus, *FLC* and genes downstream of it pleiotropically influence both flowering and germination. Here, we test whether upstream regulators of *FLC* expression, and specifically genes involved in the vernalization flowering response, also influence germination.

To examine the independence of the environmental regulation of flowering and germination, we first tested whether pre-reproductive rosette vernalization also influences seed germination. Next we tested whether genes known to regulate flowering responses to vernalization also regulate germination and how vernalization affects germination of the progeny. We also provide evidence to distinguish whether pleiotropic genes influence both flowering and germination through the same genetic pathways. We address the following specific questions: Does vernalization of maternal parents influence germination behavior in diverse ecotypes, and is this effect persistent as seeds after-ripen? Do genes known to regulate flowering responses to vernalization also influence germination? Do these genes influence germination in a manner that is consistent with their roles as regulators of *FLC* during the transition to flowering, or is there evidence that these genes influence germination through different mechanisms? To address these questions, we examined germination behavior of seeds from vernalized and nonvernalized plants using diverse ecotypes and mutants of genes in the vernalization flowering pathway, and we quantified the expression of vernalization genes both at the pre-reproductive stage and during seed maturation in plants that had been vernalized or remained nonvernalized.

Materials and Methods

Plant material and growth conditions

To test whether rosette vernalization of maternal plants influences seed germination in diverse ecotypes and in the standard laboratory accession, Columbia (hereafter Col), 21 accessions of *A. thaliana* (L.) Heynh. (Supporting Information Table S1; Fig. S1) were selected to encompass variation in both flowering time (Shindo *et al.*, 2005, 2006) and germination (Chiang *et al.*, 2009). Each ecotype was scored for *FRI* and *FLC* functionality and latitude of collection based on previously published data (Michaels *et al.*, 2003; Lempe *et al.*, 2005; Shindo *et al.*, 2005; Atwell *et al.*, 2010; Lovell *et al.*, 2013).

Seeds were sowed in 2.5-inch pots with soil (Metromix 360; Scotts Sierra, Marysville, OH, USA) and placed in EGC GCW-30 plant growth chambers (Environmental Growth Chambers, Chagrin Falls, OH, USA) in a 12 h photoperiod at 22°C for 1 wk to allow germination. Seed-sowing was staggered to synchronize

seed harvest across treatments and ecotypes – to schedule the staggered planting, bolting times were estimated in a preliminary experiment that used at least three replicate plants of each of the accessions, grown in the same conditions used in the experiment described here. Seedlings were either left in those conditions (nonvernalized plants, NV) or vernalized for 4 wk at 4°C with a 12 h photoperiod (vernalized plants, V). After the vernalization treatment, V plants were placed in the same conditions as NV plants until all plants, V and NV, bolted (floral bud emergence). After bolting, plants were transferred to a chamber set at 15°C in an 8 h photoperiod until harvest. Six plants of each ecotype for both V and NV treatments were harvested on the same date, and the seeds were kept in dry storage (Secador 4.0 Auto-Desiccator Cabinets, Bel-Art Products, Pequannock, NJ, USA) at room temperature until used for germination assays.

To test whether genes that regulate flowering responses to vernalization are also involved in germination responses to vernalization, we assessed germination in several genotypes that had altered expression or function of genes in the flowering-time vernalization pathway. These included near-isogenic lines (NILs) in the Col background that contained different combinations of functional and nonfunctional *FRI* and *FLC* alleles, as well as T-DNA insertion mutants (Col background) of inducers of *FLC* – namely *FRI* and *VIP3* – and repressors of *FLC* – namely *VIN3* and *VRN2* (Table S2). Plants of all genotypes were grown in the same conditions as described earlier, and seed-sowing was staggered to synchronize the harvest of seeds across treatments and genotypes. Seeds from 12 plants for each of the mutants/NILs and their reference genotypes in each treatment were harvested on the same date and kept in dry storage until used for germination assays.

Germination assays

For the experiments with the ecotypes, fresh seeds (3 d after harvest) and seeds after-ripened for 4, 8, 14, 27, 39 and 50 wk were used for germination assays. Six biological replicates (different mother plants), with 20 seeds per replicate for each ecotype and maternal vernalization treatment, were sowed in 0.6% (w/v) agar plates and immediately incubated in a 12 h photoperiod at 10 or 22°C in EGC Model GC8-2 Plant Growth Chambers (Environmental Growth Chambers). Germination was recorded every week for 4 wk, when germination reached a clear plateau.

For the assays with the NILs and mutant genotypes, 12 biological replicates (different mother plants), with 20 seeds per replicate, were sowed in duplicate agar plates and incubated in the same conditions as described earlier for the ecotypes. Germination was recorded every week for 3 wk after the beginning of the incubation, at which point germination had plateaued. Because synchronization failed between some of the NILs and mutants, three seed batches were harvested, with each batch including NILs or mutants with their respective reference genotype for each maternal treatment. This blocking ensured that all seeds from each genotype pair that was compared were harvested simultaneously.

For both experiments, the final germination proportion (at 4 and 3 wk after the beginning of incubation for the ecotypes and for the NILs and mutants, respectively) was calculated as the number of germinated seeds (seeds showing radicle protrusion) divided by the total number of viable seeds.

Tissue sample collection and qRT-PCR

To test whether rosette vernalization altered gene expression during life stages that are critical for germination regulation (silique development), we measured the expression of vernalization-pathway genes in vernalized and nonvernalized plants of three ecotypes that had strong germination responses to maternal vernalization. Four biological replicate samples were collected from vernalized and nonvernalized plants of Alc-0, Ra-0 and Tu-0 ecotypes. Five developmental time points were sampled: early, prebolting leaf (hereafter 'EL': collection occurred during weeks 1–4 of growth, depending on the ecotype's vegetative phase length; for vernalized plants, this was immediately after vernalization); late, prebolting leaf (hereafter 'LL': 1 wk before the estimated bolting date for both vernalized and nonvernalized plants); and three silique stages (15–20 siliques each) during the reproductive growth stage (early, 'ES': first five siliques (1–5) after fruits first became visible; medium, 'MS': silique numbers 11–15; and yellow, 'YS': mature yellow, but not dry, siliques).

RNA from three to four independent biological replicates of each ecotype from each maternal treatment was extracted with the Master Pure DNA and RNA extraction kit (Epicentre). Total RNA (500 ng) was used in the reverse transcription (RT) reaction (Superscript III, Life Technologies) and 1 µl of ½ diluted cDNA was used as template in the quantitative polymerase chain reaction (qPCR) using KAPA PROBE FAST qPCR Master Mix (KapaBiosystems, Wilmington, MA, USA). Pre-designed Taqman assays (Life Technologies) were used to quantify expression of the following genes: *TIP41-like*, AT4G34270 (probe number At02249834_g1); *PP2AA3*, AT1G13320 (At02284835_g1); *FLC*, AT5G10140 (At02272495_g1); *FRI*, AT4G00650 (At02207149_g1); *VIP3*, AT4G61150 (At02336841_g1); *VIN3*, AT5G57380 (At02267707_g1); and *VRN2*, AT4G16845 (At02222277_g1). Three technical replicates per sample were run in a StepOne cycler (Life Techonologies) in standard running conditions. If technical replicates were more variable than 1.0 Ct value, the most aberrant values were excluded. Gene expression was assessed by calculating the mean ΔΔCt value ± SE for each developmental time point using *TIP4-like* or *PP2AA3* as reference genes. For this, we first validated the assays following Livak & Schmittgen (2001), which let us assess if primers and probes for different genes had similar amplifying efficiency. As the slopes of the fitted $\log_{10}$-transformed Ct values across a dilution set for the genes of interest and the reference genes were close to 0, the assumption that the efficiencies were similar held, allowing us to calculate the ΔΔCt values and analyze our data. Late pre-bolting leaf samples from nonvernalized plants (NV-LL) were used as the reference for leaf samples, and medium silique samples from nonvernalized plants (NV-MS) were used as reference samples for siliques. Since the results that were standardized by each of the

housekeeping genes were similar, the results presented in the Results section correspond to the ΔΔCt values calculated using only *TIP41-like* as reference gene. Results based on *PP2AA3* as the reference gene are presented in the Supporting Information.

Statistical analyses

All statistical analyses were conducted using R v.3.2.3 (R Core Team, 2015). To test for effects of maternal vernalization (Vern) on germination, and to test how that effect differed among ecotypes (Eco), incubation temperatures (Temp) and after-ripening times (AR), we fit generalized linear models with logit link functions using 'GLM', and then performed type-III likelihood ratio tests using the 'ANOVA' function in the 'CAR' package (Fox & Weisberg, 2010). A logit link function was used because germination is a binomial trait, and the dependent variable was therefore in the form of proportions. A correction for multiple comparisons was conducted when appropriate, using the Holm method of 'P.ADJUST' in 'STATS' package. Vern, Temp and AR, as well as Eco, were treated as fixed factors. A full model that included all interactions was analyzed first; the four-way interaction was significant (Table S3), so we conducted submodels to interpret ecotypic differences in the interactions by analyzing each ecotype separately. As the three-way interaction Vern × Temp × AR did not improve the model for almost all the ecotypes in a preliminary test (the exceptions were Bil-7 and Tu-0), those interactions were dropped, and results for the test of the two-way interactions are presented.

To test for contributions of genes in the vernalization pathway to germination and its response to vernalization, we used the same statistical procedure as described earlier for the ecotypes, with Vern, Temp and genotype (Geno) as fixed factors. For the NILs with different combinations of *FRI* and *FLC* alleles, a full model that included all interactions was analyzed to test for a significant Geno × Vern × Temp interaction. To interpret this significant interaction (see the Results section), we tested for effects of vernalization for each genotype separately at each temperature, and we tested for differences among specific genotypes within each vernalization and temperature treatment separately. For the NILs, we tested for an effect of *FLC* variation in functional (FRI/FLC vs FRI/*flc*) or nonfunctional (*fri*/FLC vs *fri*/*flc*) *FRI* backgrounds. In addition, we tested for an effect of *FRI* variation in functional (FRI/FLC vs *fri*/FLC) or nonfunctional (FRI/*flc* vs *fri*/*flc*) *FLC* backgrounds. For the mutants, the tests compared germination of the mutant genotypes in *VIP3*, *VIN3* or *VRN2* with the wild type Col.

To test whether *FRI* and *FLC* functionality and combination predicted germination in diverse ecotypes, in addition to testing their effect in the Col laboratory strain just described, we used the same procedure as described earlier, with ecotypic mean germination in each treatment as the unit of analysis, and with Vern, Temp, AR and allele functionality as fixed factors. A full model that included all interactions was analyzed first to test for a significant four-way interaction. We next analyzed submodels at each Temp to interpret interactions, and tested for effects of *FLC* on functional and nonfunctional *FRI* backgrounds, or effects of *FRI* on functional and nonfunctional *FLC* backgrounds, as just described. See the Supporting Information for results of these analyses.

To test for significant associations between germination and flowering responses to vernalization, and between germination and latitude, we estimated correlations using the function 'COR.TEST' in the 'STATS' package. Pearson correlation estimates, 'R VALUES' and significance levels were calculated for the associations between germination response (germination of seeds from vernalized minus nonvernalized plants) and flowering response (leaf number at bolting for vernalized minus nonvernalized plants), between germination response and latitude, and between flowering response and latitude.

To test for effects of vernalization on gene expression across development, we used the 'AOV' function in the 'STATS' package to perform an ANOVA. Relative gene expression of each gene was the dependent variable, and Eco, Vern and developmental time point (DevTime) were fixed factors. We tested for Vern × DevTime × Eco interactions in a full model for each gene, and then tested for Vern effects at each developmental stage separately.

Results

Vernalization of mother plants influenced germination of the progeny in diverse ecotypes

Rosette vernalization of mother plants increased seed germination in many ecotypes (Figs 1, S2). Seeds from vernalized plants germinated more (significant effect of Vern) and lost dormancy faster (significant Vern × AR interaction) than seeds from nonvernalized plants in several ecotypes (Table 1), although this response varied greatly among them (significant Eco × Vern and Eco × Vern × AR interactions in the full model; Table S3). The effect of maternal vernalization on germination also depended on the incubation temperature, but ecotypes differed in whether the maternal effect was more pronounced when seeds were incubated at 10 or 22°C (significant Eco × AR × Vern × Temp in the full model, Table S3; significant Vern × Temp in analysis of individual ecotypes, Table 1).

The vernalization effect on germination could be observed even in ecotypes with no vernalization requirement for flowering (e.g. Ct-1, Oy-0 and Tu-0; Fig. 1), and the association between flowering and germination responses to vernalization was not significant (Table S4a). Moreover, even though the flowering response of maternal plants was strongly correlated with latitude (Pearson correlation estimate = −0.196, $P = 0.002$), the progeny's germination response to maternal vernalization was not, with the only exception being germination of seeds after-ripened for 14 wk and incubated at 22°C (Table S4b).

Genes in the flowering vernalization pathway influence progeny germination responses to maternal vernalization

To test whether *FLC* has a role in the germination response to maternal vernalization, we compared genotypes (NILs in Col background) with high or low *FLC* activity on functional or

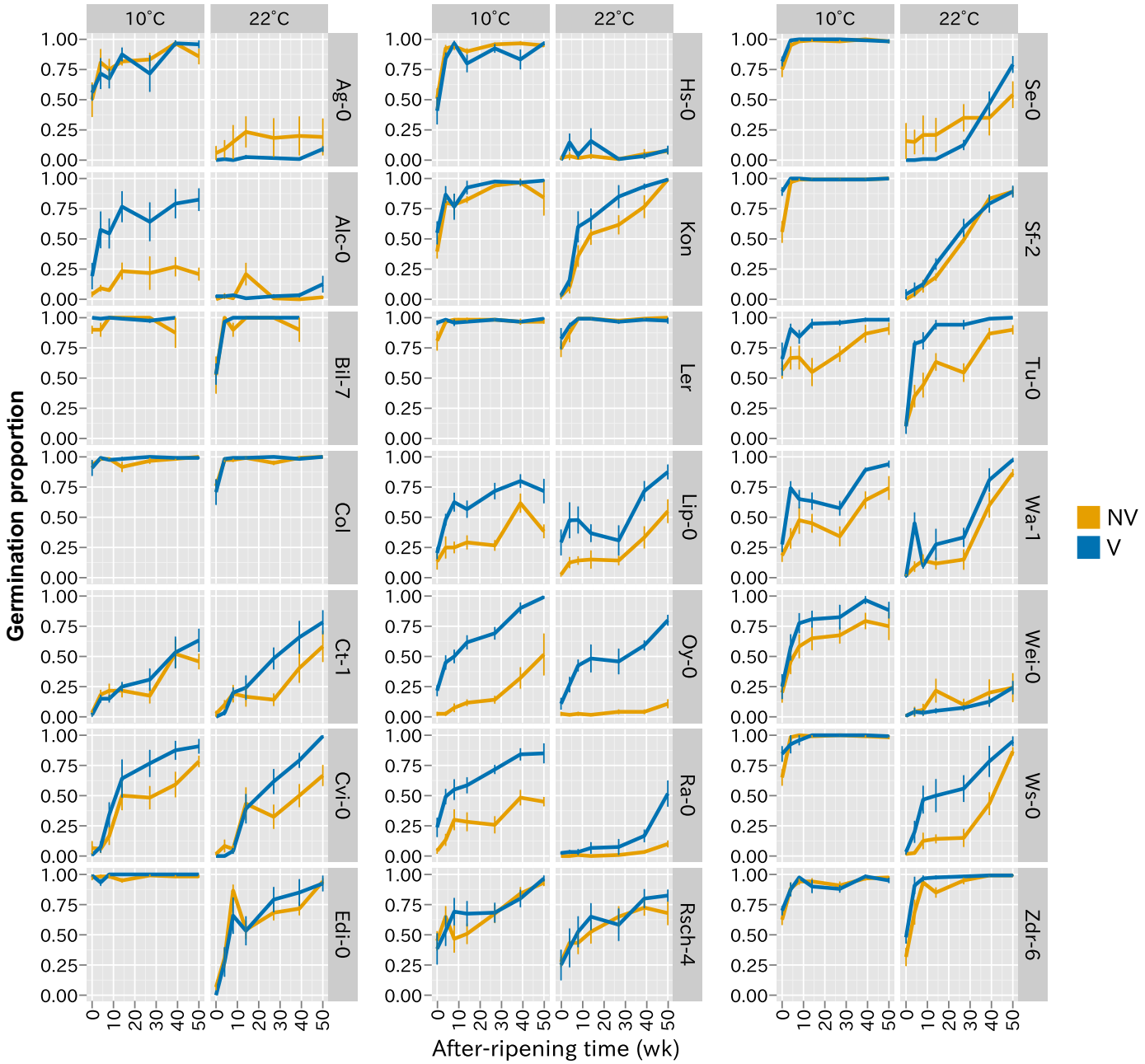

Fig. 1 Effect of vernalization of the mother plant on seed germination of the progeny in diverse *Arabidopsis thaliana* ecotypes. Graphs show the proportion of seeds that germinated (mean ± SE) from nonvernalized (NV, orange) and vernalized (V, blue) plants during the course of after-ripening (*x*-axis). Each panel shows the results of a single ecotype at each incubation temperature (10°C on left, 22°C on right).

nonfunctional *FRI* backgrounds. First, vernalization masked genotypic differences in germination at both incubation temperatures (Fig. 2; Table 2a). Second, the effect of maternal vernalization on germination depended on the genotype (Table 2a). In contrast to the response of most ecotypes described earlier (Fig. 1), seeds with both the strong $FRI_{Sf\text{-}2}$ allele and functional FLC_{Col} (FRI/FLC) germinated less when parental plants were vernalized than when they were not vernalized, and this was consistent in both incubation temperatures (closed black squares in Fig. 2; Table 2a,b). In a functional *FRI* background, disruption of *FLC* (FRI/*flc*) reduced, and even slightly reversed, the response to maternal vernalization by reducing germination of seeds from NV plants (Table 2b,c). Active *FLC* therefore appears to increase germination of seeds from NV plants. By contrast, disruption of *FLC* in the nonfunctional *fri* (*fri*/*flc*) background either had no effect on germination (at 22°C) or was associated with slightly increased germination in seeds from NV plants (marginally significant at 10°C, Table 2c). Thus, opposing the effect of vernalization observed in the diverse ecotypes discussed earlier, *FLC* appears to promote germination of seeds from NV plants when it is partnered with an active *FRI* (upregulator of *FLC*), but not if *FRI* is nonfunctional.

Table 1 Effects of maternal vernalization, incubation temperature and after-ripening time on germination of 21 natural ecotypes of *Arabidopsis thaliana*

Ecotype	Vern	Temp	AR	Vern × Temp	Vern × AR	Temp × AR
Ag-0	4.16	**276.34*****	**45.92*****	**77.33*****	7.05	5.19
Alc-0	**109.23*****	**17.72****	**19.15*****	**59.82*****	**13.61****	8.17
Bil-7	8.92	**50.15*****	0.70	5.86	0.01	**56.41*****
Col	0.16	**13.67***	4.89	0.02	6.75	**14.09***
Ct-1	4.75	**13.56***	92.86	8.75	**19.96*****	**13.88***
Cvi	0.98	**11.21***	**225.44*****	4.15	**81.10*****	1.18
Edi-0	0.43	**296.98*****	1.97	3.48	**14.66***	6.18
Hs-0	1.91	**527.47*****	**92.51*****	**27.12*****	7.69	**32.03*****
Kon	0.92	**226.93*****	**80.73*****	0.52	**22.03*****	**34.28*****
Ler	**13.14***	7.84	**21.15*****	2.87	8.67	**15.13***
Lip-0	**78.47*****	**29.01*****	**78.07*****	3.51	0.03	6.69
Oy-0	**172.65*****	6.65	**178.98*****	2.10	2.58	**24.88*****
Ra-0	**65.94*****	**179.80*****	**77.17*****	1.07	3.73	**15.88****
Rsch-4	0.04	8.57	**116.68*****	0.01	2.99	2.53
Se-0	0.08	**313.22*****	**41.08*****	**96.19*****	**123.12*****	8.46
Sf-2	**33.92*****	**311.64*****	**158.32*****	3.94	5.27	**16.96****
Tu-0	**12.42***	**81.40*****	**45.59*****	0.92	**66.49*****	**33.91*****
Wa-1	**47.87*****	**146.64*****	**108.90*****	0.12	0.29	**82.16*****
Wei-0	7.77	**171.54*****	**103.63*****	**47.99*****	**12.813***	1.52
Ws-0	3.96	**448.56*****	**122.18*****	**22.71*****	3.346	**11.05***
Zdr-6	0.35	**65.82*****	**47.07*****	10.48	0.332	**62.25*****

Tests for effects of vernalization (Vern), incubation temperature (Temp), after-ripening time (AR) and their two-way interactions on progeny germination, for each ecotype separately, based on type III analyses of likelihood ratios from a generalized linear model with a logit link function (three-way interactions were dropped from the test as preliminary analyses showed they did not improve the models). χ^2 values are shown in the table. See Supporting Information Table S3 for results of the full model. Asterisks and bold numbers show significant effects: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

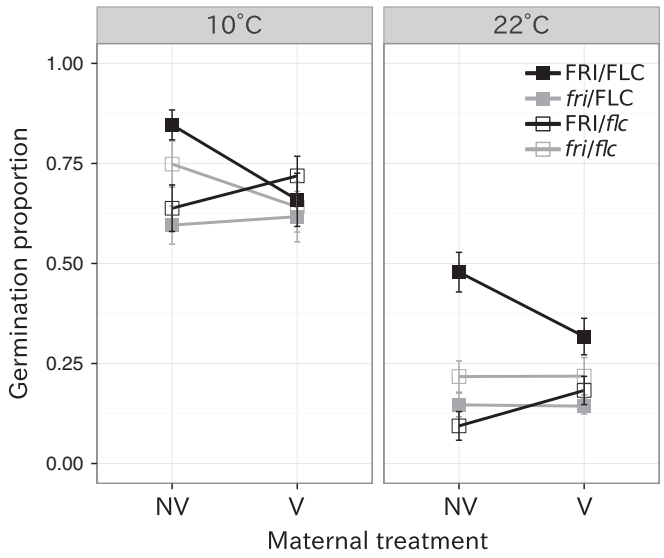

Fig. 2 Effect of vernalization of *Arabidopsis thaliana* mother plants on the germination of progeny of near-isogenic lines (NILs) with different combinations of *FRI* and *FLC* wild-type (WT) and mutant alleles. Graphs show the proportion of seeds that germinated (mean ± SE) for seeds from nonvernalized (NV) and vernalized (V) plants incubated at 10°C (left) and 22°C (right). Black closed square, FRI/FLC contains $FRI_{Sf\text{-}2}$ (strong) and FLC_{Col}; black open square, FRI/*flc* contains $FRI_{Sf\text{-}2}$ (strong) and the *flc-3* knockout mutant; gray closed square, *fri*/FLC contains both Columbia WT alleles for FRI_{Col} (nonfunctional) and FLC_{Col} (weak); and gray open square, *fri*/*flc* contains FRI_{Col} and the *flc-3* knockout mutant (see also Supporting Information Table S2).

Regarding the contribution of *FRI* to germination, *FRI* is an inducer of *FLC* expression at the pre-reproductive stage. If it also contributes to germination via regulation of *FLC*, disruption of *FRI* is expected to decrease germination (especially in nonvernalized plants, in which the effect of *FLC* is most pronounced). As predicted, disruption of *FRI* on a functional *FLC* background (closed black vs closed gray squares in Fig. 2; Table 2c) reduced germination, especially in seeds of nonvernalized plants. On a nonfunctional *FLC* background, however (open black vs open gray squares in Fig. 2; Table 2c), disruption of *FRI* was associated with increased germination, especially in seeds of NV plants (significant only at 22°C). Therefore, active *FRI* promotes germination when *FLC* is functional, but it has a slight inhibitory effect on germination when *FLC* is nonfunctional. This result suggests the existence of a weak *FLC*-independent pathway whereby *FRI* inhibits germination of seeds from nonvernalized plants – a response that reflects the vernalization response observed in the diverse ecotypes. In the ecotypes, allelic effects of *FRI* and *FLC* did not always reflect those observed in the NILs in the Col background. See the Supporting Information (Fig. S3; Tables S5, S6; Notes S1) for a discussion of these differences.

To test whether other genes in the vernalization flowering pathway influence germination, we compared the responses of T-DNA insertion mutants of the *FLC* inducer *VIP3* and the *FLC* repressors *VIN3* and *VRN2* to their wild-type Col background (Fig. 3; Table 3). The response of Col wild type to maternal vernalization varied across batches and ranged from having no response (in all batches assayed at 10°C, plus the control for *vin3*

Table 2 Effects of maternal vernalization, incubation temperature and genotype on the germination behavior of the near isogenic lines (NILs)

(a)

	LR χ^2	d.f.
Genotype	**93.00*** **	**3**
Vern	0.47	1
Temp	**215.28*** **	**1**
Geno × Vern	**56.53*** **	**3**
Geno × Temp	**19.84*** **	**3**
Vern × Temp	0.32	1
Geno × Vern × Temp	4.81	3

(b)

	LR χ^2 (Vern)	
Genotype	10°C	22°C
FRI/FLC	**44.21*** **	**26.88*** **
FRI/*flc*	**7.69***	**15.10*** **
fri/FLC	0.47	0.04
FRI/*flc*	**7.69***	**15.10*** **
fri/*flc*	**13.30****	0.001

(c)

		LR χ^2 (Geno)			
		NV		V	
Geno1	Geno2	10°C	22°C	10°C	22°C
FRI/FLC	FRI/*flc*	**9.05****	**29.79*** **	0.56	**5.25***
fri/FLC	*fri*/*flc*	**3.95***	1.98	0.066	2.34
FRI/FLC	*fri*/FLC	**15.95*** **	**30.95*** **	0.21	**12.99*** **
FRI/*flc*	*fri*/*flc*	1.79	**4.59***	1.01	0.36

Test for effects of vernalization (Vern), genotype (Geno) and their interactions with incubation temperature (Temp) on the germination behavior of the near isogenic lines (NILs) containing contrasting *FRI* and *FLC* alleles. (a) Results for the full model based on Type III analysis of likelihood ratios from a generalized linear model with a logit link function to test for effects of Vern, Temp, Geno and their interactions on progeny germination. (b) Effect of vernalization on each genotype at each incubation temperature. (c) Test for differences in germination for each pair of genotypes, for nonvernalized and vernalized maternal treatments at each incubation temperature. Asterisks and bold numbers show significant effects: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

seeds incubated at 22°C), decreased germination of seeds of vernalized plants (control to *vip3* seeds at 22°C) or increased germination of seeds of vernalized plants (control to *vrn2* at 22°C; Vern effect in Table 3). Thus, the effect of maternal vernalization on seed germination appears to be sensitive to conditions during maternal growth and/or seed germination. The effect of maternal vernalization on germination was stronger when seeds were incubated at 22°C than at 10°C (significant Vern × Temp interactions in Table S7), as were genotypic differences in response to vernalization (Fig. 3; significant Vern × Temp × Geno interactions in Table S7), so the results discussed later correspond to 22°C unless otherwise stated.

Like *FRI*, *VIP3* is an inducer of *FLC* expression at the prereproductive stage; if it behaves similarly in the germination pathway, disruption of *VIP3* is expected to decrease germination (especially for seeds from nonvernalized plants, where *FLC* has the larger influence, Fig. 2). Contrary to expectation, the *vip3* mutant germinated more than Col wild-type in seeds from vernalized plants at both incubation temperatures, although the effect was significant at 22°C only (Fig. 3). Moreover, when *VIP3* was disrupted, seeds from vernalized plants germinated more than seeds from nonvernalized plants (Table 3). Thus, functional *VIP3* appears to reduce germination of seeds from vernalized plants, which is in opposition to the effect of vernalization observed in the diverse ecotypes (namely, increased germination of seeds from vernalized plants). Moreover, the lack of effect of *vip3* on germination of seeds from nonvernalized plants, in which an effect of *FLC* was observed, suggests that *VIP3* influences germination through a mechanism that differs from its regulation of flowering.

VIN3 is upregulated by vernalization and is a repressor of *FLC* expression in the flowering pathway; if it operates in a similar manner to regulate germination, disruption of *VIN3* would be expected to increase germination, especially in seeds from vernalized plants. Consistent with this expectation, seeds of *vin3* germinated more than Col wild-type seeds at 22°C, but it did so only in seeds from vernalized plants (Fig. 3). Therefore, functional *VIN3* appears to reduce germination of seeds from vernalized plants, which is in opposition to the effect of vernalization observed in the diverse ecotypes.

VRN2 also represses *FLC* expression in the flowering pathway, and the expectation is therefore that disruption of this gene would increase germination (especially in seeds from vernalized plants). At 22°C, contrary to prediction, *vrn2* mutant seeds from vernalized plants germinated less than Col (Fig. 3). This difference reflected a pronounced enhancement of germination of seeds from vernalized plants compared with nonvernalized plants in Col but not in the mutant. Therefore, at 22°C, active *VRN2* appears to strongly promote germination of seeds from vernalized plants – the response that was observed in the diverse ecotypes. At 10°C, the *vrn2* mutant seeds from nonvernalized plants germinated significantly less than Col (Fig. 3), indicating that functional *VRN2* promotes the germination of seeds from nonvernalized plants at that temperature. Thus, *VRN2* appears to contribute to the germination response that was observed in the ecotypes at 22°C, but oppose it at 10°C.

Gene-expression responses to vernalization vary across development

If known gene expression responses to vernalization at the prereproductive stage persist throughout seed development, the expectation is that vernalized plants would exhibit lower expression of *FLC*, *FRI* and *VIP3* and higher expression (or unchanged, when gene expression that is enhanced by vernalization returns to low levels after vernalization) of *VIN3* and *VRN2* than nonvernalized plants. To test these predictions, we quantified gene expression of these genes in three ecotypes that showed a pronounced effect of maternal vernalization on progeny germination (Alc-0, Ra-0 and Tu-0; Fig. 1). Results based on the *TIP41-like* reference gene, presented here, are similar to those based on the *PP2AA3* reference gene (Fig. S4).

Consistent with expectation, *FLC* expression was in general higher in nonvernalized than in vernalized plants at most life

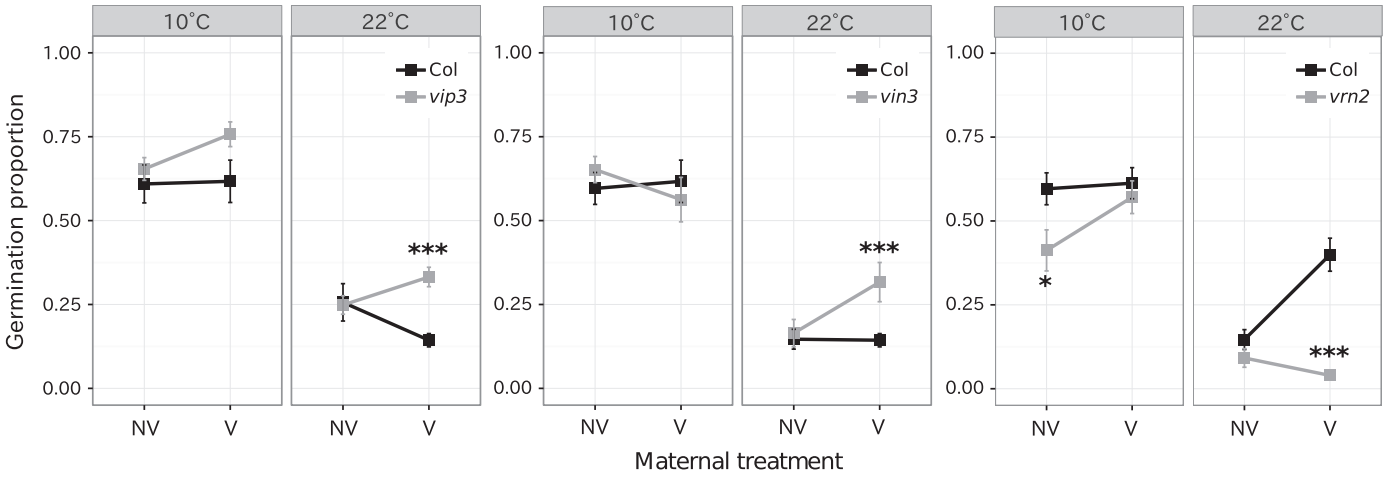

Fig. 3 Effect of vernalization of *Arabidopsis thaliana* mother plants on progeny germination of T-DNA insertion mutants of the genes *VIP3*, *VIN3* and *VRN2*. Graphs show the proportion of seeds that germinated (mean ± SE) for seeds from nonvernalized (NV) and vernalized (V) plants incubated at 10°C (left) and 22°C (right). Mutants (gray squares) are compared with the reference genotype Columbia (black squares) in the same maternal treatment. Asterisks show significant genotype effects on germination of mutants compared with Col: *, $P < 0.05$; ***, $P < 0.001$.

Table 3 Effect of maternal vernalization on the germination response of *Arabidopsis thaliana* Col, *vip3*, *vin3* and *vrn2* mutants

	LR χ^2 (Vern)	
Genotype	10°C	22°C
Col	0.07	**17.33*****
vip3	**25.00*****	**16.09*****
Col	0.47	0.04
vin3	**8.09***	**30.79*****
Col	0.28	**78.63*****
vrn2	**24.40*****	**9.51****

Effect of vernalization (Vern) on the germination response of Col and the mutants *vip3*, *vin3* and *vrn2* at each temperature based on Type III analysis of likelihood ratios from a generalized linear model with a logit link function. Asterisks and bold numbers show significant effects: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

stages, with some exceptions (Fig. 4; Table 4). This result is also consistent with our observation that disruption of *FLC* influenced seeds only from nonvernalized plants, supporting the hypothesis that *FLC* increases germination of seeds from nonvernalized plants.

We found no evidence that *FRI* expression during silique development was greater in nonvernalized plants (Fig. 4; Table 4). Instead, expression of *FRI* in siliques did not respond to vernalization, even though its levels were affected by vernalization at the pre-reproductive stage. Likewise, *VIP3* expression was not higher in siliques of nonvernalized plants, except for a small difference in the Alc-0 ecotype. Pre-reproductive expression of *VIP3* did not closely correspond to expression levels in siliques.

Counter to expectation, expression of *VIN3* in siliques was sometimes higher in nonvernalized than in vernalized plants (Fig. 4), suggesting that predicted enhancement of *VIN3* expression induced during vernalization at the pre-reproductive stage does not persist for long, and instead, vernalization appears to reduce *VIN3* expression in siliques in some ecotypes. *VRN2* expression was highly variable across ecotypes and varied over development (Fig. 4; Table 4).

In summary, with very few exceptions, the effect of vernalization on gene expression varied across development, such that enhanced or reduced gene expression at the pre-reproductive stage caused by vernalization did not persist into seed development. Only *FLC* expression in Alc-0 had a consistent response to vernalization at both the pre-reproductive and silique stage; in other cases, the response to vernalization was reversed across life stages (*FLC* in Ra-0, *VIP3* in Alc-0, and *VRN2* in Alc-0 and Ra-0); in most cases, only one life stage responded to vernalization. Therefore, gene expression in response to vernalization does not appear to be correlated across life stages.

Discussion

Vernalization of the mother plant early in the life cycle influenced progeny germination, even if developing seeds themselves did not experience cold. Thus, vernalization has a dual effect on development: a short-term effect on flowering time that is reset during fertilization, and a trans-generational effect that modifies progeny seed germination. These two effects are somewhat independent, since the maternal effect on germination is expressed even in ecotypes that do not require vernalization for flowering. Genes in the vernalization flowering pathway pleiotropically regulate seed germination. Frequently, however, the effect of these genes on germination is not consistent with their function in the flowering pathway, and the expression of these genes in response to vernalization is not correlated between early and late life stages. Therefore, despite persistent effects of vernalization on subsequent life stages, and despite pleiotropy of the regulation of flowering and germination, these vernalization genes appear to be able to regulate different life stages with some degree of independence.

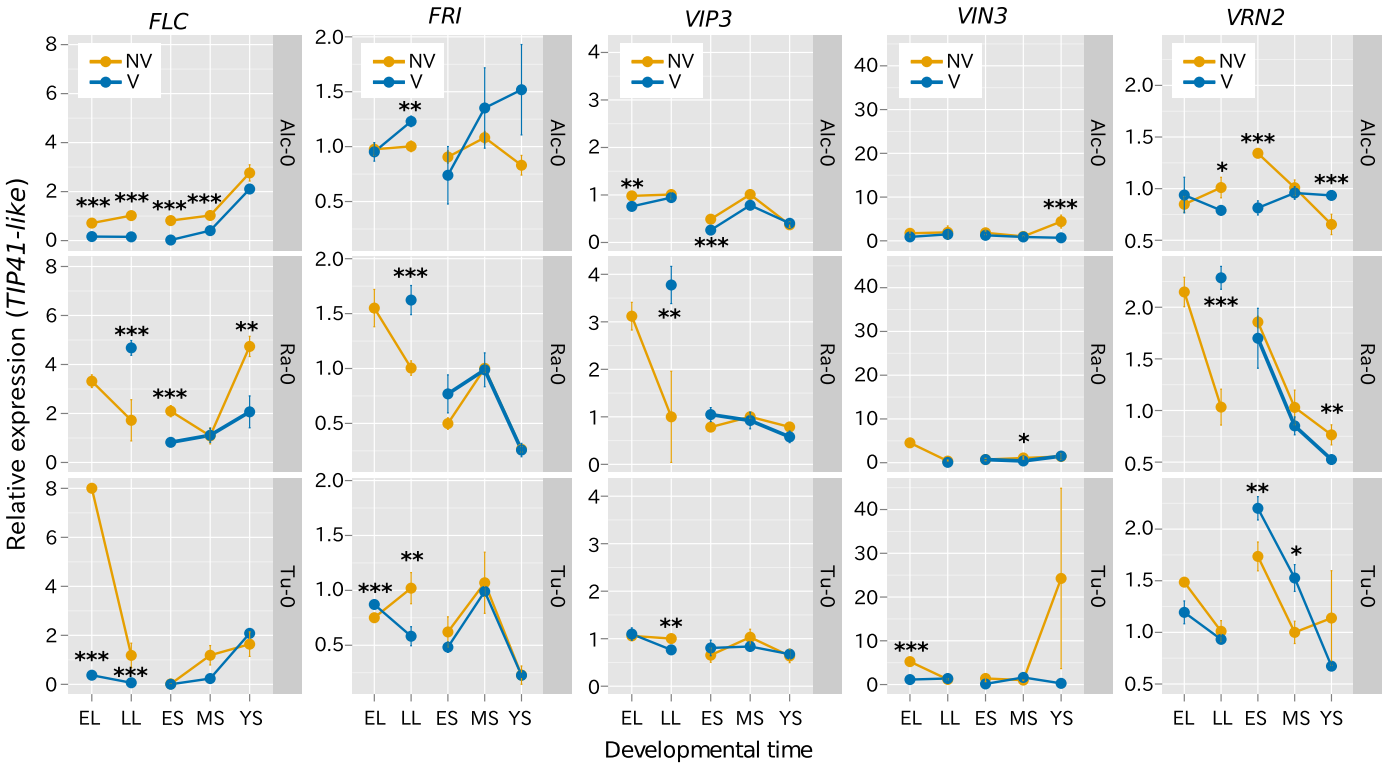

Fig. 4 *FLC*, *FRI*, *VIP3*, *VIN3* and *VRN2* expression during the course of development in nonvernalized (NV) and vernalized (V) *Arabidopsis thaliana* plants. Graphs show the mean ΔΔCt values ± SE calculated for each gene and developmental time point using *TIP41-like* expression as reference gene. NV late leaf (before bolting, NV-LL) and NV medium silique (NV-MS) were used as reference samples for leaves and siliques, respectively. Pre-reproductive life stages: EL, early leaf; LL, late leaf. Post-reproductive life stages (siliques): ES, early siliques; MS, medium siliques; YS, yellow siliques. Asterisks show significant effects of vernalization on expression: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Source	d.f.	*FLC*	*FRI*	*VIP3*	*VIN3*	*VRN2*
Vern	1	**36.02*****	0.6	0.41	2.37	0.06
DevTime	4	**27.1*****	**12.58*****	**18.11*****	1.24	**19.02*****
Eco	2	**56.5*****	**12.13*****	**32.79*****	0.14	**20.61*****
Vern × DevTime	4	**7.82*****	1.63	**8.59*****	1.26	**2.77***
Vern × Eco	2	2.45	**3.53*****	1.73	0.73	0.52
DevTime × Eco	8	**12.45*****	**5.8*****	**10.07*****	0.52	**8.64*****
Vern × DevTime × Eco	7	**20.8*****	1.78	**6.22*****	0.59	**5.87*****

Table 4 Effect of maternal vernalization, developmental time and ecotype on gene expression

Effect of vernalization (Vern), developmental time (DevTime), ecotype (Eco) and their interactions on *FLC*, *FRI*, *VIP3*, *VIN3* and *VRN2* expression, based on an ANOVA of relative gene expression. *F*-values are shown in the table. Asterisks and bold numbers show significant effects: *, $P < 0.05$; ***, $P < 0.001$.

Effects of vernalization of maternal parents persist to influence germination of progeny

Accurate cuing of individual life stages to seasonal conditions is necessary to match each life stage to appropriate seasonal conditions. Both flowering and germination are regulated by the seasonal cue of temperature (Amasino, 2004; Michaels *et al.*, 2005; Kendall & Penfield, 2012; Burghardt *et al.*, 2016). To test how independently each life stage responds to this same environmental factor, we tested whether effects of vernalization experienced at the pre-reproductive life stage persisted to influence seed germination. Although seeds did respond directly to temperatures that they themselves experienced, we found that the temperature experienced much earlier, by maternal parents, also influenced seed germination. Specifically, in a sample of diverse ecotypes, seeds from vernalized mother plants germinated more than seeds from nonvernalized plants. The germination pattern depended on the ecotype, with some responding strongly across all the tested seed conditions, while others responded more in certain incubation temperatures and after-ripening treatments. In addition, dormancy breakage via after-ripening modified the expression of the maternal vernalization effect in some ecotypes. In general, however, when a maternal effect was apparent, maternal vernalization consistently increased seed germination, and this

effect frequently persisted in progeny for several weeks of after-ripening.

The environment experienced by the mother plant frequently affects germination of the progeny by modifying dormancy levels (Penfield & Springthorpe, 2012; He *et al.*, 2014; Auge *et al.*, 2015; Burghardt *et al.*, 2016; Edwards *et al.*, 2016; Leverett *et al.*, 2016). Changes in the maternal environment can do so even if developing seeds themselves do not experience those changes, as observed here. In contrast to the pattern we observed in the diverse ecotypes, a previous study of the L*er* genotype showed that plants grown at lower temperatures before bolting produced seeds that were more dormant than seeds from plants grown at higher temperatures (Chen *et al.*, 2014). This maternal effect was shown to be mediated by the gene *FT* and by regulation of synthesis of proanthocyanidins (PAs) in the seed coats, such that increased PA synthesis was associated with decreased seed-coat permeability and higher dormancy levels (Chen *et al.*, 2014; MacGregor *et al.*, 2015). It should be noted that the temperature used in that experiment to assess maternal effects on germination (16°C) could be well above the temperature threshold for vernalization (Wollenberg & Amasino, 2012), and was much higher than that used in our study. Thus, vernalization *per se* may influence seed germination differently from lower ambient temperature.

Maternal vernalization affects the life cycle in two distinct ways: a short-term effect on flowering time, and a longer-term effect on progeny germination behavior. The combined effects probably influence the seasonal expression of life cycles under natural conditions. Based on our results, winter annuals that experience vernalization are expected to produce seeds with lower dormancy and potentially earlier germination. This would be particularly important in locations where the growing season is short and in which fast germination could permit the establishment of cold-hardy rosettes before the onset of winter. By contrast, spring germinants that do not experience vernalization are expected to produce seeds with more dormancy and potentially later germination. Plants that germinate and flower in autumn, and thereby do not experience vernalization, are expected to produce seeds with higher dormancy as well, potentially postponing germination until the following spring or later. Field studies across a geographic range are necessary to test how natural seasonal conditions influence life-cycle expression in diverse ecotypes, and the conditions and locations under which these life histories would be adaptive (if any). The lack of association between flowering and germination responses to vernalization, and the fact that not every ecotype here tested exhibited a germination response to vernalization, however, suggests some potential for the independent adaptation of vernalization responses at these two life stages.

Some flowering-time vernalization genes antagonize the observed germination response to vernalization, and others promote it

In the Col background, many genes in the flowering-time vernalization pathway seem to oppose the vernalization response that was observed in the diverse ecotypes, since active forms of those genes promoted germination of seeds from nonvernalized plants or repressed germination of seeds from vernalized plants. Specifically, active *FLC* and *FRI* enhanced germination of seeds from nonvernalized plants, in opposition to the observed effect of vernalization on germination observed in the ecotypes, but consistent with previous reports on the effect of *FLC* on germination (Chiang *et al.*, 2009). However, when *FLC* was not functional, active *FRI* weakly inhibited germination of seeds from nonvernalized plants, consistent with the effect of vernalization observed in the ecotypes.

VIP3 and *VIN3* both seem to impede germination of seeds from vernalized plants, since their disruption led to higher germination of those seeds. As with *FRI* and *FLC*, this effect is in opposition to the effect of vernalization on germination observed in the ecotypes. By contrast, *VRN2* appears to promote germination of seeds from vernalized plants when incubated at 22°C, which reflects the vernalization response of the ecotypes (but not when incubated at 10°C).

In summary, most genes in the flowering-time vernalization pathway that we studied in the Col background appear to oppose the vernalization response that we observed in the ecotypes. Only *VRN2* (at 22°C) strongly contributed to that pattern, while *FRI* contributed weakly but only when *FLC* was not functional. These results suggest that the ecotypes that exhibited increased germination after maternal vernalization may have the function of *FLC*, *VIP3* or *VIN3* disrupted. Alternatively, even if those genes are functional in those responsive ecotypes, the direction of effects of those genes may depend on alleles at other genes contained in those ecotypes, just as *FRI* suppressed the observed germination response to vernalization when *FLC* was functional, but promoted it when *FLC* was nonfunctional.

Pleiotropic vernalization genes do not always act in the same manner at different life stages

Pleiotropy can constrain adaptive evolution, since the evolution of one trait causes correlated evolution of the other trait, sometimes in a maladaptive direction (Fisher, 1958; Wagner, 1988; Barton, 1990; Crespi, 2000; Orr, 2000; Griswold & Whitlock, 2003; Walsh & Blows, 2009). For traits that are regulated by common genetic pathways of environmental perception and response, it is expected that environmental responses of one developmental transition would be associated with responses of other developmental transitions. In this manner, the coordination of environment-dependent development across the life cycle could be constrained. If those pathways diverge soon after the environmental inputs, however, or if genes are regulated differently in distinct life stages, each life stage could respond independently to the same environmental cue, allowing greater flexibility of life-cycle regulation in response to environmental conditions.

Although genes in the vernalization flowering pathway had pleiotropic effects on germination, the contribution of those genes to germination was not always consistent with their function in the flowering pathway as regulators of *FLC* expression. Previous work has demonstrated that increased *FLC* activity is

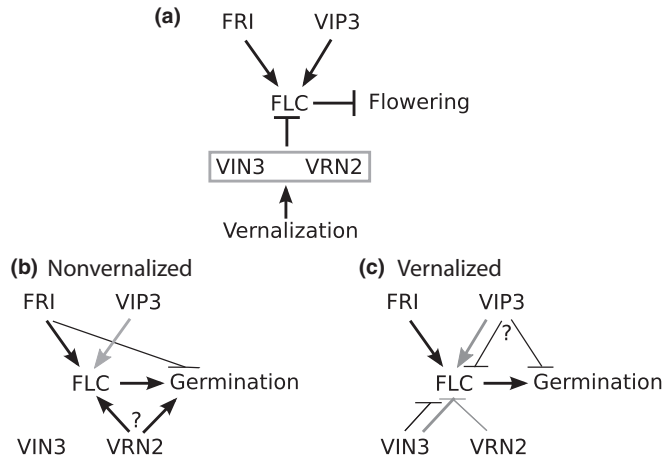

Fig. 5 The vernalization flowering-time pathway, and how it influences germination. (a) The vernalization flowering pathway, showing how the genes used in this study influence *FLC* expression during the transition to flowering, based on previously published data. (b, c) Summary of the effects of genes in the vernalization pathway on germination of seeds from nonvernalized and vernalized plants, based on this study. Gray solid arrows indicate expected results based on how the genes influence flowering. Black solid arrows indicate the pathways inferred from observed results. '?' denotes ambiguity of the results in distinguishing between the two potential pathways.

associated with higher germination (Chiang *et al.*, 2009). If vernalization genes influence germination by regulating *FLC* expression in the same manner as they do in the transitions to flowering, the expectation is that mutational disruption of enhancers of *FLC* expression – *FRI* and *VIP3* – would result in reduced germination while disruption of repressors of *FLC* expression – *VIN3* and *VRN2* – would increase germination (Fig. 5).

As predicted, disruption of *FRI* decreased germination, suggesting that *FRI* has a conserved molecular function at both the pre-reproductive and the seed-maturation stage (Fig. 5). This interpretation is supported by the observation that the effect of *FRI* was greater in nonvernalized plants, in which the effect of *FLC* was also evident. In agreement with this observation, ecotypes with functional *FRI* and *FLC* alleles showed higher germination in seeds from nonvernalized plants (Fig. S3; see Notes S1 for a more detailed account of the responses by allele functionality). However, *FRI* also appears to act through another weaker pathway that is independent of its role in regulating *FLC* expression, since mutational disruption of *FRI* altered germination even when *FLC* was nonfunctional. Interestingly, this alternative pathway influences germination in the opposite direction as *FLC*, repressing germination in nonvernalized plants. Also, as predicted, disruption of *VIN3* increased germination. However, the effect of *VIN3* was apparent only in seeds from vernalized plants, in which *FLC* did not influence germination (Fig. 5).

Contrary to prediction based on the function of *VIP3* as an enhancer (Zhang *et al.*, 2003) and *VRN2* as a repressor (Bastow *et al.* 2004) of *FLC* expression in the flowering pathway, disruption of *VIP3* increased germination, and disruption of *VRN2* decreased germination (Fig. 2). These results suggest that these genes may influence germination through pathways independently of *FLC* (Fig. 5). Alternatively, the environmental regulation of these genes may differ between pre-reproductive and dormancy-inducing life stages, a possibility that is consistent with the fact that pre-reproductive responses of gene expression to vernalization did not reflect the response of gene expression at the stage that imposes seed dormancy. Another possibility is that these genes may regulate *FLC* expression in opposing directions at the pre-reproductive vs seed-maturation stage. Further studies determining the relationship between *VIP3* or *VRN2* function and *FLC* expression would be needed to distinguish among these possibilities.

Conclusions

Environments experienced at one life stage can have persistent effects on subsequent life stages. In diverse ecotypes, vernalization experienced by maternal plants increased seed germination of progeny, even though those seeds never experienced the low temperature. Moreover, many genes in the vernalization flowering pathway also influenced seed germination and its responses to maternal vernalization. Except for *VRN2*, functional alleles of most of these genes seem to negatively affect the germination response to vernalization that was observed in the ecotypes, either by enhancing germination of seeds from nonvernalized plants or by impeding germination of seeds from vernalized plants. Some genes influenced germination in a manner that is consistent with their known effects of *FLC* regulation during the transition to flowering. Others did not, suggesting that they influence germination by regulating *FLC* differently at different life stages or by acting in pathways that are independent of *FLC*. Therefore, despite persistent effects of environmental conditions across life stages, and despite pleiotropy of genes that affect both flowering and germination, the function of these genes and the pathways through which they act can differ across development. Differences in gene functions across developmental stages, whether through differences in stage-specific regulation of gene expression or through interactions with stage-specific genetic pathways, could act to mitigate pleiotropic constraints and enable more independent expression and environmental regulation of different life stages. In seasonal environments, such independent regulation of different life stages could be critical for expressing adaptive life cycles.

Acknowledgements

We thank Brianne Edwards and numerous undergraduates for organizational and technical help. Duke University Phytotron staff provided excellent care of the plants. The work benefitted from discussions and suggestions from Lindsay Leverett, Brianne Edwards, Michelle D'Aguillo and Britany Morgan. This work was funded by grant NSF-IOS-11-46383 to K.D.

Author contributions

G.A.A. and K.D. designed experiments. G.A.A. directed the experiments. G.A.A., L.K.B. and H.N. conducted the

experiments. G.A.A. analyzed the data. G.A.A., L.K.B., H.N. and K.D. interpreted the data. G.A.A. led the writing of the manuscript. L.K.B. and K.D. contributed to the writing of the manuscript. K.D. supervised the project.

References

Amasino R. 2004. Vernalization, competence, and the epigenetic memory of winter. *Plant Cell* **16**: 2553–2559.

Atchley WR. 1984. Ontogeny, timing of development, and genetic variance–covariances structure. *American Naturalist* **123**: 519–540.

Atwell S, Huang YS, Vilhjálmsson BJ, Willems G, Horton M, Li Y, Meng D, Platt A, Tarone AM, Hu TT *et al.* 2010. Genome-wide association study of 107 phenotypes in *Arabidopsis thaliana* inbred lines. *Nature* **465**: 627–631.

Auge GA, Blair LK, Burghardt LT, Coughlan J, Edwards B, Leverett LD, Donohue K. 2015. Secondary dormancy dynamics depends on primary dormancy status in *Arabidopsis thaliana*. *Seed Science Research* **25**: 230–246.

Barton NH. 1990. Pleiotropic models of quantitative variation. *Genetics* **124**: 773–782.

Baskin CC, Baskin JM. 2001. *Seeds: ecology, biogeography, and evolution of dormancy and germination*. San Diego, CA, USA: Academic Press.

Bastow R, Mylne JS, Lister C, Lippman Z, Martienssen RA, Dean C. 2004. Vernalization requires epigenetic silencing of FLC by histone methylation. *Nature* **427**: 164–167.

Brakefield PM. 2006. Evo-devo and constraints on selection. *Trends in Ecology & Evolution* **21**: 362–368.

Burghardt LT, Edwards BR, Donohue K. 2016. Multiple paths to similar germination behavior in *Arabidopsis thaliana*. *New Phytologist* **209**: 1301–1312.

Burghardt LT, Metcalf CJE, Wilczek AM, Schmitt J, Donohue K, Gaillard AEJ-M, Kalisz ES. 2015. Modeling the influence of genetic and environmental variation on the expression of plant life cycles across landscapes. *American Naturalist* **185**: 212–227.

Chen M, MacGregor DR, Dave A, Florance H, Moore K, Paszkiewicz K, Smirnoff N, Graham IA, Penfield S. 2014. Maternal temperature history activates *FLOWERING LOCUS T* in fruits to control progeny dormancy according to time of year. *Proceedings of the National Academy of Sciences, USA* **111**: 18787–18792.

Chiang GCK, Barua D, Kramer EM, Amasino RM, Donohue K. 2009. Major flowering time gene, *FLOWERING LOCUS C*, regulates seed germination in *Arabidopsis thaliana*. *Proceedings of the National Academy of Sciences, USA* **106**: 11661–11666.

Choi J, Hyun Y, Kang M-J, In Yun H, Yun J-Y, Lister C, Dean C, Amasino RM, Noh B, Noh Y-S *et al.* 2009. Resetting and regulation of *FLOWERING LOCUS C* expression during Arabidopsis reproductive development. *Plant Journal* **57**: 918–931.

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

Crespi BJ. 2000. The evolution of maladaptation. *Heredity* **84**: 623–629.

Donohue K. 2009. Completing the cycle: maternal effects as the missing link in plant life histories. *Philosophical Transactions of the Royal Society of London B: Biological Sciences* **364**: 1059–1074.

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

Donohue K, Schmitt J 1998. Maternal environmental effects in plants: adaptive plasticity. In: Mousseau TA, Fox WC, eds. *Maternal effects as adaptations*. New York, NY, USA: Oxford University Press, 137–158.

Edwards BR, Burghardt LT, Zapata-Garcia M, Donohue K. 2016. Maternal temperature effects on dormancy influence germination responses to water availability in *Arabidopsis thaliana*. *Environmental and Experimental Botany* **126**: 55–67.

Ehrlén J. 2015. Selection on flowering time in a life-cycle context. *Oikos* **124**: 92–101.

English S, Pen I, Shea N, Uller T. 2015. The information value of non-genetic inheritance in plants and animals. *PLoS ONE* **10**: e0116996.

Fisher R. 1958. *The genetical theory of natural selection*. New York, NY, USA: Oxford University Press.

Fox J, Weisberg S. 2010. *An R companion to applied regression*. Los Angeles, CA, USA: Sage Publications.

Gendall AR, Levy YY, Wilson A, Dean C. 2001. The *VERNALIZATION 2* gene mediates the epigenetic regulation of vernalization in *Arabidopsis*. *Cell* **107**: 525–535.

Griswold CK, Whitlock MC. 2003. The genetics of adaptation: the roles of pleiotropy, stabilizing selection and drift in shaping the distribution of bidirectional fixed mutational effects. *Genetics* **165**: 2181–2192.

Gutterman Y. 1982. Phenotypic maternal effect of photoperiod on seed germination. In: Khan AA, ed. *The physiology and biochemistry of seed development, dormancy and germination*. New York, NY, USA: Elsevier Biomedical Press, 67–79.

He H, Vidigal DdeS, Snoek LB, Schnabel S, Nijveen H, Hilhorst H, Bentsink L. 2014. Interaction between parental environment and genotype affects plant and seed performance in Arabidopsis. *Journal of Experimental Botany* **65**: 6603–6615.

Holdsworth MJ, Bentsink L, Soppe WJJ. 2008. Molecular networks regulating Arabidopsis seed maturation, after-ripening, dormancy and germination. *New Phytologist* **179**: 33–54.

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

Lempe J, Balasubramanian S, Sureshkumar S, Singh A, Schmid M, Weigel D. 2005. Diversity of flowering responses in wild *Arabidopsis thaliana* strains. *PLoS Genetics* **1**: e6.

Leverett LD, Auge GA, Bali A, Donohue K. 2016. Contrasting germination responses to vegetative canopies experienced in pre- versus post-dispersal environments. *Annals of Botany* **118**: 1175–1186.

Livak KJ, Schmittgen TD. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods* **25**: 402–408.

Lovell JT, Juenger TE, Michaels SD, Lasky JR, Platt A, Richards JH, Yu X, Easlon HM, Sen S, McKay JK. 2013. Pleiotropy of *FRIGIDA* enhances the potential for multivariate adaptation. *Proceedings of the Royal Society B: Biological Sciences* **280**: 20131043.

MacGregor DR, Kendall SL, Florance H, Fedi F, Moore K, Paszkiewicz K, Smirnoff N, Penfield S. 2015. Seed production temperature regulation of primary dormancy occurs through control of seed coat phenylpropanoid metabolism. *New Phytologist* **205**: 642–652.

Marshall DJ, Uller T. 2007. When is a maternal effect adaptive? *Oikos* **116**: 1957–1963.

Michaels SD, Amasino RM. 1999. *FLOWERING LOCUS C* encodes a novel MADS domain protein that acts as a repressor of flowering. *Plant Cell* **11**: 949–956.

Michaels SD, Bezerra IC, Amasino RM. 2004. *FRIGIDA*-related genes are required for the winter-annual habit in Arabidopsis. *Proceedings of the National Academy of Sciences, USA* **101**: 3281–3285.

Michaels SD, He Y, Scortecci KC, Amasino RM. 2003. Attenuation of *FLOWERING LOCUS C* activity as a mechanism for the evolution of summer-annual flowering behavior in Arabidopsis. *Proceedings of the National Academy of Sciences, USA* **100**: 10102–10107.

Michaels SD, Himelblau E, Kim SY, Schomburg FM, Amasino RM. 2005. Integration of flowering signals in winter-annual Arabidopsis. *Plant Physiology* **137**: 149–156.

Munguía-Rosas MA, Ollerton J, Parra-Tabla V, De-Nova JA. 2011. Meta-analysis of phenotypic selection on flowering phenology suggests that early flowering plants are favoured. *Ecology Letters* **14**: 511–521.

Orr HA. 2000. Adaptation and the cost of complexity. *Evolution* **54**: 13–20.

Penfield S, Springthorpe V. 2012. Understanding chilling responses in Arabidopsis seeds and their contribution to life history. *Philosophical Transactions of the Royal Society B: Biological Sciences* **367**: 291–297.

R Core Team 2015. *R: a language and environment for statistical computing.* Vienna, Austria: R Foundation for Statistical Computing. [WWW document] URL https://www.R-project.org/ [accessed 14 March 2017]

Ream TS, Woods DP, Amasino RM. 2012. The molecular basis of vernalization in different plant groups. *Cold Spring Harbor Symposia on Quantitative Biology* **77**: 105–115.

Sheldon CC, Rouse DT, Finnegan EJ, Peacock WJ, Dennis ES. 2000. The molecular basis of vernalization: the central role of *FLOWERING LOCUS C* (*FLC*). *Proceedings of the National Academy of Sciences, USA* **97**: 3753–3758.

Shindo C, Aranzana MJ, Lister C, Baxter C, Nicholls C, Nordborg M, Dean C. 2005. Role of *FRIGIDA* and *FLOWERING LOCUS C* in determining variation in flowering time of Arabidopsis. *Plant Physiology* **138**: 1163–1173.

Shindo C, Lister C, Crevillen P, Nordborg M, Dean C. 2006. Variation in the epigenetic silencing of *FLC* contributes to natural variation in Arabidopsis vernalization response. *Genes & Development* **20**: 3079–3083.

Simpson GG, Dean C. 2002. Arabidopsis, the Rosetta Stone of flowering time? *Science* **296**: 285–289.

Sung S, Amasino RM. 2004. Vernalization in *Arabidopsis thaliana* is mediated by the PHD finger protein VIN3. *Nature* **427**: 159–164.

Wagner GP. 1988. The influence of variation and of developmental constraints on the rate of multivariate phenotypic evolution. *Journal of Evolutionary Biology* **1**: 45–66.

Wagner GP, Kenney-Hunt JP, Pavlicev M, Peck JR, Waxman D, Cheverud JM. 2008. Pleiotropic scaling of gene effects and the 'cost of complexity'. *Nature* **452**: 470–472.

Walsh B, Blows MW. 2009. Abundant genetic variation + strong selection = multivariate genetic constraints: a geometric view of adaptation. *Annual Review of Ecology, Evolution, and Systematics* **40**: 41–59.

Wollenberg AC, Amasino RM. 2012. Natural variation in the temperature range permissive for vernalization in accessions of *Arabidopsis thaliana*. *Plant, Cell & Environment* **35**: 2181–2191.

Zhang H, Ransom C, Ludwig P, van Nocker S. 2003. Genetic analysis of early flowering mutants in Arabidopsis defines a class of pleiotropic developmental regulator required for expression of the flowering-time switch *FLOWERING LOCUS C*. *Genetics* **164**: 347–358.

Supporting Information

Additional Supporting Information may be found online in the Supporting Information tab for this article:

Fig. S1 Map showing geographical origin of the Eurasian ecotypes used in this study.

Fig. S2 Heatmap showing the mean germination response of seeds from vernalized plants (V) compared with seeds from non-vernalized plants (NV) during after-ripening.

Fig. S3 Effect of vernalization of the mother plant on the germination response of the ecotypes, grouped according to the functionality and combination of *FRI* and *FLC* alleles.

Fig. S4 Gene expression levels during development in nonvernalized (NV) and vernalized (V) plants, using *PP2AA3* as reference gene.

Table S1 Information on the ecotypes used in this study

Table S2 Information on the near-isogenic lines (NILs) and mutants used in this study

Table S3 Test for effects of maternal vernalization on seed germination in diverse ecotypes

Table S4 Correlations with germination response to vernalization

Table S5 Effect of the combination and functionality of *FRI* and *FLC* alleles (FRI/FLC) and their interactions with maternal vernalization (Vern), after-ripening time (AR) and incubation temperature (Temp) in diverse ecotypes

Table S6 Test for allelic effects on seed germination of ecotypes grouped by FRI/FLC allele functionality

Table S7 Test for interactions of mutational effects and environmental treatments

Notes S1 FRI/FLC functionality in diverse ecotypes.

Please note: Wiley Blackwell are not responsible for the content or functionality of any Supporting Information supplied by the authors. Any queries (other than missing material) should be directed to the *New Phytologist* Central Office.