Major flowering time gene, *FLOWERING LOCUS C*, regulates seed germination in *Arabidopsis thaliana*

George C. K. Chiang[a,1], Deepak Barua[a], Elena M. Kramer[a], Richard M. Amasino[b], and Kathleen Donohue[a,c,1]

[a]Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, MA 02138; [b]Department of Biochemistry, University of Wisconsin, Madison, WI 53706; and [c]Department of Biology, Duke University, Durham, NC 27708

Edited by Maarten Koornneef, Wageningen University and Research Centre, Wageningen, The Netherlands, and approved May 22, 2009 (received for review February 6, 2009)

***FLOWERING LOCUS C* (*FLC*) is a major regulator of flowering responses to seasonal environmental factors. Here, we document that *FLC* also regulates another major life-history transition-seed germination, and that natural variation at the *FLC* locus and in *FLC* expression is associated with natural variation in temperature-dependent germination. *FLC*-mediated germination acts through additional genes in the flowering pathway (*FT*, *SOC1*, and *AP1*) before involving the abscisic acid catabolic pathway (via *CYP707A2*) and gibberellins biosynthetic pathway (via *GA20ox1*) in seeds. Also, *FLC* regulation of germination is largely maternally controlled, with *FLC* peaking and *FT*, *SOC1*, and *AP1* levels declining at late stages of seed maturation. High *FLC* expression during seed maturation is associated with altered expression of hormonal genes (*CYP707A2* and *GA20ox1*) in germinating seeds, indicating that gene expression before the physiological independence of seeds can influence gene expression well after any physical connection between maternal plants and seeds exists. The major role of *FLC* in temperature-dependent germination documented here reveals a much broader adaptive significance of natural variation in *FLC*. Therefore, pleiotropy between these major life stages likely influences patterns of natural selection on this important gene, making *FLC* a promising case for examining how pleiotropy influences adaptive evolution.**

life history | pleiotropy | vernalization | natural variation | *FRIGIDA*

One goal toward understanding the genetic basis of adaptation is to accurately interpret geographic patterns of genetic variation in terms of local adaptation. A major challenge in this agenda is to determine the relationship between variable genes and the phenotypes that are exposed to natural selection. A single gene may influence >1 trait, and this pleiotropy may be a considerable constraint on adaptive evolution. The extent to which pleiotropy actually constrains local adaptation depends on how divergent or convergent the complete pathways that regulate the expression of different traits are. Therefore, it is necessary to evaluate the extent of pleiotropy expressed by naturally variable genes, and to characterize the degree of convergence in pathways that regulate the expression of different traits under selection. In this study, we found that the gene *FLOWERING LOCUS C* (*FLC*) (1, 2), which has previously been shown to be associated primarily with the developmental transition to reproduction, is also strongly associated with the earlier developmental transition of germination.

Among the best characterized genetic pathways regulating a complex life-history trait in plants is the flowering pathway of *Arabidopsis thaliana* (3–6). A central gene in this pathway is *FLC* (1, 2), which represses several loci that promote flowering. *FLC* repression of flowering is relieved by an epigenetic switch that represses the expression of *FLC* itself via chromatin remodeling (5). *FLC* repression can occur after plants experience an extended period of cold (vernalization) (7, 8), or, alternatively, by the autonomous pathway, which senses plant age and ambient temperature (5, 9). It has been established that *FLC* expression is promoted by the gene *FRIGIDA* (*FRI*), although the exact biochemical basis of the function of *FRI* remains unknown (8). As a MADS-box transcription factor (so named for *MCM1*, *AGAMOUS*, *DEFICIENS*, and serum-response factor), *FLC* directly binds to and inhibits expression of the downstream genes of *SOC1* and *FT* (10, 11). The repression of these genes, in turn, delays expression of the floral meristem identity genes *LEAFY* (*LFY*) and *APETALA1* (*AP1*) to prolong the vegetative phase (12–15). Natural allelic variants of *FLC* are associated with variation in flowering time, either singly (16–18) or in combination with natural loss of function alleles of *FRI* (1, 2, 19–21). Variation in these 2 genes has been shown to account for major differences between the winter annual and the rapid-cycling life histories of *Arabidopsis*. Because *FLC* has such a large effect on flowering, the maintenance of natural variation in *FLC* has been thought to be primarily due to geographically variable natural selection on flowering time (19, 22–26). Therefore, *FLC* is unique for ecological studies in that it is one of very few genes for which we have molecular pathway data, natural variation data, and abundant hypotheses concerning how the two are related.

Although much is known about the seasonal and genetic regulation of flowering time, far less is known about the ecological and genetic basis of germination, an earlier life-history developmental transition (27, 28). However, germination timing is under intense natural selection; it has been shown to account for >70% of the variation in fitness among genotypes in an experimental population of recombinant inbred lines (29), and it is the life-stage transition that has the largest effect on projected population growth rates in natural populations of *A. thaliana* (30). Also, new evidence suggests that the seasonal timing of germination might be a stronger factor in determining the flowering time of *Arabidopsis* in the field than variation in the genetic basis of flowering time itself (31). As sessile organisms, the primary way plants determine the seasonal environment of their establishment and subsequent growth is through germination cuing. Therefore, genes associated with natural variation in germination are likely to be under strong natural selection.

Germination and flowering are both fundamental developmental transitions that require precise environmental sensing and responses to multiple seasonal cues to accurately match developmental timing to appropriate seasonal conditions. The combination of these 2 phenological traits determines the overall life cycle and generation time of many plants, including *A. thaliana* (32–35). Because both transitions respond to similar seasonal cues, it is logical to hypothesize that genetic pathways

Author contributions: G.C.K.C., D.B., E.M.K., R.M.A., and K.D. designed research; G.C.K.C. and D.B. performed research; G.C.K.C., D.B., E.M.K., and K.D. analyzed data; and G.C.K.C., D.B., E.M.K., R.M.A., and K.D. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

[1]To whom correspondence may be addressed. E-mail: ckchiang@fas.harvard.edu or k.donohue@duke.edu.

This article contains supporting information online at www.pnas.org/cgi/content/full/0901367106/DCSupplemental.

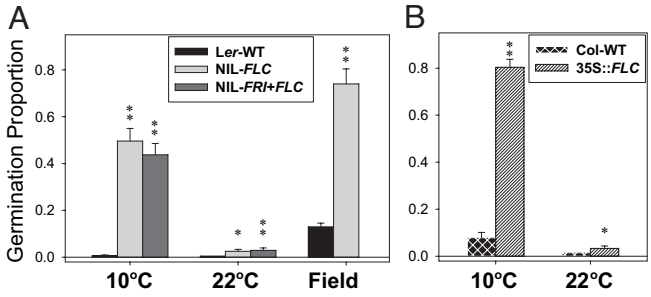

Fig. 1. Germination of high- and low-expressing *FLC* alleles. Proportion of viable seeds that germinated for different NILs (*A*), and for WT and a 35S::*FLC* overexpressor (*B*). Mean proportion of fresh seed germination (±1 SE) at 10 °C, 22 °C, or natural autumn field dispersal conditions (Field). Significance levels indicate comparisons with WT genotypes. The 2 NILs (NIL-FLC_{Cvi} and NIL-FRI_{Sf}+FLC_{Col}) do not differ under any conditions. *, $P < 0.05$; **, $P < 0.01$.

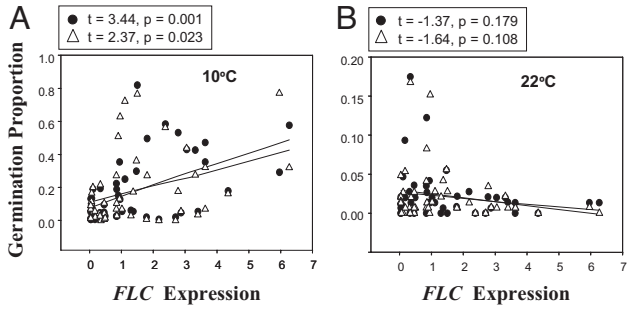

Fig. 2. Relationship of *FLC* expression to proportion germination at 10 vs. 22 °C for 52 ecotypes. Seeds were matured at short days (filled circles: 10-h light, 14-h dark) and long days (open triangles: 14-h light, 10-h dark) at 22 °C. Fresh seeds were exposed to 2 germination temperatures: 10 °C (*Left*) or 22 °C (*Right*). The photoperiod of seed maturation had no effect on germination ($P \gg 0.05$). The effect of *FLC* was significantly stronger in the 10 °C than 22 °C: short days, F(*FLC* × seed treatment) = 18.26, $P < 0.0001$; long days, F(*FLC* × seed treatment) = 18.04, $P = 0.0057$.

of these 2 life-history transitions share common elements. However, each developmental transition necessarily occurs during different seasons, so some divergence in their regulation is also required. Here, we report that a major regulator of flowering, *FLC*, controls the germination behavior of seeds in a temperature-dependent fashion, and that natural variation in *FLC* alleles and *FLC* expression is associated with natural variation in temperature-dependent germination. Also, we showed that *FLC* regulation of germination is largely maternally controlled and occurs at later stages of seed maturation, and that the signal is transmitted through the downstream pathway involving *FT*, *SOC1*, and *AP1* to connect with the abscisic acid (ABA) and gibberellins (GA) germination pathways in seeds.

Results

***FLC* Controls Temperature-Dependent Seed Germination.** Natural genetic variants and genetic constructs of *A. thaliana* with high levels of *FLC* expression exhibited significantly higher germination percentages at cool (10 °C), but not warm (22 °C), temperatures (Fig. 1). We used near isogenic lines (NILs) containing different natural alleles of *FLC* in the Landsberg *erecta* (L*er*) background that naturally has a low-expressing *FLC* allele (16) and a nonfunctional *FRI* allele. When seeds were matured under cool temperatures (15 °C), resembling ecologically realistic seed maturation conditions during spring or autumn, the L*er* background line exhibited high dormancy. This dormancy was observed when seeds were imbibed at either cool or warm temperatures (in a 12-h light/12-h dark cycle for 15 days). In contrast, a line containing an introgressed high-expressing *FLC* allele (17) from the Cape Verde Island ecotype (NIL-FLC_{Cvi}) had a significantly higher proportion of germination when seeds were imbibed at cool temperatures, but only slightly higher germination at warmer temperature (Fig. 1*A*). Although the difference of germination at 22 °C between L*er* and NIL-FLC_{Cvi} was statistically significant, the increase was extremely small so it is not likely to be biologically relevant. A similar effect on germination was seen in a second high-expressing *FLC* NIL that contained the *FLC* allele from the Columbia (Col) ecotype and a functional *FRI* allele from the San Feliu ecotype (NIL-FRI_{Sf}+FLC_{Col}). Therefore, the observed germination phenotype of increased germination at cool, but not at warm temperature may be caused by highly expressed natural *FLC* alleles. Also, *FLC*-mediated germination apparently does not require functional *FRI*, at least with the *FLC* allele from the Cape Verde Island ecotype (Cvi), as indicated by the similar germination phenotype of the 2 NILs (one that has a functional *FRI* and one that does not), even though *FLC* expression in the seedling stage is >5 times greater with the functional *FRI* allele (Fig. S1).

To examine whether this temperature-dependent, *FLC*-mediated germination behavior is also seen in a natural seasonal environment, freshly matured seeds of NIL-FLC_{Cvi} and its background L*er*-WT were dispersed into a field site in Bedford, Massachusetts, during mid October when the daily temperature was fluctuating ≈10 °C (average daily high and low: 14 and 4 °C). The NIL-FLC_{Cvi} germinated to significantly higher proportions during October and November compared with its background L*er*-WT (Fig. 1*A*). Therefore, *FLC* has the potential to be a major influence on germination of *A. thaliana* under complex natural field conditions.

We verified that *FLC* was causally implicated in germination, as opposed to another gene within the introgressed regions of the NILs, by examining the germination of a transgenic line overexpressing *FLC* under the control of the constitutive 35S promoter (35S::*FLC*) (36). The *FLC*-overexpressor exhibited much higher germination compared with the Col background genotype (Fig. 1*B*) at cool (10 °C), but not at warm (22 °C) temperatures, as in the NILs. This mediation of germination by high levels of *FLC* expression could be initiated with any combination of sequential temperature fluctuations resembling seasonal changes that involve a cold period (Fig. S2), and the germination proportion increased with an increased duration of cold, and could be saturated with 3 days of 10 °C (Fig. S3). Also, *FLC*-promoted germination required light (Fig. S2).

Natural Variation in *FLC* Expression Is Associated with Natural Variation in Germination. Further implication of *FLC* in germination at cool temperature was apparent from a survey of 52 ecotypes collected from the native range of *A. thaliana* in Eurasia and Africa. Because photoperiod and temperature both vary seasonally, we matured seeds under long day (14-h light/10-h dark) and short day (10-h light/14-h dark) conditions, and measured germination of seeds imbibed at 10 and 22 °C (in a 12-h light/12-h dark cycle). Using published data on levels of *FLC* expression in vegetative rosettes (20), and controlling for population structure (37), we found that higher *FLC* expression was significantly associated with increased germination when seeds were imbibed at 10 °C, but not at 22 °C (Fig. 2), consistent with the results from the NILs and overexpressor lines. We found no effect of photoperiod during seed maturation on the relationship between *FLC* and germination. These results suggest that variation in *FLC* expression is associated with natural variation in germination at low temperature. This knowledge greatly revises our expectations of natural selection on *FLC* and our interpretation

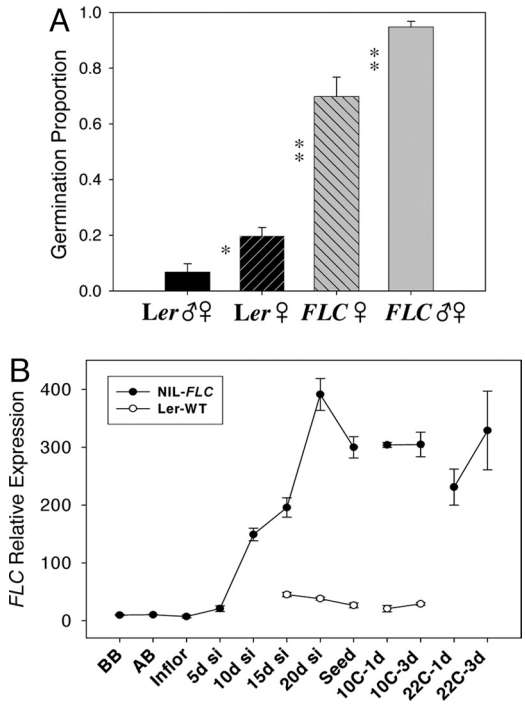

Fig. 3. Maternal control of *FLC*-mediated germination. (*A*) Proportion of viable seed germination (±1 SE) at 10 °C for fresh seeds from reciprocal crosses (L*er*♀ = L*er*-WT ♀ × NIL-*FLC*$_{Cvi}$ ♂; FLC♀ = NIL-*FLC*$_{Cvi}$ ♀ × L*er*-WT ♂; ♂♀ = self-cross). Asterisks indicate significant differences between 2 adjacent bars. *, $P < 0.05$; **, $P < 0.01$. (*B*) Relative *FLC* expression (±1 SE, normalized by *ACT8*) at different stages during development of NIL-*FLC*$_{Cvi}$ (closed circles) and L*er*-WT (open circles). BB, rosette before bolting; AB, rosette after bolting; Inflor, inflorescence; 5≈20d si, 5≈20-day-old siliques after flower opening; Seed, fresh dry seed; 10C-1d, 1 day of imbibition at 10 °C. The peak of *FLC* expression in NIL-*FLC*$_{Cvi}$ is significantly different ($P < 0.05$) from all other time points except those in seeds, 10 °C-1d, 10 °C-3d, and 22 °C-3d, based on Tukey's post hoc tests.

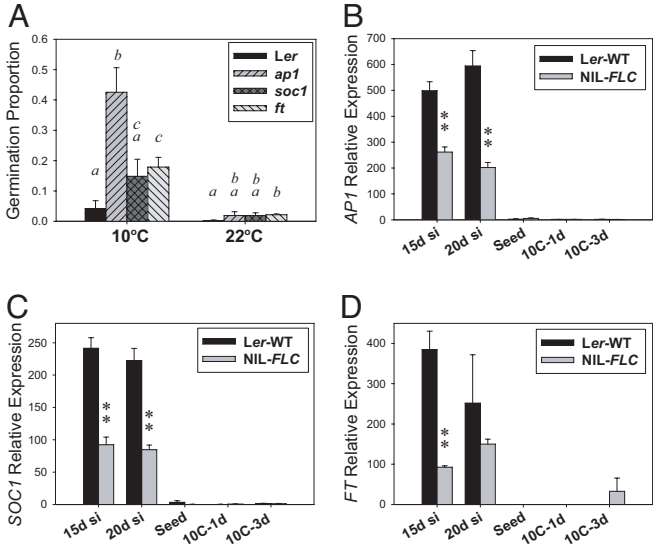

Fig. 4. A shared pathway between germination and flowering. (*A*) Proportion of viable seed germination (±1 SE) of L*er* background and mutants in *AP1*, *SOC1*, and *FT* for seeds imbibed at 10 and 22 °C. Nonoverlapping letters (a–c) indicate significant difference within each germination temperature based on Tukey's post hoc tests. (*B–D*) Relative expression normalized by *ACT8* (±1 SE) of *AP1*, *SOC1*, and *FT* in NIL-*FLC*$_{Cvi}$ compared with L*er*-WT at different stages of seed development. **, $P < 0.01$.

of the geographic distribution of *FLC* variation, which have primarily focused on the influence of *FLC* on flowering time.

Maternal Determination of the *FLC* Germination Phenotype. To determine whether *FLC*-mediated germination is controlled maternally or solely by the embryonic genotype, reciprocal crosses were performed between L*er*-WT and NIL-*FLC*$_{Cvi}$, and between Col-WT and the 35S::*FLC* overexpressor (Fig. 3*A*; Figs. S4 and S5). If *FLC* regulation of germination is exclusively under embryonic control, germination should not differ between the reciprocal heterozygous offspring, because they both would have one copy of the stronger *FLC*-allele. In contrast, if *FLC*-regulated germination is under maternal control, heterozygotes derived from the strong *FLC* maternal plants would have higher germination at cool temperatures than heterozygotes derived from weak *FLC* maternal plants. The results indicate that *FLC*-mediated germination is largely controlled by the maternal genotype, because heterozygotes containing a high-*FLC*-expressing maternal allele had higher germination at 10 °C than heterozygotes containing a high-*FLC*-expressing paternal allele. This maternal control of *FLC*-mediated germination could be through either *FLC* expression in the maternal tissue (in siliques or funiculus) or preferential expression of maternal *FLC* alleles in the seeds.

The asymmetrical contribution of the maternal *FLC* allele, observed from the reciprocal crosses, suggested that the effect of the high-expressing *FLC* allele on germination may be most prevalent during seed maturation when the physical connection between the maternal plant and the developing seed is strong. To further narrow down the possible developmental stages when this *FLC* effect might be imposed, *FLC* expression of the NIL-*FLC*$_{Cvi}$ was followed throughout the life cycle, especially during silique development (seed maturation) and seed germination. *FLC* expression increased substantially midway through silique development, and peaked at the later stage of seed development when the siliques were starting to turn yellow before seed dispersal (Fig. 3*B*). This stage is also when prominent dormancy genes, such as *DOG1*, are most highly expressed and when primary dormancy is induced (38, 39). *FLC* mRNA levels decreased subsequently, but remained relatively high in dry seeds and in seeds imbibed at both 10 and 22 °C, with no obvious pattern of change that correlated with germination differences. *FLC* expression in the L*er*-WT remained relatively low during late silique development, dry seed, and seed imbibitions stages, and the pattern of an *FLC* expression peak late in silique development was not seen, in contrast to the pattern found in NIL-*FLC*$_{Cvi}$ (Fig. 3*B*).

Parallel Pathways of *FLC*-Mediated Flowering and Germination. In the flowering pathway, *FLC* represses the genes *FT* and *SOC1*, causing low expression of *AP1* and consequently inhibiting flowering. If *FLC* regulation of germination shares this pathway, mutants in these genes should recapitulate the germination phenotype seen in high *FLC*-expressing genotypes; namely, higher germination at low, but not warm temperatures. Supporting this scenario, the *ap1-1* mutant, which is in the L*er* background, showed significantly higher germination at low, but not warm temperatures compared with the L*er*-WT (Fig. 4*A*), similar to results from the NIL-*FLC*$_{Cvi}$, NIL-*FRI*$_{Sf}$ +*FLC*$_{Col}$, and the 35S overexpressor lines. Moreover, *ft-1* and *soc1* mutants also had higher germination percentages at cool temperatures than L*er*-WT, but not as high as the *ap1-1* mutant, indicating that they might act redundantly downstream of *FLC*. Further evidence of a shared pathway between flowering and germination is demonstrated by the significantly greater down-regulation of *AP1*, *FT*, and *SOC1* in NIL-*FLC*$_{Cvi}$ late during silique development

compared with the expression of these genes in the weak *FLC* background L*er* (Fig. 4 *B–D*).

Connecting Flowering and Germination Pathways. A seed needs to break dormancy before it can successfully germinate (40). The 2 primary plant hormones regulating dormancy and germination are ABA, which induces and maintains dormancy, and GA, which stimulates germination (40–42). Endogenous levels of these 2 hormones largely determine whether a seed will germinate. Therefore, we hypothesized that *FLC* regulates germination via the biosynthesis or catabolism of one or both of these hormones.

To test whether the difference in germination of different *FLC* genotypes was due to differences in the synthesis of ABA or GA by imbibed seeds during the initial stages of germination, uniconazol and fluridone, which inhibit the synthesis of GA and ABA, respectively, were applied to imbibing seeds. Uniconazol completely prevented germination of both NIL-FLC_{Cvi} and 35S::*FLC*, as well as their WT background at 10 °C, indicating that *FLC*-mediated germination in cool temperatures cannot overcome the lack of further GA synthesis during germination (Fig. 5*A*). Fluridone did not alter the germination behavior of any genotypes under the same conditions, indicating that ABA synthesis during imbibition is not the major factor that distinguishes *FLC*-mediated germination phenotypes.

To test whether differences in *FLC* expression predict differences in downstream hormonal pathways associated with germination, we measured the expression of genes involved with ABA and GA synthesis and catabolism in developing siliques and imbibing seeds (Table S2). Several of the genes we investigated did not have significant expression differences between NIL-FLC_{Cvi} and L*er*-WT. In contrast, expression of *CYP707A2*, one of the major ABA degradation genes, was significantly higher in genotypes with a strong *FLC* allele after 1 day of imbibition at 10 °C (Fig. 5*B*). Also, the major gene in the early stages of the GA synthesis pathway *GA20ox1*, increased dramatically in NIL-FLC_{Cvi} after 3 days of imbibition at 10 °C (Fig. 5*C*). A similar trend in the differential expression of *CYP707A2* and *GA20ox1* was also observed in seeds from a 35S::*FLC* line compared with its low *FLC*-expressing Col background (Fig. S6). *GA3ox1*, a gene that regulates the conversion of inactive to bioactive GA, also was expressed to higher levels in high *FLC*-expressing lines after 3 days of imbibition at 10 °C, but this increase was not statistically significant. The results suggest that a strong *FLC* allele promotes the early degradation of endogenous ABA followed by additional GA synthesis in imbibed seeds that experience cold (Fig. 5*D*). This process could occur either through the parallel sequential control of the 2 hormonal pathways by downstream components of the *FLC* pathway, or by *FLC*-pathway-mediated ABA degradation and subsequent dormancy breakage, which then enables GA-promoted radical protrusion and germination.

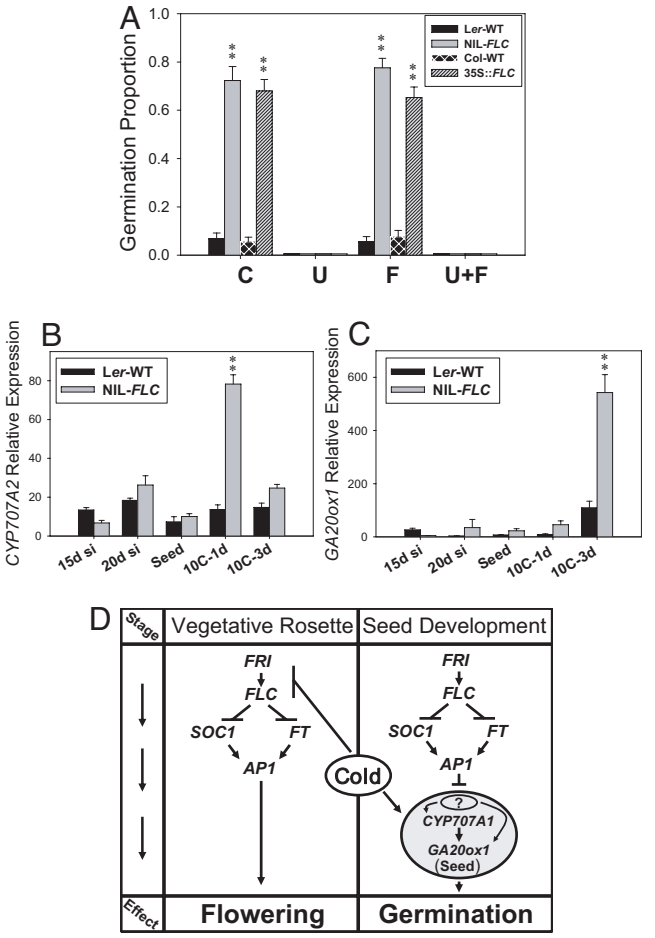

Fig. 5. *FLC*-promoted germination involves the ABA catabolic pathway (via *CYP707A2*) and GA biosynthetic pathway (via *GA20ox1*) in seeds. (*A*) Proportion of viable seed germination (±1 SE). C, control; U, uniconazol; F, flouridone; U+F, uniconazol + flouridone. Asterisks indicate significant differences between WT and their respective NIL or 35S::*FLC* overexpressor. (*B*) The expression (±1 SE) of *CYP707A2* in NIL-FLC_{Cvi} and *Ler* seeds at different stages. (*C*) Expression of *GA20ox1* (±1 SE) at different stages. **, $P < 0.01$. (*D*) Simplified pathway model of the hypothesized connections between flowering and germination pathways, and the possible signal transduction during flowering (*Left*) and germination (*Right*). *AP1* repression by high *FLC* expression during seed development directly or indirectly influences *CYP707A2* and *GA20ox1* through unknown pathways in germinating seeds.

Discussion

The major flowering time gene *FLC* promotes temperature-dependent seed germination. Therefore, significant pleiotropy exists between the 2 fundamental developmental transitions of germination and flowering, both of which require precise sensing and responses to seasonal cues. Shared pathways of flowering and germination occur, with *FLC*-mediation of germination acting through *FT*, *SOC1*, and *AP1*, similar to what occurs in *FLC*-mediated flowering. *FLC* regulation of germination subsequently acts through the pathway of ABA degradation and GA synthesis in imbibed seeds. Significant components of the same genetic pathway are shared between flowering and germination regulation, but downstream divergence may occur. Identifying precisely when the pathways diverge would contribute significantly to our understanding both of the genetic basis of pleiotropy of these life-history traits and of the importance of pleiotropy as a constraint on adaptive phenotypic expression of multiple traits.

It is interesting to note that functional *FRIGIDA* (*FRI*) does not appear to be required for *FLC*-mediated germination for some *FLC* alleles, because the 2 NILs that contained functional *FLC* both had similarly increased germination even though one of them had nonfunctional *FRI*. Therefore, some difference in the pathways of flowering and germination may also exist upstream of *FLC*. It is possible that the threshold of *FLC* expression for germination is lower in the L*er* background. Alternatively, the genes regulating *FLC* in rosettes during the transition to reproduction might differ or might have different effects on *FLC* expression from those acting during seed maturation. Vegetative *FLC* expression level was nevertheless a good predictor of germination in a sample of natural ecotypes.

FLC in vegetative rosettes is epigenetically silenced through chromatin remodeling during an extended period of cold, and *FLC* activity is reset during the early reproductive phase (9, 43–46). In contrast, to elicit germination, both high *FLC* ex-

pression and cool temperatures are required. The temporal separation of high *FLC* expression (during seed maturation) and cool temperature (during imbibition) can account for the requirements for both high *FLC* and cool temperature; *FLC* itself need not be expressed during low-temperature imbibition. It appears that increased *FLC* expression may impede both flowering and dormancy. We propose that high *FLC* expression during seed maturation renders seeds able to overcome primary dormancy during imbibition, most likely via lowering endogenous ABA levels, followed by GA-promoted germination (Fig. 5*D*).

FLC regulation of germination is largely maternally controlled. This maternal control could be imposed during the late stages of seed development, when *FLC* expression peaked and corresponding levels of *FT*, *SOC1*, and *AP1* decreased. Interestingly, *FLC* expression during seed development seems to alter the expression of hormonal genes in imbibed seeds, long after any physical connection between the maternal plant and seeds. One possibility for the maternal control of *FLC*-mediated germination is that mRNA and proteins involved in dormancy and germination are synthesized directly in seeds via the expression of maternal *FLC* alleles. Alternatively, high maternal *FLC* expression during silique development may prime seeds to be responsive to cold in terms of promoting germination through chromatin remodeling or other means of epigenetic manipulation. Also, mRNA transcripts, hormones, or proteins could have been provisioned to seeds during late stages of seed maturation, and those compounds may either degrade (reducing dormancy maintenance) or become active (promoting germination) during imbibition (47, 48). Stored RNAs have been shown to degrade during seed germination as a result of transcription during imbibition (49), but seeds are metabolically active even before imbibition, because transcription (50–52), protein synthesis (53), and metabolism (54, 55) occur even in dry seeds. The role of stored compounds in regulating dormancy and germination is perhaps one of the least understood phenomena in seed biology; *FLC* regulation of germination may offer a promising system for investigating these processes in a comparatively well-characterized genetic pathway.

The major role of *FLC* in temperature-dependent germination documented here indicates that a reevaluation of the adaptive significance of natural variation in *FLC* is required. Evolutionary ecological studies of *FLC* have focused almost exclusively on the role of *FLC* and its epistatic interactions with *FRI* to produce variation in flowering time, and they have interpreted natural variation in *FLC* in terms of variable natural selection on flowering time. Although evidence for natural selection on flowering phenology has been mixed, selection on germination phenology has been shown to be among the strongest documented for a single life history trait (29, 30, 56). It is likely that natural variation in *FLC* and other genes in its pathway may reflect a history of natural selection on germination as much as, if not more than, natural selection on flowering. This hypothesis needs testing in the field.

FLC regulation of flowering and germination provides an excellent system for investigating how pleiotropy contributes to or constrains adaptive evolution. Although we could predict that one gene that regulates 2 life history traits with different phenotypic optima may necessarily impose adaptive challenges, pleiotropy may actually contribute to the coordinated expression of different traits. For example, many of the ecotypes with high *FLC* expression are late-flowering winter annuals from colder climates. In these winter annuals, seeds likely germinate in cool temperatures of autumn and postpone flowering until after vernalization. As such, high *FLC* expression would enable both germination in autumn and flowering in spring, and thus, may actually be an example of adaptive pleiotropy that contributes to local adaptation to colder climates. Further study of the convergence and divergence of *FLC*-mediated flowering and germination pathways, combined with explicit tests of the adaptive value of pleiotropic control of these fundamental developmental transitions, would provide insight into the genetic basis of adaptation, given the constraints of shared genetic pathways across multiple traits.

Materials and Methods

NIL-FLC_{Cvi} (named LCN5-1) and its L*er* background were previously described (57–59). The NIL-FRI_{Sf2}+FLC_{Col} in the L*er* background and 35S::*FLC* in the Col background, as well as their corresponding WTs, were previously described (36). Table S1 lists the ecotypes used in this study and their original sites of collection. All of the ecotypes and the flowering time mutants *ft-1* (CS56), *soc1* (CS27278), and *ap1-1* (CS28) were obtained from the Arabidopsis Biological Resource Center seed stock center. Experimental seeds were matured in 10-h light/14-h dark at 15 °C. Germination assays were conducted with fresh seeds placed on 0.5% agar. Three independent biological replicates were used for qRT-PCR analysis (Table S2). RNA extractions were performed according to ref. 60, with the following modification: only 1 round of phenol extraction was performed. Transcript levels were calculated and presented with normalization to *ACT8* using a cDNA dilution series for each primer set in each experiment. Similar expression patterns were reconfirmed with normalization to *TUB2*.

For more details, see *SI Materials and Methods*.

ACKNOWLEDGMENTS. We thank Leonie Bentsink and Faye Rosin for critical comments on the manuscript; Melanie Schwab and Anji Ballerini for assistance with qRT-PCR; Johanna Schmitt for general discussion; Maarten Koornneef (Max Planck Institute for Plant Breeding Research, Cologne, Germany) for his generous supply of materials, critical reading of this manuscript, and extensive discussion; and Arabidopsis Biological Resource Center (ARBC) for supplying seed stocks. This work was supported by National Science Foundation Grants DEB 0807973 (to G.C.K.C. and K.D.) and ISO 0544053 (to K.D.).

1. Koornneef M, Blankestijndevries H, Hanhart C, Soppe W, Peeters T (1994) The phenotype of some late-flowering mutants is enhanced by a locus on chromosome-5 that is not effective in the Landsberg *erecta* wild-type. *Plant J* 6:911–919.
2. Lee I, Michaels SD, Masshardt AS, Amasino RM (1994) The late-flowering phenotype of FRIGIDA and mutations in LUMINIDEPENDENS is suppressed in the Landsberg erecta strain of Arabidopsis. *Plant J* 6:903–909.
3. Ausin I, Alonso-Blanco C, Martinez-Zapater JM (2005) Environmental regulation of flowering. *Int J Dev Biol* 49:689–705.
4. Baurle I, Dean C (2006) The timing of developmental transitions in plants. *Cell* 125:655–664.
5. Dennis ES, Peacock WJ (2007) Epigenetic regulation of flowering. *Curr Opin Plant Biol* 10:520–527.
6. Simpson GG, Dean C (2002) Flowering-Arabidopsis, the rosetta stone of flowering time? *Science* 296:285–289.
7. Lee I, Amasino RM (1995) Effect of vernalization, photoperiod, and light quality on the flowering phenotype of *Arabidopsis* plants containing the FRIGIDA gene. *Plant Physiol* 108:157–162.
8. Michaels SD, Amasino RM (2001) Loss of FLOWERING LOCUS C activity eliminates the late-flowering phenotype of FRIGIDA and autonomous pathway mutations but not responsiveness to vernalization. *Plant Cell* 13:935–941.
9. Amasino RM (2005) Vewrnalization and flowering time. *Curr Opin Biotechnol* 16:154–158.
10. Helliwell CA, Wood CC, Robertson M, Peacock WJ, Dennis ES (2006) The Arabidopsis FLC protein interacts directly in vivo with SOC1 and FT chromatin and is part of a high-molecular-weight protein complex. *Plant J* 46:183–192.
11. Hepworth SR, Valverde F, Ravenscroft D, Mouradov A, Coupland G (2002) Antagonistic regulation of flowering-timegene SOC1 by CONSTANS and FLC via separate promoter motifs. *EMBO J* 21:4327–4337.
12. Mandel MA, Yanofsky MF (1995) A gene triggering flower formation in *Arabidopsis*. *Nature* 377:522–524.
13. Sessions A, Yanofsky MF, Weigel D (2000) Cell-cell signaling and movement by the floral transcriptional factors LEAFY and APETALA1. *Science* 289:779–781.
14. Simon R, Igeno MI, Coupland G (1996) Activation of floral meristem identity genes in Arabidopsis. *Nature* 384:59–62.
15. Wagner D, Sablowski RWM, Meyerowitz EM (1999) Transcriptional activation of APETALA1 by LEAFY. *Science* 285:582–584.
16. 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. *Proc Natl Acad Sci USA* 100:10102–10107.
17. Gazzani S, Gendall AR, Lister C, Dean C (2003) Analysis of the molecular basis of flowering time variation in *Arabidopsis* accessions. *Plant Physiol* 132:1107–1114.
18. Werner JD, et al. (2005) FRIGIDA-independent variation in flowering time of natural Arabidopsis thaliana accessions. *Genetics* 170:1197–1207.

19. Caicedo AL, Stinchcombe JR, Olsen KM, Schmitt J, Purugganan MD (2004) Epistatic interaction between Arabidopsis FRI and FLC flowering time genes generates a latitudinal cline in a life history trait. *Proc Natl Acad Sci USA* 101:15670–15675.
20. Shindo C, et al. (2005) Role of FRIGIDA and FLOWERING LOCUS C in determining variation in flowering time of Arabidopsis. *Plant Physiol* 138:1163–1173.
21. Stinchcombe JR, et al. (2004) A latitudinal cline in flowering time in Arabidopsis thaliana modulated by the flowering time gene FRIGIDA. *Proc Natl Acad Sci USA* 101:4712–4717.
22. Stinchcombe JR, et al. (2005) Vernalization sensitivity in Arabidopsis thaliana (Brassicaceae): The effects of latitude and FLC variation. *Am J Bot* 92:1701–1707.
23. Le Corre V (2005) Variation at two flowering time genes within and among populations of *Arabidopsis thaliana*: Comparison with markers and traits. *Mol Ecol* 14:4181–4192.
24. Le Corre V, Roux F, Reboud X (2002) DNA polymorphism at the FRIGIDA gene in Arabidopsis thaliana: Extensive nonsynonymous variation is consistent with local selection for flowering time. *Mol Biol Evol* 19:1261–1271.
25. 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 Dev* 20:3079–3083.
26. Korves TM, et al. (2007) Fitness effects associated with the major flowering time gene FRIGIDA in Arabidopsis thaliana in the field. *Amer Nat* 169:E141–E157.
27. Finch-Savage WE, Leubner-Metzger G (2006) Seed dormancy and the control of germination. *New Phytol* 171:501–523.
28. Holdsworth MJ, Finch-Savage WE, Grappin P, Job D (2008) Post-genomic dissection of seed dormancy and germination. *Trends Plants Sci* 13:7–13.
29. Donohue K, et al. (2005) The evolutionary ecology of seed germination of Arabidopsis thaliana: Variable natural selection on germination timing. *Evolution* 59:758–770.
30. Donohue K (2009) Completing the cycle: Maternal effects as the missing link in plant life cycles. *Philos Trans R Soc London* 364:1059–1074.
31. Wilczek AM, et al. (2009) Effects of genetic perturbation on seasonal life history plasticity. *Science* 323:930–934.
32. Effmertova E (1967) The behaviour of ''summer annual'', ''mixed'', and ''winter annual'' natural populations as compared with early and late races in field conditions. *Arabidopsis Information Service* 4.
33. Evans J, Ratcliffe D (1972) Variation in ''after-ripening'' of seeds of *Arabidopsis thaliana* and its ecological significance. *Arabidopsis Information Service* 9:3–5.
34. Nordborg M, Bergelson J (1999) The effect of seed and rosette cold treatment on germination and flowering time in some Arabidopsis thaliana (Brassicaceae) ecotypes. *Am J Bot* 86:470–475.
35. Griffith C, Kim E, Donohue K (2004) Life-history variation and adaptation in the historically mobile plant Arabidopsis thaliana (Brassicaceae) in North America. *Am J Bot* 91:837–849.
36. 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.
37. Falush D, Stephens M, Pritchard JK (2007) Inference of population structure using multilocus genotype data: Dominant markers and null alleles. *Mol Ecol Notes* 7:574–578.
38. Kucera B, Cohn MA, Leubner-Metzger G (2005) Plant hormone interactions during seed dormancy release and germination. *Seed Sci Res* 15:281–307.
39. Bentsink L, Jowett J, Hanhart CJ, Koornneef M (2006) Cloning of *DOG1*, a quantitative trait locus controlling seed dormancy in *Arabidopsis*. *Proc Natl Acad Sci USA* 103:17042–17047.
40. Koornneef M, Bentsink L, Hilhorst H (2002) Seed dormancy and germination. *Curr Opin Plant Biol* 5:33–36.
41. Finkelstein R, Reeves W, Ariizumi T, Steber C (2008) Molecular aspects of seed dormancy. *Annu Rev Plant Biol* 59:387–415.
42. Holdsworth MJ, Bentsink L, Soppe WJJ (2008) Molecular networks regulating Arabidopsis seed maturation, after-ripening, dormancy and germination. *New Phytol* 179:33–54.
43. He YH, Amasino RM (2005) Role of chromatin modification in flowering-time control. *Trends Plants Sci* 10:30–35.
44. Sheldon CC, et al. (2008) Resetting of FLOWERING LOCUS C expression after epigenetic repression by vernalization. *Proc Natl Acad Sci USA* 105:2214–2219.
45. Sung S, Amasino RM (2006) Molecular genetic studies of the memory of winter. *J Exp Bot* 57:3369–3377.
46. Sung S, et al. (2006) Epigenetic maintenance of the vernalized state in Arabidopsis thaliana requires LIKE HETEROCHROMATIN PROTEIN 1. *Nat Genet* 38:706–710.
47. Almoguera C, Jordano J (1992) Developmental and environmental concurrent expression of sunflower dry-seed-stored low-molecular-weight heat-shock protein and Lea messenger-RNAs. *Plant Mol Biol* 19:781–792.
48. Dure L, Waters L (1965) Long-lived messenger RNA-Evidence from cotton seed germination. *Science* 147:410–412.
49. Li Q, Feng JX, Han P, Zhu YX (2006) Parental RNA is significantly degraded during Arabidopsis seed germination. *J Integr Plant Biol* 48:114–120.
50. Bove J, et al. (2005) Gene expression analysis by cDNA-AFLP highlights a set of new signaling networks and translational control during seed dormancy breaking in Nicotiana plumbaginifolia. *Plant Mol Biol* 57:593–612.
51. Leubner-Metzger G (2005) beta-1,3-glucanase gene expression in low-hydrated seeds as a mechanism for dormancy release during tobacco after-ripening. *Plant J* 41:133–145.
52. Nakabayashi K, Okamoto M, Koshiba T, Kamiya Y, Nambara E (2005) Genome-wide profiling of stored mRNA in Arabidopsis thaliana seed germination: Epigenetic and genetic regulation of transcription in seed. *Plant J* 41:697–709.
53. Chibani K, et al. (2006) Proteomic analysis of seed dormancy in Arabidopsis. *Plant Physiol* 142:1493–1510.
54. Bailly C (2004) Active oxygen species and antioxidants in seed biology. *Seed Sci Res* 14:93–107.
55. Fait A, et al. (2006) Arabidopsis seed development and germination is associated with temporally distinct metabolic switches. *Plant Physiol* 142:839–854.
56. Donohue K (2002) Germination timing influences natural selection on life-history characters in Arabidopsis thaliana. *Ecology* 83:1006–1016.
57. Alonso-Blanco C, El-Assal SE, Coupland G, Koornneef M (1998) Analysis of natural allelic variation at flowering time loci in the landsberg erecta and cape verde islands ecotypes of Arabidopsis thaliana. *Genetics* 149:749–764.
58. Edwards KD, et al. (2006) FLOWERING LOCUS C mediates natural variation in the high-temperature response of the Arabidopsis circadian clock. *Plant Cell* 18:639–650.
59. Keurentjes JJB, et al. (2007) Development of a near-isogenic line population of Arabidopsis thaliana and comparison of mapping power with a recombinant inbred line population. *Genetics* 175:891–905.
60. Vicient CM, Delseny M (1999) Isolation of total RNA from Arabidopsis thaliana seeds. *Anal Biochem* 268:412–413.