Antagonistic pleiotropic effects reduce the potential adaptive value of the FRIGIDA locus

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Although the occurrence of epistasis and pleiotropy is widely accepted at the molecular level, its effect on the adaptive value of fitness-related genes is rarely investigated in plants. Knowledge of these features of a gene is critical to understand the molecular basis of adaptive evolution. Here we investigate the importance of pleiotropy and epistasis in determining the adaptive value of a candidate gene using the gene *FRI* (FRIGIDA), which is thought to be the major gene controlling flowering time variation in *Arabidopsis thaliana*. The effect of *FRI* on flowering time was analyzed in an outbred population created by randomly mating 19 natural accessions of *A. thaliana*. This unique population allows the estimation of *FRI* effects independent of any linkage association with other loci due to demographic processes or to coadapted genes. It also allows for the estimation of pleiotropic effects of *FRI* on fitness and inflorescence architecture. We found that *FRI* explains less variation in flowering time than previously observed among natural accessions, and interacts epistatically with the *FLC* locus. Although early flowering plants produce more fruits under spring conditions, and nonfunctional alleles of *FRI* were associated with early flowering, variation at *FRI* was not associated with fitness. We show that nonfunctional *FRI* alleles have negative pleiotropic effects on fitness by reducing the numbers of nodes and branches on the inflorescence. We propose that these antagonistic pleiotropic effects reduce the adaptive value of *FRI*, and helps explain the maintenance of alternative life history strategies across natural populations of *A. thaliana*.

Arabidopsis thaliana | *FLC* | flowering time | pleiotropy | gene-by-environment interaction

Despite decades of research, we still know surprisingly little about the molecular basis of adaptation (but see refs. 1 and 2). To understand the genetic basis of adaptive evolution it is necessary to combine knowledge of the molecular basis of fitness-related traits with information about how selection acts on the available genetic variation (1). A number of candidate genes underlying adaptive traits have been identified in studies by using QTL analysis or forward genetic approaches (3–5). The role of candidate genes on past adaptive evolution is then often investigated by looking at rates and patterns of nucleotide substitution for evidence of past selection. Although a number of studies have shown that the rate of synonymous-to-nonsynonymous substitution in candidate genes is compatible with natural selection (6–8), demographic dynamics also can cause departure from the neutral expectation (9, 10). Thus, direct evidence for selection at the molecular level is ultimately needed, and such data are still very limited.

Determining the adaptive value of candidate genes is further complicated by the fact that the relationship between genotype and fitness is complex and results from the combination and/or interaction of multiple traits and genes. Consequently, understanding the relationship between molecular variation and fitness requires an understanding of the patterns of the pleiotropic effects of alleles of candidate genes and how these effects depend on epistatic interactions with other loci. These features of genetic architecture are important because they can significantly affect evolutionary dynamics, usually constraining response to selection or even preventing adaptive evolution (11–13). Thus, to improve our understanding of the mechanism of adaptive evolution, it is important to determine (*i*) the overall phenotypic effects of candidate genes, including epistatic and pleiotropic effects, and (*ii*) the mechanism's interaction with the environment. Although these effects are of paramount importance with respect to understanding evolutionary processes, they have been mostly neglected in studies of the adaptive value of candidate-genes.

The gene *FRI* (FRIGIDA) is an ideal candidate with which to investigate adaptive evolution because it is thought to be the major genetic factor determining flowering time in *Arabidopsis thaliana* (14–16), a trait expected to be under strong selection. The ability to flower at the appropriate time given local environmental conditions can strongly affect plant fitness (2, 17). Studies of the molecular variation in *FRI* support the idea that the molecular variation at this locus has been shaped by adaptive evolution (6, 18, 19). These studies show that nonfunctional alleles of *FRI* that result in early flowering have evolved from late-flowering functional alleles through a number of independent loss-of-function mutations (20–22).

Although natural populations of *A. thaliana* show a range of variation in life cycles, they generally fall into two main life-cycle strategies (23). Winter annual accessions typically germinate in the fall, overwinter as rosettes, and flower early in spring. Spring annual or "rapid-cycling" accessions germinate early in spring and complete seed production in the same growing season. Plants can sense local environmental conditions via a number of cues, such as day length, temperature, and light quality. Accordingly, dozens of genes and genetic pathways have been identified in *A. thaliana* that affect flowering time (24, 25). Even so, *FRI* explains a large proportion of the variation in flowering time among *A. thaliana* accessions (23–70% as estimated in refs. 16, 22, and 26). However, because natural populations of *A. thaliana* are mainly homozygous and have very low rates of recombination (27), the effect of *FRI* might be overestimated due to coadaptation of multiple linked loci. Its apparent strong effect on flowering time could be due to other, correlated loci.

FRI is part of the vernalization pathway that promotes flowering after a long period of cold. It is thought that functional alleles of *FRI* are favored in northern latitudes and that a recently observed increase in nonfunctional *FRI* alleles is the result of natural selec-

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Abbreviation: MANOVA, multivariate ANOVA.

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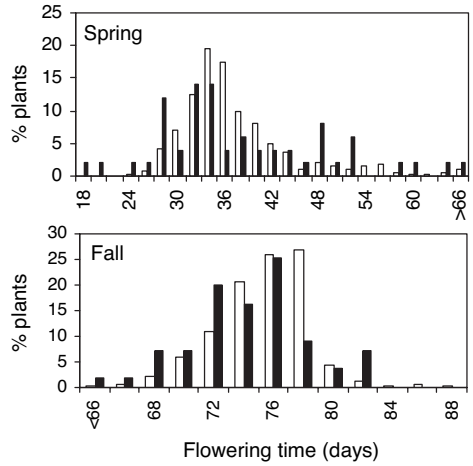

Fig. 1. Distribution of flowering time in the F_5 population (white bars) and among the 19 parental accessions (black bars) under spring and fall treatments.

tion for early-flowering during *A. thaliana*'s range expansion (14). However, expected latitudinal clines in flowering time and in the frequency of *FRI* alleles have not been observed (14–16). Furthermore, evidence that loss-of-function alleles confer higher fitness in the absence of severe winters or short growing seasons is still lacking (but see ref. 28).

Epistasis and pleiotropy are likely to be important for *FRI* because an epistatic interaction between *FRI* and *FLC* (FLOWERING LOCUS C) has been previously observed (22, 29), and pleiotropic effects of *FRI* on drought resistance have been reported (30). Antagonistic pleiotropy, where a gene affects two or more traits with opposite effects on fitness, is central to the study of life history evolution (31) and is the primary explanation for the existence of trade-offs in life history strategies (32, 33). Moreover, the combination of antagonistic pleiotropy and gene by environment interaction has been shown to maintain genetic diversity (12).

Here we investigate the adaptive value of *FRI* in an outbred population of *A. thaliana* produced by intermating 19 accessions. This outbred population allows the estimation of the average effect of *FRI* alleles in a heterogeneous and recombined genetic background, where any linkage disequilibrium between coadapted genes would have been ameliorated by recombination. Thus, we can analyze the effect of three common natural alleles of *FRI* on flowering time, fitness and other life history traits, as well as *FRI*'s epistatic interaction with *FLC*. To better relate our findings to the most common life history variation in *A. thaliana*, we performed these analysis under simulated spring and winter annual conditions.

Results

Phenotypic Variation in the Outbred Population. The average for all traits measured in the derived outbred population and among the 19 founder ecotypes [supporting information (SI) Table 5] was not significantly different under simulated winter annual or spring annual growth conditions (which hereafter will be referred to as "fall" and "spring" treatments) (Fig. 1). Moreover, there was no evidence of transgressive segregation (i.e., no outbred plant had trait values that were more extreme than the most extreme parental phenotype ± 2 SD), except for the number of branches in the fall treatment. Histograms for all traits are presented in SI Fig. 4. All markers genotyped are in Hardy–Weinberg equilibrium, and no linkage disequilibrium was observed between loci (SI Tables 6 and 7). These results suggest that the outbred population created by intermating the 19 accessions for five generations captures the full range of diversity of the original parents and does not show unintended phenotypic selection.

To determine the effect of growth conditions and family on traits, we performed a two-way multivariate ANOVA (MANOVA) (Table 1). We found that plants grown under spring treatment tend to bolt faster and are overall more vigorous: Inflorescences are on average taller, with more nodes, branches, and fruit. We also detected a significant effect of family on all traits, indicating heritable variation. There also is a significant interaction among growth conditions and family for flowering time, plant height, number of inflorescence nodes, and fruit production, indicating gene-by-environment interactions. These results indicate that all traits measured are affected by both genetic and environmental factors and that families respond differently to the different growing conditions (G × E effects).

Selective Value of Life History Traits. Because spring and fall treatments give rise to plants with very different phenotypes, we tested whether the treatments favor different life history strategies. A multivariate regression of all traits on fruit production indicates that similar trait values are favored in both environments, with the exception of flowering time (Table 2). Plants that flowered earlier had higher fruit production in the spring treatment. In contrast, there is no relationship between flowering time and fruit produc-

Table 2. Multivariate regression of all traits measured on fruit production

	Spring treatment		Fall treatment	
Trait	$\beta \pm$ SE	*P*	$\beta \pm$ SE	*P*
Flowering time	−1.04 ± 0.19	**<0.001**	0.05 ± 0.40	0.898
Rep. dev.	−0.67 ± 0.66	0.307	−0.92 ± 0.60	0.128
No. of nodes	−1.86 ± 0.69	**0.007**	−1.86 ± 0.63	**0.004**
No. of branches	7.34 ± 0.79	**<0.001**	6.47 ± 0.60	**<0.001**
Height	1.02 ± 0.34	**0.003**	1.52 ± 0.29	**<0.001**

The directional selection gradient (β) was estimated from the partial regression coefficients of number of fruits on the phenotypic traits. Significant *P* values are shown in bold. Rep. dev., reproductive development.

Table 1. Results for the two-way MANOVA on the effect of family and growth conditions on all traits measured

	Family			Env.			Family × Env.		
Trait	*F*	df	*P*	*F*	df	*P*	*F*	df	*P*
Multivariate test (Roy's greatest root)	4.0	197	**<0.001**	4,305.2	6	**<0.001**	2.8	187	**<0.001**
Univariate tests									
Flowering time	2.6	197	**<0.001**	16,680.8	1	**<0.001**	1.6	187	**<0.001**
Rep. dev.	1.2	197	**0.025**	10,541.0	1	**<0.001**	1.0	187	0.634
No. of nodes	3.4	197	**<0.001**	51.6	1	**<0.001**	2.0	187	**<0.001**
No. of branches	1.8	197	**<0.001**	432.9	1	**<0.001**	1.2	187	0.054
Height	2.6	197	**<0.001**	361.7	1	**<0.001**	1.4	187	**0.001**
No. of fruits	2.8	197	**<0.001**	790.6	1	**<0.001**	2.1	187	**<0.001**

Significant *P* values are shown in bold. Env., environment; Rep. dev., reproductive development.

Table 3. Results for the three-way MANOVA on the effect of growth conditions and *FRI* and *FLC* genotype on all traits measured

	FRI			*FLC*			Env.			*FRI* × *FLC*			*FRI* × Env.			*FLC* × Env.			*FRI* × *FLC* × Env.		
Trait	*F*	df	*P*	*F*	df	*P*	*F*	df	*P*	*F*	df	*P*	*F*	df	*P*	*F*	df	*P*	*F*	df	*P*
Multivariate test (Roy's greatest root)	6.9	6	**<0.001**	4.5	6	**0.001**	978.4	6	**<0.001**	4.2	6	**0.001**	2.4	6	**0.031**	1.8	6	0.110	2.9	24	**0.010**
Univariate test																					
Flowering time	11.7	2	**<0.001**	3.9	2	**0.022**	3495.1	1	**<0.001**	3.2	4	**0.013**	5.3	2	**0.006**	4.8	2	**0.009**	2.5	4	**0.046**
Rep. dev.	0.5	2	0.614	1.0	2	0.367	705.8	1	**<0.001**	1.0	4	0.413	0.7	2	0.495	0.1	2	0.988	1.0	4	0.430
No. of nodes	10.5	2	**<0.001**	5.0	2	**0.007**	8.0	1	**0.005**	3.2	4	**0.013**	2.0	2	0.188	0.7	2	0.480	1.1	4	0.382
No. of branches	3.5	2	**0.033**	8.2	2	**<0.001**	138.7	1	**<0.001**	2.5	4	**0.042**	2.6	2	0.080	1.2	2	0.315	1.1	4	0.337
Height	2.2	2	0.116	2.8	2	0.061	139.3	1	**<0.001**	3.9	4	**0.004**	0.1	2	0.989	1.6	2	0.203	1.5	4	0.204
No. of fruits	0.3	2	0.710	3.4	2	**0.036**	188.9	1	**<0.001**	1.1	4	0.338	2.0	2	0.139	0.9	2	0.393	0.3	4	0.856

Significant *P* values are shown in bold. Env., environment; Rep. dev., reproductive development.

tion in the fall treatment. However, under both environments, the trait most strongly associated with fitness is number of branches: plants with more branches produced more fruits.

Effect of *FRI* and *FLC* on Flowering Time. Plants grown in spring and fall conditions were assayed for their genotypes at the *FRI* locus and at the epistatically interacting locus *FLC*. At the *FRI* locus, plants were divided into three genotypes: homozygous for putatively functional alleles (FRI^{+}/FRI^{+}), homozygous for nonfunctional alleles ($FRI^{\Delta}/FRI^{\Delta}$), or heterozygous ($FRI^{+}/FRI^{\Delta}$). At the *FLC* locus, plants were homozygous or heterozygous for the FLC^{A} and FLC^{B} alleles (28). A three-way univariate ANOVA on the effect of growth conditions and the *FRI* and *FLC* genotypes indicates that all three factors have significant effects on flowering time (Table 3). The observed interaction between *FRI* and *FLC* loci confirms the epistatic relationship previously observed. Furthermore, we found that the two-way interactions between growth conditions and genotypes, and the three-way growth conditions by *FRI* by *FLC* interactions were all significant. This means that the effects at each locus and the effects of epistatic interactions between the loci depend on the growing conditions.

To better understand the nature of these interactions, we performed two-way ANOVA separately for each environment. In the spring treatment we found significant effects of *FRI* ($F = 9.5$, df $= 2, P < 0.001$), *FLC* ($F = 4.7$, df $= 2, P = 0.010$), and their interaction ($F = 3.12$, df $= 4$, $P = 0.017$) on flowering time. Together, the two loci explain 18% of the variation in flowering time, a much smaller percentage than observed in previous studies that compared inbred natural accessions. As expected, plants homozygous for FRI^{+} alleles flowered later than any other plants (Fig. 2). However, the magnitude of this effect depended on the genotype at the *FLC* locus: the effect of *FRI* is stronger on the FLC^{A} background than on the FLC^{B} background (where the *FRI* effect is not statistically significant). Overall, FLC^{B} alleles result in weaker flower repression, and plants homozygous for FLC^{B} alleles bolt earlier than plants homozygous for FLC^{A} (Fig. 2). However, the genotype at the *FLC* locus is statistically significant only for plants homozygous FRI^{+}.

Under fall conditions, the genotype of *FRI* is nearly significant ($F = 2.8$, df $= 2$, $P = 0.066$), whereas *FLC* ($F = 1.9$, df $= 2$, $P = 0.153$) and the interaction of *FRI* and *FLC* ($F = 1.0$, df $= 4$, $P = 0.428$) do not significantly affect flowering time. Albeit of smaller magnitude, the effect of *FRI* and *FLC* genotypes are qualitatively similar to what we observed in spring conditions: FRI^{+}/FRI^{+} plants flowered significantly later than $FRI^{\Delta}/FRI^{\Delta}$ plants (post hoc test; $F = 4.3$, df $= 2, P = 0.019$). Thus, the observed interaction between *FRI* and growth conditions in the three-way MANOVA (Table 3) is mostly due to changes in the magnitude of effects and not in their direction.

Pleiotropic Effects of *FRI* and *FLC*. A three-way MANOVA shows that *FRI*, *FLC*, and their interaction affect plants' overall phenotype (Table 3). The growth conditions significantly affected all measured traits, and significantly interacts with *FRI*, and *FRI* × *FLC*. The univariate ANOVA (Table 3) indicates the traits that contribute mostly to the observed effects: *FRI* affects mainly number of nodes and branches, whereas *FLC* also affects fruits. The interaction between *FRI* and *FLC* had significant effects on number of nodes and branches, suggesting that their epistatic interaction extends to other traits.

To further understand the effects of the growth conditions on *FRI* and *FLC*, we performed two MANOVAs for each treatment separately. In the spring treatment, the pleiotropic effect of *FRI* is mainly on the number of nodes and branches (Table 4): Plants homozygous for FRI^{+} produce inflorescences with more nodes and more branches. Notably there is no overall significant effect of *FRI* genotypes on number of fruits. In contrast, the effect of *FLC* genotypes is mainly on the number of branches, but a significant effect was also detected on the number of nodes and fruits. The interaction between *FRI* and *FLC* significantly affects the number of branches and height. There is no significant effect of *FRI*, *FLC*, or their interaction on plants' overall phenotype under fall conditions.

Pathways Through Which *FRI* and *FLC* Affects Fitness. We have shown that *FRI* and *FLC* genotypes significantly affect flowering time and that flowering time is significantly associated with fruit production

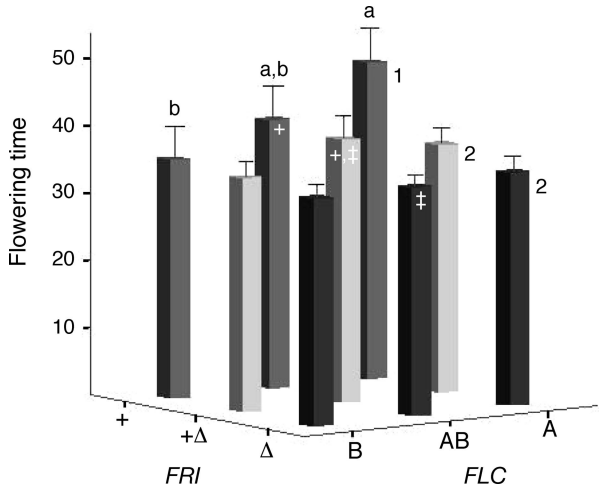

Fig. 2. Effect of *FRI* and the *FLC* genotypes on flowering time under spring treatments. Letters (a and b), numbers (1 and 2), or symbols (+ and ‡) indicate groups that are significantly different based on Tukey's post hoc tests. Error bars represent the 95% confidence interval.

Table 4. Results of the two-way MANOVA for the effect of *FRI* and *FLC* on plants' overall phenotype in the spring treatment

	FRI			*FLC*			*FRI* × *FLC*		
Trait	*F*	df	*P*	*F*	df	*P*	*F*	df	*P*
Multivariate test (Roy's greatest root)	6.4	6	**<0.001**	5.7	6	**<0.001**	5.2	6	**<0.001**
Univariate test									
Rep. dev.	0.5	2	0.611	0.5	2	0.629	1.1	4	0.368
No. of nodes	9.8	2	**<0.001**	3.1	2	**0.049**	2.0	4	0.101
No. of branches	4.3	2	**0.015**	9.6	2	**<0.001**	4.6	4	**0.002**
Height	1.8	2	0.168	4.9	2	**0.008**	2.5	4	**0.043**
No. of fruits	0.6	2	0.530	3.9	2	**0.022**	0.3	4	0.892

Significant *P* values are shown in bold. Rep. dev., reproductive development.

in the spring treatment, yet no direct effect of *FRI* on fruit production has been detected. Because we detected pleiotropic effects for both *FRI* and *FLC*, we used a path analysis to investigate the relative contribution of these pleiotropic effects to fitness (fruit production).

The best fit model for plants in the spring treatment (Fig. 3*A*) shows that the lack of an effect of *FRI* on fruit production is due its pleiotropic effect on branching. Although FRI^{Δ} increases fruit production by reducing flowering time, it also decreases fruit production by reducing the number of branches. These antagonistic effects cancel each other out, resulting in no overall effect of *FRI* genotype on fitness. Surprisingly, *FLC* explains more variation in branching than in flowering time, although it is usually considered a "flowering time" locus. The fact that the increase in branching is not fully counteracted by the antagonistic increase in flowering time explains the significant effect of *FLC* on fitness observed in Table 4. Under the fall treatment, the best fit model (Fig. 3*B*) shows that most of the same paths are significant and in the same direction, but the contribution of *FRI* and *FLC* to flowering, branching, and height are of much smaller magnitude. These results suggest that other loci affecting branching might play a more important role on fitness under fall treatment.

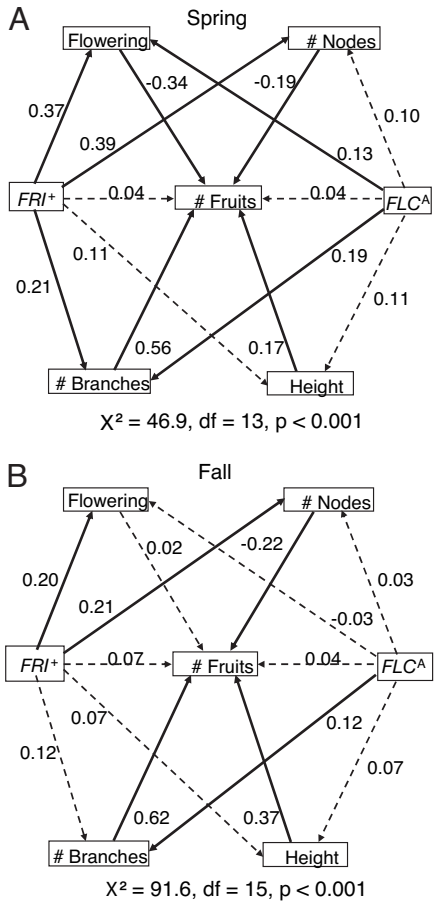

Fig. 3. Path coefficients (standardized partial regression coefficients) from *FRI* and *FLC* to flowering time, inflorescence traits, and fruit production. Dashed lines represent nonsignificant paths. The overall significance of the model is indicated below each diagram and was calculated including only significant paths.

Discussion

To further advance our knowledge about the mechanism of adaptation, it is necessary that we understand the phenotypic effects of candidate genes. It is well known that epistasis and pleiotropy are common and that these effects can affect the evolutionary process (33, 34). However, phenotypic effects of candidate genes have been primarily focused on one particular trait and often on simple genetic backgrounds. Using an outbred population of *A. thaliana*, we have detected a complex relationship between *FRI* and fruit production, which depends on epistatic, pleiotropic, and G × E effects. *FRI* has been proposed as the main candidate gene to explain variation in flowering time among natural accessions of *A. thaliana.* However, most of the studies that analyzed the effect of *FRI* on flowering time were conducted with either inbred natural accessions or mutant lines (e.g., refs. 15, 16, 22, 26, and 29). Here, we confirm that *FRI* affects flowering time in the absence of cold temperatures, but its effect was much smaller than previously estimated (12.6% compared with 23–70% as estimated in refs. 16, 22, and 26). We believe this difference is most likely due to recombination that occurred in the production of the F_5 plants. Due to their typically low rate of outcrossing (27), *A. thaliana* populations and stock accessions are mainly homozygous (35), and genetic variation among populations is highly structured (36). Interlocus associations are common within geographical areas (29, 36), which may cause coadaptation of multiple genes and the overestimation of the effect of specific candidate genes. It is also possible that *FRI* explains a smaller proportion of the variation in flowering time here than in other studies due to the particular environmental conditions used. Unlike field conditions, past studies used constant growth conditions with fixed day length and temperature (14, 16). By using growth conditions where day length and temperatures change to simulate the different seasons, we have attempted to make more ecologically relevant growth conditions, while avoiding a number of uncontrollable variables typical of field conditions (such as severity of winter, drought, and herbivores, among others). Because flowering time in *A. thaliana* also is known to respond to light quality and photoperiod (25, 37), it is possible that the overall effect of *FRI* is smaller in our study because the effect of other genes involved in the response to other cues is increased.

Our results suggest that the genetic background also affects intralocus dominance relationships. The *FRI* allele usually is con-

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sidered a dominant allele (14, 38), but in our study *FRI* is codominant with heterozygous plants flowering at an intermediate time and being significantly different from both homozygotes (Fig. 2). Dominance relationships can significantly alter the evolutionary dynamics of an allele (38) and the economic value of a particular locus to plant breeding. These results further highlight the importance of investigating gene function and its adaptive value in a heterogeneous and recombined genetic background. The effects of an allele are often strongly conditional on the genetic and environmental context in which it is embedded.

Because new mutations arrive at random and in a heterozygous context, the best predictor of the adaptive value of any mutation is the average effect of the new allele independent of (i.e., averaged across) genetic backgrounds. Because nonfunctional *FRI* alleles are hypothesized to have evolved multiple times in *A. thaliana* from a late-flowering ancestral (6), an adaptive explanation for its increase in frequency after the postglacial recolonization of Europe requires a positive association with fitness across multiple genetic backgrounds. It has been proposed that FRI^{Δ} has increased in frequency due to its effect in reducing time to flowering, a trait thought to be adaptive in environments with short or unpredictable seasons or in regions where multiple generations are possible. This scenario requires that FRI^{Δ} reduces flowering time and that this reduction is associated with higher fitness. Consistent with this hypothesis, we found that FRI^{Δ} reduces flowering time. However, we could not detect any evidence that FRI^{Δ} alleles were associated with higher fruit production. The lack of association between *FRI* genotype and fruit production is particularly surprising because early flowering plants had higher fruit production under spring annual conditions. However, the only other study that investigated the effect of *FRI* on fitness (28) also failed to find an association. Here, we propose that the lack of an association between *FRI* and fitness is due to antagonistic pleiotropic effects of *FRI* on the number of nodes and branches. Flowering time is an important part of a plants' developmental program and it is expected that some of the regulatory factors that affect flowering time also will affect other aspects of plant development. Although pleiotropic effects of *FRI* on inflorescence architecture have not been previously reported, it should not be surprising, considering that a number of genetic mutations that regulate flowering time also have shown pleiotropic effects on leaf shape, trichome density, and inflorescence architecture (39–41). In addition, mutants of genes that affect inflorescence development, such as *tfl1* and *emf2*, have also been shown to affect flowering time (42, 43).

Theoretical models suggest that pleiotropic effects can affect the adaptive process, usually reducing the rate of evolution (11, 44). The number of branches produced is one of the strongest predictors of *A. thaliana*'s fitness, as shown here and in previous laboratory- and field-based studies (41, 45, 46). Thus, the effect of *FRI* as mediated by branching eliminates the adaptive value gained through early flowering time. The magnitude of these pleiotropic effects depends on epistatic interactions with *FLC* and on the environmental growth conditions. It is likely that the balance between the relative importance of flowering time and number of branches on fitness will vary according to local environmental conditions. Although we expect that under most circumstances the antagonistic relationship between these effects will result in a lack of selection on *FRI*, it also is possible that a small negative or positive selective value will be observed, which would explain the maintenance of the two types of alleles over *A. thaliana*'s geographical range.

Although the hypothesis that FRI^{Δ} has evolved through adaptive selection to reduce flowering time has strong support from molecular studies (19), the expected latitudinal clines in flowering time mediated by *FRI* or in its allele frequency are not evident (15). Furthermore, direct evidence for selection at the *FRI* locus through its effect on flowering time remains elusive (28). We propose that a combination of pleiotropic and epistatic effects that modify the effect of *FRI* on fitness explain these inconsistent results. The detection of such an effect would not have been possible without the outbred population of *A. thaliana*, indicating that this resource provides improved power to investigate the function and adaptive value of candidate genes in a complex context.

Materials and Methods

Outbred Population. Seeds for the 19 accessions of *A. thaliana* (listed in SI Table 5) used to create the outbred population were obtained from the *Arabidopsis* stock center and chosen to maximize genetic and phenotypic diversity based on previously reported patterns of genetic variation (47–49). Intermating was initiated with a complete diallel cross for which each accession was crossed with all other accessions as a maternal and paternal parent, yielding 342 F_1 progeny. These F_1 were then further intermated through four generations of random mating to produce 342 F_5 families. In every generation, two plants from each family were randomly chosen to be paternal and maternal parents (providing an effective population size $N_e = 684$). Assortative mating between plants that have similar flowering time was prevented by staggering planting schedules and planting the same families multiple times.

Experimental Design. Two hundred F_5 families were randomly selected and planted into ArabiPatches (Lehle Seeds, Round Rock, TX). Each ArabiPatch contains eight pots (2.5 cm in diameter) and a water reservoir, which ensures constant and equally distributed amounts of water. Pots were filled with John Innes #1 soil treated with Intercept 70 WG (Bayer, Wuppertal, Germany) to avoid fungus gnats during the experiment. Each pot was sowed with two seeds and placed in the dark at 4°C for 3 days to promote germination. Germination date for each seed was monitored daily, and pots where more than one seed germinated had one seedling removed at random 3 weeks later. Each family was planted into six pots, and these were randomly split into a spring (which simulated a spring annual cycle) or a fall (simulating winter annual conditions) treatments. Pots were randomly assigned to patches, and the position of the patches in the growth chamber was randomly assigned every week. Growth treatments were simulated in AR66L growth chambers (Percival Scientific, Perry, IA) using the programs described in the following two sections.

Spring Treatment. This cycle started with 14 days of spring-like conditions (14°C during the day/10°C during the night and 8 h of light per day). Conditions were then transitioned over to summer-like conditions over a period of 14 days by increasing temperature by 0.5°C/day (0.6°C/night) and day length by 34 min/day. Chamber was then held at summer conditions (21°C during the day/18°C at night and 16 h of light per day) for the next 3 months.

Fall Treatment. This cycle started with 21 days of fall-like conditions (16°C during the day/10°C during the night and 8 h of light per day). Environmental conditions were then transitioned over to winter-like conditions over a period of 7 days by decreasing temperature by 1.7°C/day (0.9°C/night) and day length by 15 min/day. Winter conditions (4°C during day and night and 6 h of light per day) were then held for the next 3 weeks and then transitioned to spring-like conditions over a period of 7 days by increasing temperature by 1.4°C/day (0.9°C/night) and day length by 15 min/day. After these steps, the program follows the same steps as the spring treatment.

Phenotypic Data. We inspected plants daily and recorded the bolting date (when floral buds are first observed) and the day the first mature fruit were observed. After plants senesced, we recorded the number of nodes and extended branches, the height of the main stem, and the total number of fruits produced. Flowering time was calculated as the number of days between germination and bolting. Reproductive development time was calculated as the number of days between bolting and first mature fruit.

Genotypic Data. We collected leaves from one plant of each family in each environment ($n = 175$ in spring, and $n = 168$ in fall) after they had bolted. Their DNA was extracted by following the protocol of Edwards *et al.* (50). The *FRI* genotype was determined by assaying for the presence or absence of the two common deletions typical of the Landsberg (L*er*) and Columbia (Col) accessions that are known to cause *FRI* to become nonfunctional (described in ref. 14). The PCR primers used are described in ref. 14, and products were ran on 5% agarose gels. Although six possible genotypes exist, previous studies found that the effect of the two deletions are similar (14). In addition, we did not find significant differences in flowering time between FRI^{Col}/FRI^{Col} and FRI^{Ler}/FRI^{Ler} (t test; $t = -1.00$, df = 37, $P = 0.323$) or between FRI^{+}/FRI^{Ler} and FRI^{+}/FRI^{Col} (t test; $t = 1.46$, df = 67, $P = 0.150$). Thus, we reduce all genotypes into three functional classes: homozygous functional (FRI^{+}/FRI^{+}), homozygous nonfunctional ($FRI^{\Delta}/FRI^{\Delta} = FRI^{Ler}/FRI^{Ler}$, FRI^{Ler}/FRI^{Col}, or FRI^{Col}/FRI^{Col}), and heterozygous ($FRI^{+}/FRI^{\Delta} = FRI^{+}/FRI^{Ler}$ or FRI^{+}/FRI^{Col}).

FLC genotypes were determined by using the PCR primers described by Caicedo *et al.* (29), and the products were ran on 5% agarose gels. Natural molecular variation in *FLC* previously has been shown to be divided into two major haplotypes, FLC^{A} and FLC^{B} (29), which can be distinguished by a single nucleotide substitution. We assayed all samples for their *FLC* genotype in terms of A or B haplotype and for the presence or absence of two insertions previously identified in accessions L*er* and Col (29, 51). All alleles that had a Col or a L*er* deletion were of the FLC^{A} haplotype. Because we found no differences in flowering time within FLC^{A} due to the presence of these deletions, we performed all analyses considering only whether the alleles were FLC^{A} or FLC^{B}.

Plants were also assayed at four additional markers (Indels 1, 2, 3, and 5) to ensure the F_5 population were in Hardy–Weinberg equilibrium. These indels were identified in the resequencing of 96 accessions described in Nordborg *et al.* (52), and they were chosen by their chromosomal position. The primers used to genotype these accessions are described in SI Table 8). The genotype at each of these locations was determined after running PCR products in 5% agarose gels, with two alleles being possible for each marker (with or without the insertion).

Data Analysis. To test whether the outbred population was in Hardy–Weinberg equilibrium, we calculated F_{is} and pairwise linkage disequilibrium using Genepop 3.4 (53).

The effect of family and growth conditions on all traits measured was tested with a two-way MANOVA. The potential adaptive value of these traits under the different growing conditions was evaluated by using a multivariate regression as proposed by Lande and Arnold (54). This analysis estimates the association between each trait and the total number of fruits produced per plant. The partial regression coefficient (β) of each trait and its significance indicates the adaptive potential of these traits given our growth conditions.

The effect of the *FRI* and *FLC* genotypes and their interaction with growing conditions on flowering time and life history traits was tested by using ANOVA and MANOVA, respectively. The independent variables in both analyses were growth conditions (spring vs. fall) and the *FRI* and *FLC* genotypes (entered as categorical variables). Tests on the effect of *FRI* within each *FLC* genotype and the effect of *FLC* within each *FRI* class were done by using a Tukey post hoc test.

To determine the relative importance of different pathways through which *FRI* and *FLC* can affect fruit production, we performed a path analysis as implemented by the Amos 6.0 software package (55). The initial model allows for pathways from *FRI* and *FLC* to flowering time, number of nodes, number of branches, height, and number of fruits produced; we also included pathways from flowering to all inflorescence traits. Finally, we allowed pathways between all variables and fruit production. The relative importance of each path in the model is evaluated by the path coefficient, which corresponds to the standardized partial regression coefficient. The best model was chosen by backward model selection, where all statistically nonsignificant paths were removed one at a time, and its importance for the overall model was evaluated by comparing χ^2 values.

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