Pleiotropy of *FRIGIDA* enhances the potential for multivariate adaptation

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An evolutionary response to selection requires genetic variation; however, even if it exists, then the genetic details of the variation can constrain adaptation. In the simplest case, unlinked loci and uncorrelated phenotypes respond directly to multivariate selection and permit unrestricted paths to adaptive peaks. By contrast, ‘antagonistic’ pleiotropic loci may constrain adaptation by affecting variation of many traits and limiting the direction of trait correlations to vectors that are not favoured by selection. However, certain pleiotropic configurations may improve the conditions for adaptive evolution. Here, we present evidence that the *Arabidopsis thaliana* gene *FRI* (*FRIGIDA*) exhibits ‘adaptive’ pleiotropy, producing trait correlations along an axis that results in two adaptive strategies. Derived, low expression *FRI* alleles confer a ‘drought escape’ strategy owing to fast growth, low water use efficiency and early flowering. By contrast, a dehydration avoidance strategy is conferred by the ancestral phenotype of late flowering, slow growth and efficient water use during photosynthesis. The dehydration avoidant phenotype was recovered when genotypes with null *FRI* alleles were transformed with functional alleles. Our findings indicate that the well-documented effects of *FRI* on phenology result from differences in physiology, not only a simple developmental switch.

1. Introduction

Populations of a species are frequently distributed across climatic gradients, where natural selection can lead to adaptation to local conditions. The environmental conditions that cause local adaptation have been well documented through reciprocal transplants and studies of clines [1–6]. These experiments show that divergent patterns of selection cause shifts in the mean values of many traits leading to a multivariate response. Such a response to selection improves fitness and promotes successful adaptation to local conditions. Despite a large body of research, it remains a challenge to determine the specific genetic loci that respond to selection and confer local adaptation [7–10].

Long-term breeding programmes and quantitative genetic studies have demonstrated variation in nearly all traits, and thus a simple lack of additive genetic variation is not expected to constrain adaptation [11]. Instead, a limited amount of genetic variation along vectors of selection has been shown to limit adaptive evolution [12–14]. Theoretically, independence of all loci and phenotypes will improve the potential for adaptation by optimizing evolvability [15] and the response to selection (R) [14,16,17]. However, certain genetic correlations can disrupt the optimal genetic architecture by reducing the amount of genetic variation which is available to selection and causing correlated responses of...
non-adaptive traits [12,18]. Although this maladaptive role for genetic correlations is not universal [19], genetic correlations may affect R by limiting the dimensionality of the genetic (co)variance matrix or restricting genetic variation to vectors which are not aligned with selection [12–13,18,20].

The combined effects of the pleiotropic loci, which cause genetic correlations, may have a profound impact on patterns of local adaptation. As pleiotropy can constrain multivariate adaptation and cause correlated evolution of adaptive and deleterious phenotypic values, these loci are typically considered ‘antagonistic’ [21–25]. Adaptation is especially constrained when pleiotropic gene action limits phenotypic correlations along a vector orthogonal to that of selection and reduces R [13]. Antagonistic pleiotropy is well documented and has led to the belief that all pleiotropy is maladaptive [26]. However, recent theoretical work has countered this viewpoint by demonstrating that intermediate levels of pleiotropy may actually improve the conditions for adaptation and evolution of complexity [27–29].

To study the adaptive value of pleiotropic loci, it is necessary to assess the effects of genetic variation on the structure of many phenotypes which are subject to correlational selection in nature. Adaptation to drought in plants provides an ideal system to achieve this goal [30–33]. In natural and agricultural systems, annual plants can be adapted to local drought conditions by either growing and reproducing before the onset of drought (drought escape) [31,34–36] or by delaying reproduction, increasing water use efficiency (WUE) and conserving resources (dehydration avoidance) [37–39]. For example, accessions which exhibit early-flowering time (FT) and low WUE were selected for in consistently wet soil and late-season drought conditions [36,40], whereas direct selection on increased WUE favoured a dehydration avoidance strategy in environments with early-season drought [41]. Therefore, adaptation to different local soil moisture conditions and seasonal rainfall patterns contributes to the observed strong correlations between FT, growth rate and WUE within and among species [6,32,38,41–43]. Several studies have suggested that pleiotropy may also affect this correlation [38,44,45].

Here, we provide empirical evidence for an adaptive role of pleiotropy. Using genome-wide approaches, allelic variants and transgenic manipulation, we demonstrate that the ‘FT’ gene, *FRIGIDA* (*FRI*) pleiotropically affects phenotypic variation in growth rate, WUE and FT. Derived, null *FRI* alleles produce a drought escape phenotype (decreased WUE, increased growth rate, decreased FT) relative to the ancestral adaptive strategy. This phenomenon, which we term ‘adaptive pleiotropy’, enhances the likelihood of adaptation by increasing adaptive responses to selection.

2. Methods

(a) *Arabidopsis thaliana* genetic resources

We used four sets of genetic variants: TK RILs, a panel of 317 physiologically diverse *A. thaliana* accessions, a nearly isogenic line (FRI-NIL) and *FRI* transgenic overexpression lines (tr-FRI). The TK RILs are the product of a bi-directional cross between two physiologically divergent accessions: TSU-1 (low WUE, short FT) and KAS-1 (high WUE, long FT) [46]. The TK RILs mapping population consists of 343 F9 lines each genotyped at 166 genomic loci. In addition to the published loci, all RILs were genotyped at *FRI* via fragment analysis of PCR product generated across the promoter (primer F: 5′-AGTACTCACAAGTCACAAC-3′; primer R: 5′-GAAGATCATAATTGCC-3′) [47]. The 317 accession panel was genotyped at this marker (FR Ide1) and two additional markers: FR Ide2 (primer F: 5′-AGATTTGCAGATTGATAAGG-3′; primer R: 5′-ATATTTGTTGCTCTCC-3′) and FR LCap (primer F: 5′-CCATAGCGAATTAGCTGC-3′; primer R: 5′-AGACTCCGATATAAAGG-3′). The 317 accessions and TK RILs are listed in the electronic supplementary material, tables S1 and S2 and are available from the Arabidopsis stock center (http://www.arabidopsis.org/).

The FRI-NIL was generated by introgressing a functional *FRI* allele from the SI-2 line into wild-type (WT) Col-0, the reference *A. thaliana* accession with a null *FRI* allele [47–49]. The tr-FRI transgenic over expressed line was generated by ligating FRIGFP into the XmaI and Xhol sites of 35S:PBARN vector and then transformed into a Col-0 background using the floral dip method. We used only FRI transgenic lines that exhibited a late flowering phenotype. The FRI-NIL and the transgenic line ‘FRI-GFP Col T2 #20’ are available from S.D.M. and X.Y. We also present WUE data from FRI-NIL and Columbia genotypes with knocked-out FLC alleles. See Michaels & Amasino [50] for details on these lines.

(b) Plant growth and phenotypic analysis

Phenotypic analyses of the TK RILs, the FRI-NIL, tr-FRI and Col-0 were conducted in a Conviron ATC60 growth chamber (Controlled Environments, Winnipeg, MB, Canada) at Colorado State University (CSU). All plants were grown at 12 h, 40 per cent humidity, 23°C days and 12 h, 50 per cent humidity, 18°C nights. Photosynthetic photon flux density during daylight was approximately 330 μmol m⁻² s⁻¹. All plants except those analysed for gas exchange were grown in 2 litre plastic pots containing Fafard 4p mix (Conrad Fafard Inc. Agawam, MA). Gas exchange measurements were taken on plants grown in the same conditions in modified Cone-tainer pots (Stueve and Sons, Tangent, OR). A 195-line subset of the 317 line panel was grown at University of Texas, Austin in promix BT potting soil and 164 ml Cone-tainer pots under long-day photoperiod conditions (16 L:8 D) at approximately 18–21°C. Consistent with previous studies, these long-day environmental conditions induced flowering much more quickly than the 12/12 h conditions at CSU.

Gas exchange physiology was measured with an LI-6400 photosynthesis system (LiCor Inc, Lincoln, NB) equipped with a custom whole-plant gas exchange cuvette. A total of 20 measurements were taken over a 2 min period for each of 20 plants (10 replicates/genotype) at two time points (14 and 21 days post-germination). The photosynthetic parameters (*A*, *ci* and *gsc*) were estimated following von Caemmerer & Farquhar [51]. Gas exchange data were analysed in a mixed-model framework where genotype was fixed, and measurement and date were nested within individual as a random effect in JMP GENOMICS v. 5.0 (SAS Institute, Cary, NC). We also generated *A/ci* curves by measuring photosynthetic rate across nine levels of external CO2 concentrations using a different set of plants grown hydroponically. We compared *A* between the FRI-NIL and Col-0 controlling for variation in *ci* with a mixed effect ANOVA. The genotype was a fixed effect, and *ci* was a continuous, random covariate.

We measured WUE, growth rate and FT for each plant (n/genotype = 10). Flowering initiation was recorded when a visible bolting structure first appeared at the apical meristems; FT is calculated as the number of days between germination and initiation of flowering. We analysed carbon isotope composition ([δ13C]), a surrogate measure of WUE [38,52], on lyophilized, finely ground rosette leaves at the Stable Isotope Facility at University of California, Davis (UCD; http://stableisotopefacility.
were on average 87 per cent accurate in cross-validation. By \( n = 20 \), models were 93 per cent accurate in cross-validation. This signifies that the SNP associations with \( \text{FRI} \) functionality are easily observable in even small samples of accessions. Using this model, we then imputed the allelic state (binned into functional or null categories) for all accessions in the SNP database.

We calculated genome-wide \( F_{\text{ST}} \) in \texttt{PLINK} [59] by classifying the accessions as ‘functional’ or ‘non-functional’ \( \text{FRI} \) and calculating the molecular variance between and within these allele classes. We generated 5000 random divisions at the same frequency as the \( \text{FRI} \) allele classes. These permutations allow us to assess the significance of the \( F_{\text{ST}} \) measure compared with random evolution. We conducted two additional analyses with subsets of the available accessions. Ten of the 574 sites sampled by Horton et al. [37] showed within-population variation at \( F_{\text{RI}} \). Using these populations and geographical clusters at the country level [57], we calculated an average heterozygosity over SNPs sampled at 50 kb intervals (\( H_l \)). Then, we split the population based on \( \text{FRI} \) phenotype, calculated an average heterozygosity within each subpopulation (\( H_s \)) in the same way and took the mean of those. We used these \( H_l \) and \( H_s \) values to calculate genome-wide \( F_{\text{ST}} \) based on the \( \text{FRI} \) phenotype. We bootstrapped to calculate significance by dividing the data at a random subset of 500 SNPs with similar frequency to \( F_{\text{RI}} \) and recalculating \( F_{\text{ST}} \).

### 3. Results and discussion

#### (a) Mapping the water use efficiency—flowering time correlation

We measured FT and WUE of 195 \( A. \text{thaliana} \) accessions in a common garden. The genetic correlation between WUE and FT is positive and significant: WUE explains nearly 40 per cent of FT variation (\( n = 195, r^2 = 0.395, p < 0.0001 \); electronic supplementary material, figure S1). If this correlation results from many loci independently affecting each phenotype, then recombination between differently adapted lines will break down this favourable correlation. To test the cause of the WUE–FT correlation, we used TK RILs from two phenotypically divergent accessions, TSU-1 (low WUE, short FT) and KAS-1 (high WUE, long FT) [46] (see the electronic supplementary material, figure S1). Experimental crosses induce recombination and break up linkage disequilibrium across these genomes. Despite a large reduction in linkage disequilibrium, FT and WUE remained significantly correlated (\( n = 304, r^2 = 0.138, p < 0.0001 \); figure 1a) in the TK RILs, demonstrating that either tight genetic linkage or pleiotropy caused WUE and FT to covary.

To determine the genetic basis of the remaining WUE–FT correlation in the RILs, we conducted a QTL analysis by simultaneously scanning for genomic loci significantly associated with both phenotypes. Stepwise model selection (\( \alpha = 0.05 \)) revealed a total of 11 different QTLs across both traits (see
After adding the RILs (see the electronic supplementary material, table S2).

For each pairwise locus combination across the first 20 cM of Ch4. Grey bars on the axes indicate the point where the maximum score is achieved.

The genetic correlation between WUE and FT is well documented in agricultural breeding populations and studies of local adaptation in nature [38,44–45]. The genetic correlation between WUE and FT is affected, in part, by resource availability [60,61], a physiological connection between WUE and FT is plausible.

(b) Cloning the water use efficiency—flowering time quantitative trait locus

To identify all possible causal variants underlying the main QTL, we re-sequenced both parents and analysed gene expression in the TK RILs for loci within a 100 kb region surrounding the QTL. Within this region, only FRI (FRIGIDA) is differentially expressed between TSU-1 and KAS-1 (figure 1d). Re-sequencing of both parents revealed a 376 bp deletion within the promoter of the TSU-1 FRI allele, but a functional allele in KAS-1. We genotyped the FRI allele in all TK RILs (see the electronic supplementary material, table S1). After adding the FRI polymorphism to the linkage map, we conducted a multi-trait position refinement analysis. The QTL maps to a single pleiotropic locus at the nearest pseudo-marker to FRI; chromosome 4, position 4.0 cm (figure 1c).

FRI is a particularly good candidate gene underlying the FT QTL. Derived mutations that reduce expression have been detected in the evolution of spring annual types from the ancestral state of a fully functional FRI and a winter annual life history [47,62]; allelic variation at FRI contributes to variation in FT across diverse accessions [49,63–65]. FRI is also a candidate for WUE [38,66]. Biogeographic analyses have associated lines with functional FRI alleles, such as KAS-1, to regions with lower precipitation; these environments would favour drought adaptation via dehydration avoidance [67,68].

To further assess the pleiotropic effects of the FRI locus, we also genotyped 195 Arabidopsis accessions at FRI to determine functionality (see the electronic supplementary material, table S1). Consistent with pleiotropy and our observation in the TK RILs (figure 1b), phenotypic variation in both WUE (ANOVA d.f. = 1, F34,105, p < 0.0001) and FT (ANOVA d.f. = 1, F34,105, p < 0.0001) is predicted by functional variation at FRI in natural populations (figure 1b). In the 195 accessions, FRI explains 30.2 per cent and 24.7 per cent of the total phenotypic variation of WUE and FT, respectively. Interestingly, this ‘FT’ gene explains less phenotypic variation in FT than WUE in wild accessions. Null FRI alleles represent a derived state of early FT and lower WUE relative to functional alleles; a drought escape strategy.

(c) Physiological pleiotropy of FRI

To test for the phenotypic effects of FRI, we compared the phenotypes of a near isogenic line with a functional FRI
Consistent with the epistasis observed to affect FT, FRI confers increased WUE only in the presence of a functional FLC (contrast d.f. = 1, $F_{1,477} = 0.00001$) but not when associated with a null flc allele (contrast d.f. = 1, $F_{0,279} = 0.38$; electronic supplementary material, figure S3).

The FRI-NIL (also referred to as 'Sf-2 FRI in Col' or 'Col-FRI') is one of the most used genetic resources in the FT literature [38,49,71]. These studies assume that the FRI-NIL carries a single, narrow, introgression of the Sf-2 genome which contains a functional FRI allele; however, this assumption has never been tested. To assess the size of the Sf-2 introgression, we re-sequenced the FRI-NIL, aligned the reads to the TAIR9 Columbia genome, called SNPs and mapped SNP density to the reference genome. Many SNPs exist between Sf-2 and Col-0 (data are publicly available at http://msu.msu.edu/superfies/19genomes [72]). High SNP density between the FRI-NIL and Col-0 exists solely on proximate Chr. 4 (see the electronic supplementary material, figure S4a,b). The region of elevated SNP density represents a single 1.070 Mb ($\pm$ 10 kb) Sf-2 introgression that contains FRI as well as the other 329 gene models between AT4G00005 and AT4G02710. Although most studies that use the FRI-NIL assume the only genotypic divergence exists at FRI, this is obviously not the case.

To unambiguously determine whether the effects observed in the FRI-NIL were due to FRI, we compared WUE, FT and GRla between WT Col-0 and transgenic lines (Col-0 overexpressing FRI: tr-FRI). Under well-watered conditions, tr-FRI had greater WUE (d.f. = 1, $F_{1,275} = 0.00001$), decreased GRla (d.f. = 1, $F_{22,32} = 0.00001$) and later FT (d.f. = 1, $F_{1,79} = 0.00001$) than WT (figure 3d). As FRI functionality is the only DNA sequence difference between these lines, FRI is pleiotropic and controls covariation of three traits along a vector shown to be adaptive. Our conclusion is supported by QTL, natural accession, NIL and transgenic comparisons.

(d) The population genetics of adaptive pleiotropy

Population genetic models are at odds about the role of pleiotropy in maintaining variation within and among populations. Pleiotropic gene action may cause non-adaptive and adaptive phenotypes to covary, thus reducing the efficacy of correlational selection and permitting the persistence of multiple allelic states within populations [73]. However, where the effects of pleiotropy are more aligned with the direction of selection, within-population variation can be purged by strong directional selection [74]. Therefore, we predicted low levels of within-population variation at FRI, because multivariate selection would favour either a functional (drier habitats) or non-functional allele (wetter habitats). In addition, if variation at FRI can lead to local adaptation, then we predicted increased population structure (across the entire genome) between functional and non-functional FRI classes.

A population genetic test for adaptive pleiotropy is complicated in our study as FRI may cause population structure through both adaptive pleiotropy and allochrony: FRI-NILs and tr-FRI lines flowered at least 28 and 32 days later than Col-0, respectively. All main-raceme Col-0 flowers had been pollinated and produced fruits before any FRI-NIL or tr-FRI lines produced open flowers. In the greenhouse environment, single mutations at FRI can produce a reproductive isolation index near 1.0. However, assortative mating owing to variation at FRI may be tempered in nature as the environment has a profound effect on phenology [71].
To test for evidence of reproductive isolation between accessions and populations that differ at \(FRI\), we first imputed \(FRI\) functionality of 1188 accessions [57], then compared the group of individuals with derived, weak alleles (i.e. null Col-0 nonsense and Ler deletion alleles) to the group of individuals with functional, ancestral-type \(FRI\). We then calculated \(F_{ST}\) between \(FRI\) allele functional classes in PLINK [59]. \(F_{ST}\) values averaged across 216 130 SNPs are significantly greater between the \(FRI\) functionality classes than is expected from genome-wide subsampling \((p < 0.0001\); electronic supplementary material, figure S5). To control for geographical population structure, we divided the global sample into 11 geographical regions according to Horton et al. [57]. Ten of 11 geographical regions showed elevated \(F_{ST}\) at \(FRI\) compared with a genome-wide sample of sites with the same allele frequencies as the \(FRI\) functional variants (see the electronic supplementary material, table S5). Functional alleles tend to be present in areas with lower growing season precipitation than non-functional alleles \((l = -3.68, p = 0.0003\); electronic supplementary material, figure S6).

We have demonstrated that lines that diverged only at \(FRI\) exhibit altered positions along an adaptive phenotypic correlation. Scarcelli et al. [25] found antagonism between the floral morphology traits affected by \(FRI\), and we cannot rule out that a portion of \(FRI\)'s pleiotropic gene action is maladaptive. However, analyses presented here demonstrate a strong adaptive role of the physiological and phenological phenotypic correlations conferred by \(FRI\). Given our results, it is not surprising that \(FRI\) is associated with strong population genetic signatures of diversifying selection [65,75,76]. Studies demonstrating historical selection on \(FRI\) invoke the timing of flowering as the phenotype under selection [65]. Our results indicate that the observed signature of selection is not only an effect of FT variation, but may also be due to upstream physiological effects.

4. Conclusions

We have presented a mechanistic understanding of how \(FRI\) alters physiology, phenology and confers local adaptation. Phenology, growth rate and water-use physiology have been mapped to similar genomic loci or correlated in natural or experimental populations [31,38,46,52,66]. Here, we have demonstrated that \(FRI\) causes these adaptive correlations to be heritable. Although we present a situation where pleiotropy controls phenotypic variation along a vector known to be adaptive, we have not measured the efficacy of or response to selection in the field. Fitness measures in diverse common gardens with watering treatments would allow for direct inference of the adaptive value of \(FRI\).

To date, most gene annotation and characterization is conducted by forward or reverse genetics whereby a single gene or trait is under consideration. Our results indicate that a more holistic approach to phenotyping and whole plant, integrative approaches for annotating gene function
may reveal complex patterns of pleiotropy among ecologically correlated phenotypes. It is possible that many trait associations are not purely a product of correlational selection, but also affected by adaptive pleiotropy.

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References


