

Published in final edited form as:

Nat Genet. 2006 June ; 38(6): 711–715.

The *PHYTOCHROME C* photoreceptor gene mediates natural variation in flowering and growth responses of *Arabidopsis thaliana*

Sureshkumar Balasubramanian^{1,†}, Sridevi Sureshkumar^{1,†}, Mitesh Agrawal¹, Todd P. Michael^{2,3}, Carrie Wessinger⁴, Julin N. Maloof⁴, Richard Clark¹, Norman Warthmann¹, Joanne Chory^{2,3}, and Detlef Weigel^{1,2}

¹ Department of Molecular Biology, Max Planck Institute for Developmental Biology, D-72076 Tübingen, Germany.

² Plant Biology Laboratory, and

³ Howard Hughes Medical Institute, Salk Institute for Biological Studies, La Jolla, CA 92037, USA.

⁴ Section of Plant Biology, University of California, Davis, CA 95616, USA.

Abstract

Light plays an important role in modulating seedling growth and flowering time¹. We show that allelic variation at the *PHYTOCHROME C* (*PHYC*) photoreceptor locus affects both traits in natural populations of *A. thaliana*. Two functionally distinct *PHYC* haplotype groups are distributed in a *FRIGIDA*-dependent latitudinal cline that is stronger than the one reported for *FLOWERING LOCUS C*, which together with *FRIGIDA* explains a large portion of the variation in *A. thaliana* flowering time². In a genome-wide scan for association of 65 loci with latitude, there was an excess of significant *p*-values, indicative of population structure. Nevertheless, *PHYC* was the most strongly associated locus across 163 strains, suggesting that *PHYC* alleles are under diversifying selection in *A. thaliana*. Our work, together with previous findings^{3–6}, suggests that photoreceptor genes are major agents of natural variation in plant flowering and growth response.

Arabidopsis thaliana occurs across a wide range of latitudes in the northern hemisphere, and wild strains display vary extensively in light sensitivity and flowering responses. *FRI* and *FLC*, which mediate the effects of exposure to winter-like temperatures (vernalization), account for about 23% of the phenotypic variation in flowering time of *A. thaliana* in short days^{7–9}. In contrast to *FRI*, alleles of other loci that modulate variant flowering responses tend to be rare^{3–5,10}. In addition, most of the characterized natural alleles affect either seedling growth or flowering time, even though both traits are light dependent. Linkage disequilibrium mapping studies suggest that common allelic variation at the *CRYPTOCHROME2* (*CRY2*) photoreceptor locus is associated with flowering time variation, but this has not yet been experimentally verified⁶.

We have recently identified an accession from Frankfurt, Germany, Fr-2, which responds only weakly to a change in photoperiods. Compared to standard laboratory strains such as Col-0, Fr-2 flowers early in short days. This phenotype shows simple segregation in an F₂ population derived from a cross of Fr-2 to Col-0⁸. By performing whole-genome scans of 140 early flowering individuals selected from 900 F₂ plants, we mapped the earliness to a 1 Mb region on chromosome 5 (Supplementary Fig. 1 online). Within this interval, we found 11 genes that

Correspondence should be addressed to DW (weigel@weigelworld.org).

[†]These authors contributed equally to the work.

were differentially expressed between Col and Fr-2 (Fig. 1a), as deduced from AtGenExpress microarray data of 34 wild strains⁸. Because none of the other strains showed an Fr-2 like flowering behavior, and because the early flowering phenotype was recessive, we asked whether any of the 11 genes was specifically underexpressed in Fr-2. Only one gene, which encodes the red-light receptor *PHYC*, fulfilled this criterion (Fig. 1b).

PHYC knockout alleles not only cause early flowering in short days, but also confer reduced sensitivity to red light during seedling growth^{11,12}. Consistent with a defect in *PHYC*, Fr-2 seedlings responded to red light across a range of fluences less strongly than Col-0 seedlings, similar to plants with a mutation in the canonical red-light receptor *PHYB* (Fig. 1c). In an F₁, Fr-2 alleles complemented the flowering phenotypes of *phyA* and *phyB* mutants induced in the *Ler* background, indicating that *PHYA* and *PHYB* alleles of Fr-2 are functional (not shown). In contrast, Fr-2 failed to complement the flowering phenotype of a *phyC* knockout allele in Col-0, confirming that the early flowering of Fr-2 is largely due to a defect in *PHYC* (Fig. 1d).

Sequencing of a *PHYC* cDNA from Fr-2 revealed a nonsense change in the first exon, which converts the K299 codon to a stop codon. The predicted Fr-2 *PHYC* protein therefore lacks half of the GAF domain, and the entire PHY, PAS and histidine kinase domains, all of which are typically required for phytochrome function. Premature stop codons can trigger non-sense mediated mRNA decay¹³, which may explain the reduced levels of *PHYC* mRNA detected on microarrays¹⁴.

In addition to the stop codon, the Fr-2 *PHYC* allele is highly polymorphic with 12 nonsynonymous changes compared to Col-0. Ten of these substitutions are present in another common laboratory strain, *Ler*¹⁵. Many screens for seedlings with reduced light responses have been carried out in the *Ler* background, but *phyC* alleles were never recovered, suggesting that the *Ler* *PHYC* allele may have limited activity. Earlier overexpression studies of *PHYC* are in agreement with this hypothesis. Overexpression of Col-0 *PHYC* in *Arabidopsis* resulted in reduced hypocotyl length under red light. In contrast, while overexpression of *Ler* *PHYB* in transgenic tobacco plants also lead to shorter hypocotyls, *Ler* *PHYC* had no effect on hypocotyl length in red light^{16,17}.

If the *Ler* *PHYC* allele is less active than that of Col-0, *PHYC* should at least partially explain the earlier flowering of *Ler* in short days. Indeed, a quantitative trait locus (QTL) that maps to the *PHYC* region has been detected in *Ler* x Col recombinant inbred lines (RILs) grown under natural conditions in fall, when days are short¹⁸. Consistent with a similar genetic basis of early flowering under short days in Fr-2 and *Ler*, a *Ler* x Fr-2 F₂ population showed a near normal distribution of flowering time in short days, which contrasts with the behavior of a Col-0 x Fr-2 F₂ population (Supplementary Fig. 2a online). Genotyping of F₂ populations derived from Fr-2 x Col or Fr-2 x *Ler* revealed that the average flowering time of plants that carry Col-0 *PHYC* was much higher than that of plants with Fr-2 or *Ler* alleles (Supplementary Fig. 2b online). Furthermore, the *PHYC* allele of *Ler* failed to fully complement the *phyC* knockout in the Col-0 background. Fr-2 x *Ler* F₁ plants flowered early in short days, in agreement with the limited activity of *Ler* *PHYC* (Supplementary Fig. 2c online). Finally, quantitative complementation analyses showed that phenotypic differences due to allelic variation between Fr-2 and *Ler* *PHYC* were less than those observed between Col-0 and either Fr-2 or *Ler* (Fig. 2).

Since both the sequence of *PHYC* in Col, *Ler*, and Fr-2 and the genetic data suggested functionally distinct *PHYC* alleles, we studied the haplotype structure at the *PHYC* locus in more detail. We sequenced *PHYC* from a randomly chosen sample of 29 wild strains. Phylogenetic analysis, using the sister species *A. lyrata* as outgroup, indicated two major haplotype classes, with *Ler* in one class and Col-0 in the other (Fig. 3). A total of 40 SNPs that

result in eight informative amino acid changes were in complete linkage disequilibrium among the two haplotype groups (Fig 3). In addition, the two haplotype groups were distinguished by an indel 500 bp upstream of the start codon.

Mapping experiments have detected QTL for flowering time and hypocotyl length in many RIL populations across multiple environments. Haplotype analysis on multiple crosses has been previously used to identify candidate genes for a QTL¹⁹. In a reverse approach, we predicted that if the two *PHYC* haplotypes are distinguished by their activity, variation in flowering time and hypocotyl length should map to *PHYC* in crosses derived from parental lines with contrasting haplotypes. Using the promoter indel as a diagnostic marker for the two haplotype groups, we assessed the *PHYC* haplotype of more than 220 Eurasian strains (Supplementary Table 1 online), and then examined published QTL maps from six different crosses for flowering time and hypocotyl length^{10,18,20–24}. As predicted, only populations in which the parents carried different haplotypes at *PHYC* showed a QTL near *PHYC* (Table 1). The direction of the QTL effect in all crosses was consistent with the *Ler*-like haplotype being less active than the *Col*-like haplotype. In agreement with the known function of *PHYC*, the QTL depend on the environment, with the light sensitivity QTL being detected under red light and the flowering time QTL in short days.

We also asked whether *PHYC* haplotype groups could account for variation in hypocotyl lengths of 115 wild strains, which we had previously analyzed in several different light conditions⁵. We found that *PHYC* could explain 8% of the variation in white light, and that *PHYC* was significantly associated with hypocotyl lengths across several conditions (Table 2). All these results support the hypothesis that *PHYC* is responsible for the flowering time and hypocotyl length QTL that have been reported in this genomic region. We note, however, that a tightly linked locus could contribute to these results.

That the less active *PHYC* haplotype group is quite common suggests that variation at this locus may be adaptive in the wild. We therefore asked whether the frequency of *PHYC* haplotypes varies with latitude, since co-variation with environmental factors can be evidence of adaptation²⁵. Latitudinal clines in light sensitivity and flowering time are known in *A. thaliana*^{5,8,26}. Indeed, we found that the more active *Col-0* *PHYC* haplotype group was more frequent at northern latitudes ($p = 0.0001$, $n = 221$). This is particularly striking among strains that do not carry obvious lesions in *FRI* (Fig. 4a). In addition, the contribution of *PHYC* to flowering time is latitude-dependent. A *PHYC* x latitude interaction can explain 10% of the residual variation in short day flowering, after accounting for effects of *FRI*, suggesting that *PHYC* partially contributes to the late flowering of *FRI* positive strains at northern latitudes (Supplementary Table 2 online). Since latitudinal associations could also be due to population structure, we asked how often significant interaction terms were observed in 163 strains for a set of 65 SNPs that have comparable allele frequencies and that are spaced throughout the genome²⁷. The same analysis was carried out for *FLC*, which has been reported to show a *FRI*-dependent latitudinal cline independent of population structure²⁸. The p -values for the association of *PHYC* ranked first for latitude (Fig. 4b) and fourth for the *FRI*-dependent interaction with latitude (Fig. 4c). In addition, it always ranked ahead of the corresponding p -values for *FLC*. *PHYC* was also the highest ranking marker for association with hypocotyl length after gibberellin treatment⁵ (Supplementary Table 3 online). While there is an excess of significant p -values among the random loci, which is consistent with the effect of population structure, the relative ranks of the *PHYC* p -values are suggestive of adaptive selection²⁹ (Supplementary Table 3 online). A similar analysis with an independent set of 69 SNP markers, even with a much smaller sample size of 56 strains, yielded comparable results (Supplementary Table 4 online).

Of the informative SNPs that distinguish the *PHYC* haplotype groups, 20 Col-0 SNPs are shared with *A. lyrata*, as are 20 *Ler*-SNPs. Of the 8 amino acid changes that characterize the Col-0 and *Ler* haplotypes, four are clustered in a small region that seems to have regulatory activity in both *PHYA* and *PHYB*^{30,31}. These changes could in principle be responsible for the functional difference between the haplotypes, although both specify unique amino acids at positions that are conserved in other phytochromes, not only in *Arabidopsis*, but also in land plants in general.

PHYC appears to have arisen by duplication of *PHYA*, which encodes a far-red light receptor. *phyC* knock out alleles in Col-0 and *Ws* backgrounds have phenotypes mostly in red and blue light^{11,12}, but we found phenotypic associations even in far-red light. Our results could indicate differential cross talk among photoreceptors, dependent on *PHYC* haplotype. Alternatively, our understanding of *phyC* mediated light perception may have been limited by having only knock out alleles in Col-0 and *Ws*, which share the same haplotype.

While *PHYC* is mostly far-red sensing in rice, in *Arabidopsis* it is functionally more similar to the red-light sensing *PHYB*^{11,12,32,33}. Nevertheless, loss of *PHYC* has similar consequences in *Arabidopsis* and rice, namely early flowering under non-inductive conditions^{12,33}. This suggests that the functional divergence of *PHYC* from *PHYA* varies in different taxa. Consistent with this idea, *PHYA* evolves faster than *PHYC* in some species, but *PHYC* sequences are more divergent than those of other phytochrome genes in *Arabidopsis*, tomato and sorghum^{34–36}. *PHYC* has even been suggested as a target for adaptive evolution in sorghum³⁵. In *Arabidopsis*, allelic variation with demonstrated functional effects appears to be more pervasive at *PHYC* than at other photoreceptor loci^{3–5}. The functional role of allelic variation at *PHYC* in other species is therefore an important question for future research.

Materials and Methods

Plant material and phenotyping

Seed stocks and growth conditions have been described⁸. *phyC* insertion lines were obtained from the Nottingham Arabidopsis Stock Centre. Typically, 120 F₂ plants were used to analyze flowering time, as measured by counting total leaf number on the main shoot. For complementation studies, 30–50 F₁ plants each were examined. For hypocotyl length measurements, about 20 seeds were stratified on Murashige-Skoog medium for four days at 4°C in dark, and then transferred to growth chambers at 23°C. A week later, plants were flattened and imaged on a flatbed scanner. Hypocotyls lengths were measured using NIH Image.

DNA Analyses

Genomic DNA was amplified with Pfu polymerase (Fermentas), and products pooled from two to four independent reactions were directly sequenced. See Supplementary Table 5 online for oligonucleotide primers. A 4.9 kb region covering the promoter and almost the entire coding region was obtained from 26 *A. thaliana* strains, the same fragment without the 1.5 kb promoter from three *A. thaliana* and one *A. lyrata* strain (GenBank accession numbers XXXX-XXXXX).

Phylogenetic Analysis

Sequences were aligned using Seqman (DNA Lasergene Inc, USA) and alignment was verified manually. Diversity measurements were obtained with DnaSP v4.10 (<http://www.ub.es/dnasp>)³⁷. Sequence alignments were imported into PAUP³⁸ and a heuristic search with maximum likelihood was performed using the settings for HKY model specified through Modeltest³⁹. A maximum parsimony search was performed with ACCTRAN character state optimization. In both methods, initial trees were generated through step-wise

addition, TBR branch swapping option was used. 1000 bootstrap permutations were performed using the same search settings used for parsimony search with the full heuristic search option in PAUP. Parsimony and maximum likelihood resulted in similar trees. The maximum parsimony tree is shown in Fig. 3. The same split in the tree was obtained when using coding or non-coding regions.

Statistical Analyses

Data were analyzed using JMP (version 5.1, SAS Institute), Excel (Microsoft) or R (<http://www.r-project.org>). For the latitudinal association studies, only Eurasian strains were used. The larger set made use of SNP genotyping information published in ref. ²⁷. Information on the independent SNPs used in the second, smaller analysis is available at <http://naturalvariation.org>. A nominal logistic regression model with genotype as the response and latitude as a factor was used to compute p-values for latitudinal cline. For testing the interaction with *FRI*, a simple linear regression model with latitude as the response, and *FRI* and the genotype of *PHYC* or a random SNP as interaction term was used. The intrinsic multiple testing problem associated when comparing *PHYC* with random SNPs is somewhat mitigated by the fact that of 43 SNPs in *PHYC*, 40 are fixed in the two haplotype groups. For association with flowering time, residuals on regression with flowering time as the response and *FRI* functionality as a factor, were regressed using *PHYC* haplotype group and latitude as factors with interaction. *PHYC* x latitude interaction accounted for about 10% of the variation in the residuals. For association with hypocotyl length, a single factor ANOVA was performed with *PHYC* haplotype group as the factor and hypocotyl lengths as response. Previously published flowering time and hypocotyls length measurements were used for association studies^{5,8}.

Acknowledgements

We thank the Nottingham Arabidopsis Stock Center for seed stocks of SALK T-DNA lines donated by Joe Ecker and colleagues. We thank Christa Lanz for help with sequencing, and Kirsten Bomblies and Ya Long Guo for help with phylogenetic analysis. We thank Kirsten Bomblies, Vava Grbic, Yasushi Kobayashi, Janne Lempe and Stephen Russell for discussion and critical reading of the manuscript, and the anonymous reviewers for insightful comments that led to improvements of the manuscript. Supported by an EMBO Long-Term Fellowship to S.B., an NIH Postdoctoral Fellowship to T.P.M., an NIH grant (GM62932) to J.C. and D.W., and by the Max Planck Society, of which D.W. is a director. J.C. is an HHMI Investigator.

S.B., J.C. and D.W. conceived the experiments; S.B., S.S., M.A., T.P.M., C.W., and J.N.M. performed the experiments; S.B., S.S., J.N.M., R.C., N.W., J.C. and D.W. analyzed the data and wrote the manuscript.

References

1. Chen M, Chory J, Fankhauser C. Light signal transduction in higher plants. *Annu Rev Genet* 2004;38:87–117. [PubMed: 15568973]
2. Johanson U, et al. Molecular analysis of *FRIGIDA*, a major determinant of natural variation in *Arabidopsis* flowering time. *Science* 2000;290:344–347. [PubMed: 11030654]
3. Aukerman MJ, Amasino RM. Molecular genetic analysis of flowering time in *Arabidopsis*. *Sem Cell Dev Biol* 1996;7:427–433.
4. El-Assal SED, Alonso-Blanco C, Peeters AJ, Raz V, Koornneef M. A QTL for flowering time in *Arabidopsis* reveals a novel allele of *CRY2*. *Nat Genet* 2001;29:435–40. [PubMed: 11726930]
5. Maloof JN, et al. Natural variation in light sensitivity of *Arabidopsis*. *Nat Genet* 2001;29:441–6. [PubMed: 11726931]
6. Olsen KM, et al. Linkage disequilibrium mapping of *Arabidopsis* *CRY2* flowering time alleles. *Genetics* 2004;167:1361–9. [PubMed: 15280248]
7. Shindo C, et al. Role of *FRIGIDA* and *FLOWERING LOCUS C* in determining variation in flowering time of *Arabidopsis*. *Plant Physiol* 2005;138:1163–73. [PubMed: 15908596]
8. Lempe J, et al. Diversity of flowering responses in wild *Arabidopsis thaliana* strains. *PLoS Genet* 2005;1:e6.

9. Werner JD, et al. *FRIGIDA*-independent variation in flowering time of natural *A. thaliana* accessions. *Genetics*. 2005in press
10. Werner JD, et al. Quantitative trait locus mapping and DNA array hybridization identify an *FLM* deletion as a cause for natural flowering-time variation. *Proc. Natl. Acad. Sci. USA* 2005;102:2460–5.
11. Franklin KA, Davis SJ, Stoddart WM, Vierstra RD, Whitelam GC. Mutant analyses define multiple roles for phytochrome C in *Arabidopsis* photomorphogenesis. *Plant Cell* 2003;15:1981–9. [PubMed: 12953105]
12. Monte E, et al. Isolation and characterization of *phyC* mutants in *Arabidopsis* reveals complex crosstalk between phytochrome signaling pathways. *Plant Cell* 2003;15:1962–80. [PubMed: 12953104]
13. Conti E, Izaurralde E. Nonsense-mediated mRNA decay: molecular insights and mechanistic variations across species. *Curr Opin Cell Biol* 2005;17:316–25. [PubMed: 15901503]
14. Dybbs M, Ngai J, Kaplan JM. Using microarrays to facilitate positional cloning: identification of tomosyn as an inhibitor of neurosecretion. *PLoS Genet* 2005;1:e2.
15. Cowl JS, et al. The *PHYC* gene of *Arabidopsis*. Absence of the third intron found in *PHYA* and *PHYB*. *Plant Physiol* 1994;106:813–4. [PubMed: 7991704]
16. Qin M, Kuhn R, Moran S, Quail PH. Overexpressed phytochrome C has similar photosensory specificity to phytochrome B but a distinctive capacity to enhance primary leaf expansion. *Plant J* 1997;12:1163–72. [PubMed: 9418054]
17. Halliday KJ, Thomas B, Whitelam GC. Expression of heterologous phytochromes A, B or C in transgenic tobacco plants alters vegetative development and flowering time. *Plant J* 1997;12:1079–90. [PubMed: 9418049]
18. Weinig C, et al. Novel loci control variation in reproductive timing in *Arabidopsis thaliana* in natural environments. *Genetics* 2002;162:1875–84. [PubMed: 12524356]
19. Wang X, Korstanje R, Higgins D, Paigen B. Haplotype analysis in multiple crosses to identify a QTL gene. *Genome Res* 2004;14:1767–72. [PubMed: 15310659]
20. Alonso-Blanco C, El-Assal SE, Coupland G, Koornneef M. Analysis of natural allelic variation at flowering time loci in the Landsberg *erecta* and Cape Verde Islands ecotypes of *Arabidopsis thaliana*. *Genetics* 1998;149:749–764. [PubMed: 9611189]
21. Borevitz JO, et al. Quantitative trait loci controlling light and hormone response in two accessions of *Arabidopsis thaliana*. *Genetics* 2002;160:683–96. [PubMed: 11861571]
22. Loudet O, Chaillou S, Camilleri C, Bouchez D, Daniel-Vedele F. Bay-0 x Shahdara recombinant inbred line population: a powerful tool for the genetic dissection of complex traits in *Arabidopsis*. *Theor Appl Genet* 2002;104:1173–1184. [PubMed: 12582628]
23. El-Lithy ME, Clerks EJ, Ruys GJ, Koornneef M, Vreugdenhil D. Quantitative trait locus analysis of growth-related traits in a new *Arabidopsis* recombinant inbred population. *Plant Physiol* 2004;135:444–58. [PubMed: 15122039]
24. Wolyn DJ, et al. Light-response quantitative trait loci identified with composite interval and eXtreme array mapping in *Arabidopsis thaliana*. *Genetics* 2004;167:907–17. [PubMed: 15238539]
25. Endler, JA. Princeton University Press; Princeton, NJ: 1977. *Geographic Variation, Speciation, and the Clines*; p. 262
26. Stinchcombe JR, et al. A latitudinal cline in flowering time in *Arabidopsis thaliana* modulated by the flowering time gene *FRIGIDA*. *Proc. Natl. Acad. Sci. USA* 2004;101:4712–7.
27. Schmid KJ, et al. Evidence for a large-scale population structure of *Arabidopsis thaliana* from genome-wide single nucleotide polymorphism markers. *Theor Appl Genet* 1:11.2006
28. Caicedo AL, Stinchcombe JR, Olsen KM, Schmitt J, Purugganan MD. Epistatic interaction between *Arabidopsis FRI* and *FLC* flowering time genes generates a latitudinal cline in a life history trait. *Proc. Natl. Acad. Sci. USA* 2004;101:15670–15675.
29. Aranzana MJ, et al. Genome-wide association mapping in *Arabidopsis* identifies previously known flowering time and pathogen resistance genes. *PLoS Genet* 2005;1:e60. [PubMed: 16292355]
30. Wagner D, Quail PH. Mutational analysis of phytochrome B identifies a small COOH-terminal-domain region critical for regulatory activity. *Proc. Natl. Acad. Sci. USA* 1995;92:8596–8600.

31. Xu Y, Parks BM, Short TW, Quail PH. Missense mutations define a restricted segment in the C-terminal domain of phytochrome A critical to its regulatory activity. *Plant Cell* 1995;7:1433–43. [PubMed: 8589627]
32. Reed JW, Nagpal P, Poole DS, Furuya M, Chory J. Mutations in the gene for the red/far-red light receptor phytochrome B alter cell elongation and physiological responses throughout *Arabidopsis* development. *Plant Cell* 1993;5:147–157. [PubMed: 8453299]
33. Takano M, et al. Distinct and cooperative functions of phytochromes A, B, and C in the control of deetiolation and flowering in rice. *Plant Cell* 2005;17:3311–25. [PubMed: 16278346]
34. Mathews S, Donoghue MJ. The root of angiosperm phylogeny inferred from duplicate phytochrome genes. *Science* 1999;286:947–50. [PubMed: 10542147]
35. White GM, Hamblin MT, Kresovich S. Molecular evolution of the phytochrome gene family in sorghum: changing rates of synonymous and replacement evolution. *Mol Biol Evol* 2004;21:716–23. [PubMed: 14963106]
36. Devos KM, Beales J, Ogihara Y, Doust AN. Comparative sequence analysis of the *Phytochrome C* gene and its upstream region in allohexaploid wheat reveals new data on the evolution of its three constituent genomes. *Plant Mol Biol* 2005;58:625–41. [PubMed: 16158239]
37. Rozas J, Sanchez-DelBarrio JC, Messeguer X, Rozas R. DnaSP, DNA polymorphism analyses by the coalescent and other methods. *Bioinformatics* 2003;19:2496–7. [PubMed: 14668244]
38. Swofford DL. PAUP: A computer program for phylogenetic inference using maximum parsimony. *J Gen Physiol* 1993;102:9A.
39. Posada D, Crandall KA. MODELTEST: testing the model of DNA substitution. *Bioinformatics* 1998;14:817–8. [PubMed: 9918953]

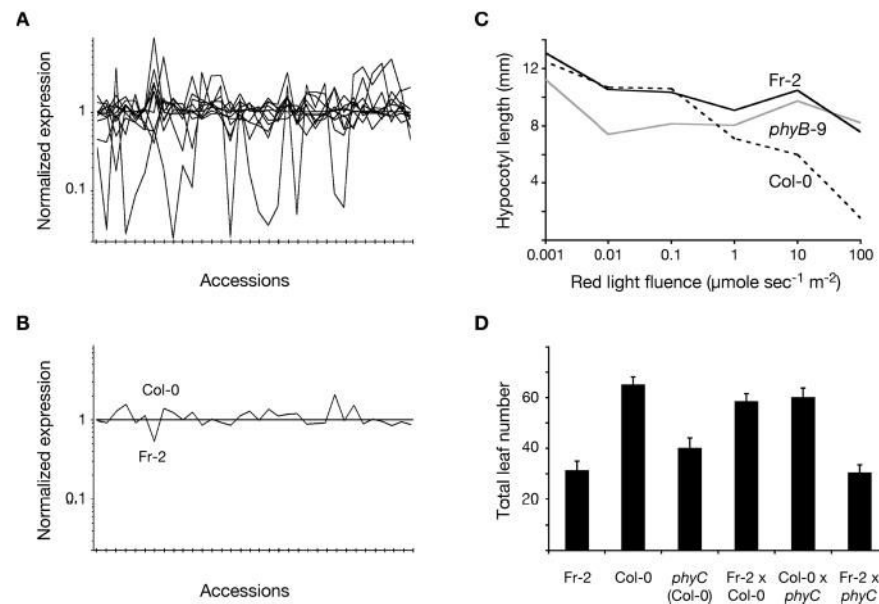


Figure 1.

Identification of a defective *PHYC* allele in Fr-2. (a) Expression profiles of genes that are differentially expressed between Fr-2 and Col across 34 wild strains. The genomic interval that co-segregates with early flowering of Fr-2 were analyzed for differentially expressed genes using the AtGenExpress dataset of expression data from 34 strains⁸. Of 112 genes in this interval represented on the ATH1 microarray, 11 were differentially expressed between Col-0 and Fr-2, and are shown here. (b) Expression profile of *PHYC* (At5g38540) in the 34 strains shown in (a). (c) Red light response of Fr-2, Col-0 and *phyB-9* in Col-0. The response of Fr-2 is similar to that of *phyB-9*, indicating reduced red light sensitivity. (d) Flowering under short days of different parental lines and their F_1 progeny.

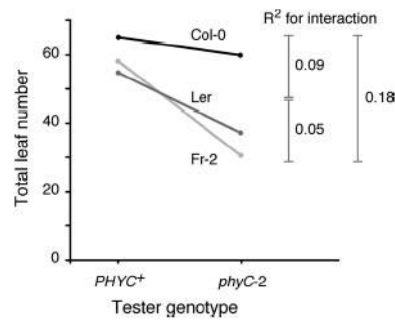


Figure 2.

Quantitative complementation analysis with different parental lines. Col-0 was used as the wild type and the null allele *phyC-2* in the Col-0 background as a tester for the crosses. An ANOVA was performed with the following model: $TLN \sim \text{Line} + \text{Cross} + \text{Line} \times \text{Cross}$. The Line \times Cross interaction was significant in all three combinations ($p < 0.0001$). However, the proportion of total variance accounted for by the Line \times Cross interaction varies (shown as R^2), which is consistent with the *Ler* allele being intermediate in activity between the Col-0 and Fr-2 alleles.

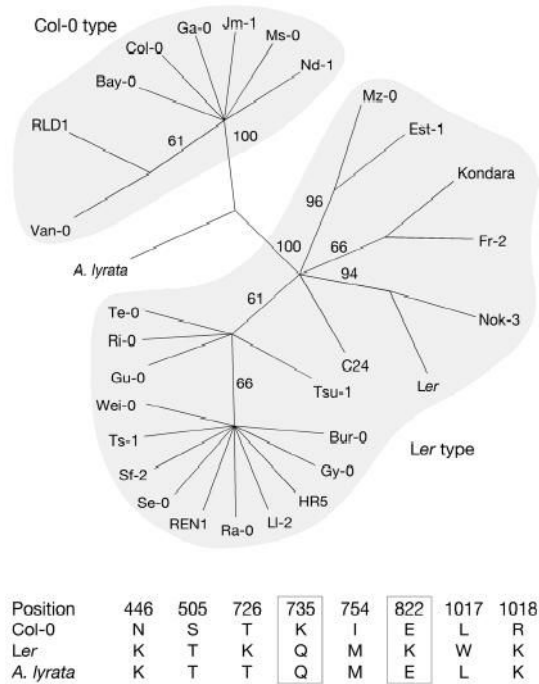
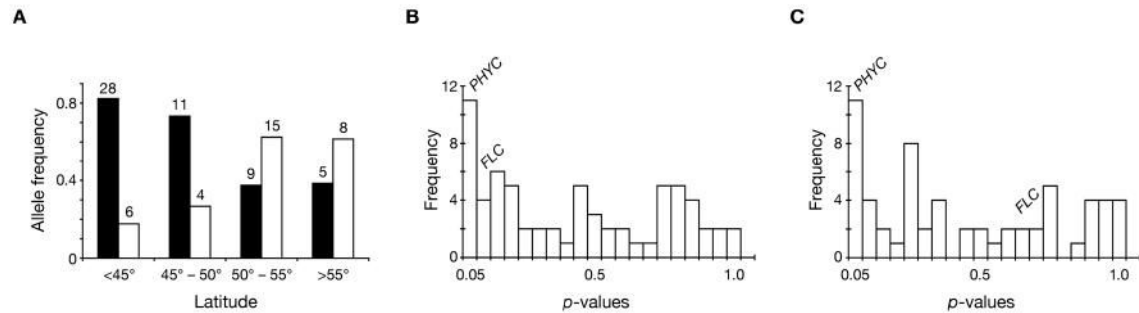
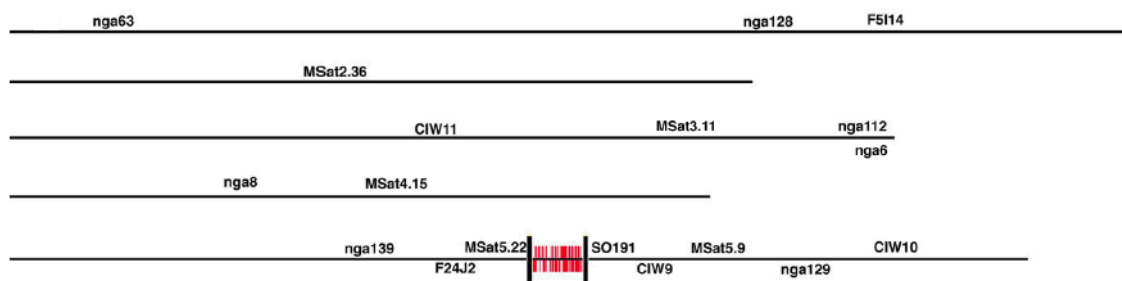


Figure 3. *PHYC* haplotypes. A representative unrooted phylogenetic tree generated from *PHYC* coding sequences is shown on top. Numbers indicate bootstrap values above 60. The amino acid changes that delineate the two haplotype groups are given below. Unique changes compared to the outgroup *A. lyrata* are found in both haplotypes. Amino acids that are conserved in other phytochromes (Q or E at position 735, and E at position 822), but changed in either the Col-0- or Ler-type haplotype, are boxed. See Supplementary Table 6 online for provenance of strains.

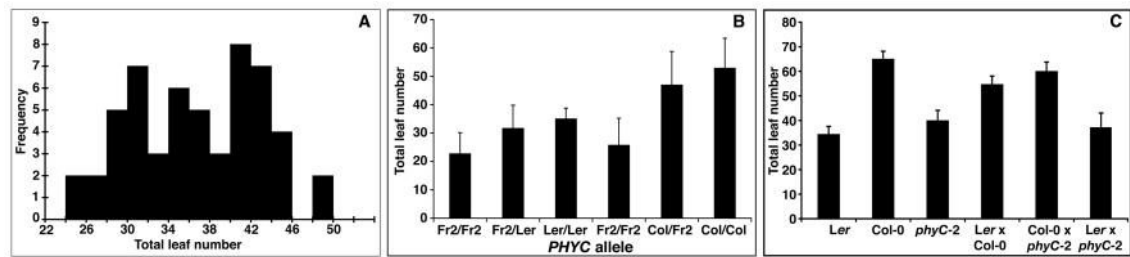
**Figure 4.**

Latitudinal cline of *PHYC* alleles. (a) Proportion of *Ler*-type (black) and *Col-0*-type (white) *PHYC* alleles at different latitudes among apparently *FRI* functional strains. The absolute numbers for each of the classes is given on top of the histograms. (b) Distribution of *p*-values of a nominal logistic regression model with latitude as a factor and genotypes as response. Allele information of 65 random SNP markers with similar allele frequency as that of *PHYC* was available in a set of 163 strains. This information was used as a response. Note genome-wide skew towards small *p*-values. (c) Distribution of *p*-values for interaction of a given random marker with *FRI* in an interaction model with latitude as the response and *FRI* and marker genotypes as factors with interaction.



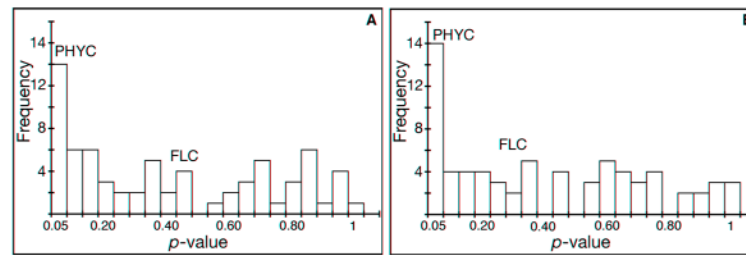
Supplementary Figure 1.

Markers used for mapping of the early flowering phenotype in Fr- 2. About 80 early plants were genotyped for all markers and linkage of the phenotype to MSat 5.22 and SO191 was confirmed with 60 additional early plants.



Supplementary Figure 2.

Flowering behavior of populations segregating for different *PHYC* alleles. (a) Distribution of flowering time in an F₂ population derived from *Ler* x *Fr-2* cross showing a continuous distribution, in contrast to a bimodal distribution observed in an F₂ population derived from the *Col* x *Fr-2* cross (see Lempe, J. et al. Diversity of flowering responses in wild *Arabidopsis thaliana* strains. PLoS Genet. 1, e6 [2005]). (b) Average flowering times of plants with different allelic combinations at *PHYC* in F₂ populations derived from *Ler* x *Fr-2* and *Col* x *Fr-2*. A single copy of the *Col-0* allele delays flowering much more than a single *Ler* allele. (c) Genetic complementation analysis with *Ler*. Flowering times of F₁ progeny in short days along with parental lines are shown.



Supplementary Figure 3.

Latitudinal cline of *PHYC* alleles. (a) Distribution of p -values of a nominal logistic regression model with latitude as a factor and genotype as response. Allele information of 69 random SNP markers with similar allele frequency as that of *PHYC* was available in a set of 56 strains. This information was used as a response. Note genome-wide skew towards small p -values. (c) Distribution of p -values for interaction of a given random marker with *FRI* in an interaction model with latitude as the response and *FRI* and marker genotypes as factors with interaction. *PHYC* falls in the top bin for both associations and the p -values for *PHYC* are smaller than those for *FLC*.

Table 1

Summary of QTL in existing RIL sets.

RIL set	Trait	<i>PHYC</i> haplotype contrast	QTL at <i>PHYC</i>	Reference
<i>Ler</i> x Col-0	Flowering time in fall cohorts	Yes	Yes	18
Bay-0 x Shahdara	Flowering time in short days	Yes	Yes	22
Kas-1 x Col-0	Hypocotyl length in red light	Yes	Yes	24
<i>Ler</i> x Cvi-0	Flowering time in short days; hypocotyl length in red light	No	No	20; 21
Nd-1 x Col-0	Flowering time in short days	No	No	10
<i>Ler</i> x Shahdara	Flowering time in short days	No	No	23

Table 2Associations of *PHYC* with hypocotyl lengths across range of conditions.

Environment	Average hypocotyl length (mm)*		<i>p</i> -value
	Ler haplotype group	Col-0 haplotype group	
White	7.11	6.09	0.001
Blue	6.42	5.78	0.02
Red	10.42	9.43	0.02
Far red	5.40	4.77	0.02
Gibberellin (GA)	8.42	7.03	0.0003
Brassinazole (BRZ)	5.62	5.08	0.05
Dark	12.45	12.00	0.21

* from ref. ⁵

Supplementary Table 1

PHYC haplotypes of *A. thaliana* strains.

No	Accessions	Stock [#]	Country	Latitude	<i>PHYC</i>	<i>FRI</i> * [†]	<i>FLC</i> [†]
1	Aa-0	CS900	Germany	51	Ler	Del	B
2	Ag-0	CS901	France	45	Ler	Wt	A
3	Ak-1	N939	Germany	48.6	Ler	Del	A
4	An-1	CS6603	Belgium	51.5	Ler	Del	A
5	Ang-1	N951	Belgium	50.62	Ler	Del	A
6	Bay-0	N955	Germany	49.95	Col	Del	B
7	Bch-1	N957	Germany	53.37	Col	Del	A
8	Bch3	N959	Germany	53.37	Ler	Del	B
9	Bch4	N961	Germany	53.37	Col	Del	A
10	Be-1	N967	Germany	49.5	Ler	Del	B
11	Ber-0	CS8068	Denmark	55	Ler		
12	Bl-1	CS6615	Italy	44.5	Col	Wt	B
13	Bla-1	N971	Spain	41.68	Ler	Wt	A
14	Bla-11	N985	Spain	41.68	Ler	Wt	A
15	Bla-12	N987	Spain	41.68	Col	Del	A
16	Bla-14	N989	Spain	41.68	Col	Del	
17	Bla-2	N973	Spain	41.68	Ler	Wt	A
18	Bla-3	N975	Spain	41.68	Ler	Wt	A
19	Bla-5	N6620	Spain	41.68	Col	Wt	
20	Bla-6	N6621	Spain	41.68	Ler	Wt	A
21	Blh-1	CS6645	Czech	48.83	Ler	Wt	
22	Bor-4	CS22591	Czech	49.5	Ler	Wt	
23	Br-0	N995	Czech	49.2	Ler	Wt	A
24	Bs-1	N997	Switzerland	47.55	Ler	Del	B
25	C24	CS906	Portugal	40.2	Ler	Wt	A
26	Ca-0	CS6658	Germany	50.5	Ler	Del	B
27	Can-0	N1065	Spain	28	Ler	Wt	
28	Cha-0	N1069	Switzerland	46.03	Col	Wt	
29	Chi-0	N1073	Russia	54	Col	Wt	
30	Co-1	N1085	Portugal	40.2	Ler	Wt	A
31	Co-2	N1087	Portugal	40.2	Col	Wt	
32	Co-3	N1089	Portugal	40.2	Col	Wt	A
33	Col	Lab Stock			Col	Del	A
34	Ct-1	CS6674	Italy	37.5	Ler	Del	
35	Da(1-12)	CS917	Czech	49.45	Ler	Wt	A
36	Da-0	CS6676	Germany	50	Ler	Del	B
37	Db-0	N1101	Germany	50	Col	Del	B
38	Db-1	CS6678	Germany	50	Col	Del	B
39	Db-2	N6679	Germany	50	Col	Del	B
40	Di-1	N1109	France	47.42	Col	Del	B
41	Di-2	N1111	France	47.42	Col	Del	A
42	Di-g	CS910	France	47.2	Ler	Del	A
43	Dr-0	N1115	Germany	51.05	Ler	Del	B
44	Dra-0	CS6685	Czech	49.42	Ler	Wt	A
45	Dra-1	N1119	Czech	49.42	Ler	Del	A
46	Dra-2	N1121	Czech	49.42	Col	Del	A
47	Ei-2	CS6689	Germany	50.25	Ler	Wt	A
48	Ei-5	N6691	Germany	50.25	Ler	Wt	
49	Ei-6	N1131	Germany	50.25	Ler	Del	B
50	Eil-0	N1133	Germany	51.5	Col	Del	A
51	El-0	N1135	Germany	51.5	Ler	Del	B
52	En-1	N1137	Germany	50.15	Ler	Del	B
53	En-2	N1139	Germany	50.15	Col	Del	
54	Ep-0	N1141	Germany	50.5	Ler	Del	
55	Er-0	N1143	Germany	49.58	Col	Del	A
56	Est-1	N1151	Estonia	59	Ler	Del	A
57	Et-0	N1153	France	44.63	Ler	Wt	
58	Fei-0	CS22645	Portugal	39	Ler	Del	
59	Fi-0	N1157	Germany	50.5	Col	Del	A
60	Flo-0	CS6044	Italy	43.8	Ler	Wt	A
61	Fr-2	N1169	Germany	50.2	Ler	Del	B
62	Fr-3	N1171	Germany	50.12	Ler	Del	B
63	Fr-4	N1173	Germany	50.12	Ler	Del	B
64	Fr-5	N1175	Germany	50.12	Col	Del	
65	Fr-6	N1177	Germany	50.12	Ler	Del	A
66	Fr-7	N1179	Germany	50.12	Ler	Del	B
67	Ga-0	N1181	Germany	50.3	Col	Del	A
68	Gd-1	N1185	Germany	53.55	Ler	Del	
69	Gie-0	N1193	Germany	50.5	Col	Del	B
70	Go-0	N1195	Germany	51.5	Ler	Del	
71	Go-2	N1197	Germany	51.5	Ler	Del	B
72	GOT1	N22277	Germany	51.5	Ler	Wt	

No	Accessions	Stock [#]	Country	Latitude	PHYC	FRI* [†]	FLC [†]
73	GOT10	N22286	Germany	51.5	Ler	Wt	
74	GOT7	CS22608	Germany	51.5	Ler	Wt	
75	Gr-1	N1199	Austria	47.06	Ler	Del	A
76	Gr-3	N1203	Austria	47.06	Col	Wt	A
77	Gr-5	N1207	Austria	47.06	Col	Wt	B
78	Gr-6	N6728	Austria	47.06	Ler	Wt	B
79	Gu-0	N1213	Germany	50.4	Ler	Del	B
80	Gu-1	N1215	Germany	50.4	Ler	Del	B
81	Gy-0	N1217	France	49	Col	Del	A
82	H55	CS932	Czech	50	Col	Del	A
83	Ha-0	N1219	Germany	52.5	Col	Del	A
84	Hau-0	N1221	Denmark	55.67	Ler	Del	
85	Hi-0	CS6736	Netherlands	52.5	Ler	Del	B
86	HI-2	N1231	Germany	52.38	Ler	Del	B
87	HI-3	N1233	Germany	52.38	Ler	Del	A
88	Hn-0	N1235	Germany	51.5	Ler	Del	B
89	Is-0	N1241	Germany	50.48	Ler	Del	B
90	Jl-3	CS6745	Czech	50.1	Col	Del	A
91	Jm-0	CS6748	Czech	49.04	Ler	Del	B
92	Jm-1	N1261	Czech	49.04	Col	Del	A
93	Ka-0	CS6752	Austria	46.5	Col	Del	A
94	Kas-2	CS6751	India	34	Ler	Wt	B
95	Kb-0	CS6753	Germany	50.5	Col	Del	A
96	Kl-0	N1275	Germany	50.93	Ler	Del	B
97	Kl-1	N1277	Germany	50.93	Ler	Del	B
98	Kl-5	CS1284	Germany	50.93	Ler	Del	A
99	Kn-0	N1287	Lithuania	54.9	Col	Wt	B
100	Ko-2	N1289	Denmark	55.5	Ler	Wt	
101	Kondara	N6175	Tajikistan	38.81	Ler	Wt	B
102	Kro-0	N1301	Germany	50.2	Col	Del	B
103	KZ10	N22442	Kazakhstan	50.42	Col	Wt	
104	KZ11	N22443	Kazakhstan	50.42	Col	Wt	
105	La-1	N1303	Poland	52.73	Ler	Wt	
106	Ler	Lab Stock			Ler	Del	
107	Ler-K	Lab Stock			Ler	Del	
108	Li-10	N6911	Germany	50.38	Col	Wt	B
109	Li-2	N6908	Germany	50.38	Col	Wt	
110	Li2:1	N1315	Germany	50.38	Col	Wt	A
111	Li-7	CS6878	Germany	50.38	Ler	Del	B
112	Lip-0	N1337	Poland	53.47	Col	Wt	A
113	Ll-0	N1339	Spain	41.82	Ler	Wt	A
114	Ll-1	N1341	Spain	41.82	Ler	Wt	A
115	Ll-11	N1341	Spain	41.82	Col	Wt	
116	Ll-2	N1343	Spain	41.82	Ler	Del	A
117	Lm-2	CS6784	France	48	Ler	Del	A
118	Lo-1	N1347	Germany	47.62	Col	Del	B
119	Lo-2	N1349	Germany	47.62	Ler	Del	B
120	Lov-5	CS22575	Sweden	62	Ler	Wt	
121	Lz-0	CS6788	France	46	Ler	Del	A
122	Ma-0	N1357	Germany	50.5	Ler	Del	A
123	Mh-0	N1367	Poland	53.78	Ler	Del	B
124	Mh-1	N1369	Poland	53.78	Ler	Del	B
125	Mir-0	N1379	Italy	45.65	Ler	Wt	
126	Mnz-0	N1371	Germany	50	Ler	Del	
127	Mr-0	N1373	Italy	44.5	Col	Wt	A
128	Mrk-0	N1375	Germany	49	Ler	Del	B
129	Ms-0	N1377	Russia	55.75	Col	Wt	B
130	Mz-0	N1383	Germany	50.85	Ler	Del	A
131	Na-1	CS6801	France	47.5	Ler	Del	B
132	Nc-1	N1389	France	48.6	Ler	Del	A
133	Nd-0	N1391	Germany	50.47	Col	Del	
134	Nd-1	N1680	Germany	50.47	Col	Del	A
135	No-0	N1395	Germany	51	Col	Del	A
136	Nok-0	N1399	Netherlands	52.23	Col	Wt	B
137	Nok-3	N1405	Netherlands	52.23	Ler	Wt	B
138	Np-0	N1397	Germany	52.68	Col	Del	
139	Nw-0	N1409	Germany	50.32	Ler	Del	B
140	Nw-2	N1413	Germany	50.32	Ler	Del	B
141	Nw-3	N1415	Germany	50.32	Col	Del	B
142	Ob-0	N1419	Germany	50.2	Ler	Del	B
143	Ob-1	N1421	Germany	50.2	Col	Del	
144	Ob-2	N1423	Germany	50.2	Ler	Del	B
145	Ob-3	N1425	Germany	50.2	Col	Del	
146	Old-1	N1427	Germany	53.03	Ler	Del	B
147	Old-2	N1429	Germany	53.03	Ler	Del	B

No	Accessions	Stock [#]	Country	Latitude	PHYC	FRI* [†]	FLC [†]
148	Or-0	N1433	Germany	50.5	Col	Wt	B
149	Ost-0	N1431	Sweden	60.5	Col	Wt	
150	Ove-0	N1435	Germany	53.33	Col	Del	B
151	Oy-0	N1437	Norway	60.5	Ler	Del	B
152	Oy-1	N6929	Norway	60.5	Col	Del	A
153	Pa-2	N1441	Italy	37.83	Ler	Wt	A
154	Pa-3	N1443	Italy	37.83	Ler	Del	B
155	Per-1	N1445	Russia	58.02	Col	Wt	B
156	Per-2	N1449	Russia	58.02	Col	Wt	
157	Per-3	N1451	Russia	58.02	Col	Wt	
158	Pf-0	N1453	Germany	48.5	Ler	Del	B
159	Pi-0	N1455	Austria	47.12	Ler	Del	B
160	Pi-2	N1457	Austria	47.12	Ler	Del	B
161	Pla-1	N1459	Spain	41.82	Ler	Wt	A
162	Pla-2	N1463	Spain	41.82	Ler	Wt	
163	Pn-0	N1469	France	48	Ler	Wt	A
164	Po-1	N1473	Germany	50.5	Col	Wt	A
165	Pr-0	N1475	Germany	50.5	Ler	Del	B
166	PUZ16	N22451	Czech	49.12	Ler	Wt	
167	Ra-0	N1481	France	46.02	Ler	Del	
168	Rak-2	CS6846	Czech	49.03	Col	Del	A
169	Rd-0	N1483	Germany	50.5	Ler	Del	B
170	REN1	N22253	France	46.02	Ler	Del	
171	REN11	N22263	France	46.02	Ler	Del	
172	RLD1	N913	Russia	56.5	Col	Del	B
173	Rou-0	N1489	France	49.5	Ler	Wt	A
174	Rsch-0	N1491	Russia	56.86	Col	Wt	B
175	Rsch-4	N1495	Russia	56.86	Col	Del	B
176	Sav-0	N1515	Czech	50	Col	Del	
177	Se-0	N1503	Spain	42.5	Ler	Wt	A
178	Sei-0	N6853	Italy	46.5	Ler	Del	B
179	Sf-1	N1513	Spain	42.05	Ler	Wt	A
180	Sf-2	N1517	Spain	42.05	Ler	Wt	
181	Sf-2e	N1675	Spain	42.05	Ler	Wt	
182	Sg-1	N1519	Germany	48.12	Ler	Del	A
183	Sg-2	N1521	Germany	48.12	Ler	Del	A
184	Shakdara	N6180	Tajikistan	37.48	Ler	Wt	B
185	Sn(5)-1	N6181	Czech	50	Col	Del	A
186	Sorbo	CS931	Tajikistan	39	Ler	Wt	B
187	Sp-0	N1531	Germany	52.5	Col	Wt	A
188	St-0	N1535	Sweden	59	Ler	Wt	A
189	Stw-0	N1539	Russia	52.96	Ler	Wt	A
190	Ta-0	N1549	Czech	49.42	Col	Wt	A
191	Tamm-2	CS22604	Finland	60	Col	Wt	
192	Te-0	N1551	Finland	60	Ler	Wt	A
193	Ts-1	N1553	Spain	41.72	Ler	Wt	
194	Ts-5	N6871	Spain	41.72	Ler	Wt	A
195	Ts-6	N1561	Spain	41.72	Ler	Wt	A
196	Ts-7	N1563	Spain	41.72	Ler	Wt	B
197	Tu-1	N1569	Italy	45	Ler	Del	B
198	Uk-1	N1575	Germany	48.03	Ler	Del	A
199	Uk-2	N1579	Germany	48.03	Ler	Del	A
200	Uk-3	N1577	Germany	48.03	Ler	Del	A
201	Uk-4	N1581	Germany	48.03	Ler	Del	A
202	ULL2-5	CS22586	Sweden	56.09	Ler	Wt	
203	Var2-6	CS22581	Sweden	55.33	Col	Wt	
204	Wa-1	N1587	Poland	52.5	Col	Wt	B
205	Wc-1	N1589	Germany	52.6	Col	Del	A
206	Wc-2	N1591	Germany	52.6	Col	Del	A
207	Wei-0	N6182	Switzerland	47.42	Ler	Del	A
208	Wei-1	N1683	Switzerland	47.42	Ler	Del	B
209	Wil-0	CS6888	Lithuania	54.68	Col		
210	Wil-1	N1595	Lithuania	54.68	Col	Wt	A
211	Wil-3	N1599	Lithuania	54.68	Col	Wt	A
212	Wl-0	N1631	Germany	48.75	Ler	Del	B
213	Ws	N6924	Ukraine	52.5	Col	Del	
214	Ws-0	CS6891	Ukraine	52.5	Ler	Wt	A
215	Ws-2	CS2360	Ukraine	52.5	Col	Del	B
216	Wt-1	N1605	Germany	52.67	Ler	Del	A
217	Wt-4	N1611	Germany	52.67	Ler	Del	A
218	Wt-5	N1613	Germany	52.67	Ler	Del	B
219	Wu-0	N1615	Germany	49.78	Ler	Del	B
220	Zu-0	N1627	Switzerland	47.42	Ler	Wt	A
221	Zu-1	N1629	Switzerland	47.42	Ler	Del	A

No	Accessions	Stock [#]	Country	Latitude	<i>PHYC</i>	<i>FRI</i> [†]	<i>FLC</i> [†]
----	------------	--------------------	---------	----------	-------------	-------------------------	-------------------------

* "Wt" denotes no lesion known, thus putatively wild type.

[†] Genotype of *FRI* and *FLC* from: Lempe, J. et al. Diversity of flowering responses in wild *Arabidopsis thaliana* strains. PLoS Genet. 1, e6 (2005); Shindo, C. et al. Role of *FRIGIDA* and *FLOWERING LOCUS C* in determining variation in flowering time of Arabidopsis. Plant Physiol. 138, 1163-73 (2005); Caicedo, A.L., Stinchcombe, J.R., Olsen, K.M., Schmitt, J. & Purugganan, M.D. 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 (2004).

Supplementary Table 2

ANOVA of latitude by *PHYC* haplotype group interaction on residual variation in flowering time at 23°C in short days after accounting for *FRI* functionality. The significance for each of the factors is given.

Factor	DF	Sum of Squares	FValue	Probability (>F)
Latitude	1	2144.61	6.374	0.013
<i>PHYC</i> haplotype group	1	484.79	1.44	0.23
<i>PHYC</i> haplotype group x latitude interaction	1	5344.66	15.88	0.0001

Multiple R-Squared: 0.12, Adjusted R-squared: 0.10, p-value = 0.0017

Supplementary Table 3

p-values obtained from for different models testing for association with latitude (Latitude), *FRI* dependent interaction with latitude (Interaction), hypocotyl length under GA treatment (GAHypo) and total leaf number in short days (TLNSD) for 67 markers across 163 strains. The rankings for each of the markers in each of the association is given (A) Model: Logistic regression model of SNP ~ Latitude. (B) Model: Latitude ~ *FRI* functionality + SNP + SNP * *FRI* functionality. The *p*-values for the whole model, *FRI* functionality, SNP, and for interaction with *FRI* are tabulated. Ranks are given for the interaction with *FRI*. (C) Model: Hypocotyl Length ~ SNP (D). Model: TLNSD ~ SNP. The table is sorted according to the rankings obtained for the logistic regression model for association with latitude. While the *p*-value ranking for flowering time (D) does not appear to be significant after background correction, the relative ranking is still consistent with a true association.

S.No	SNP_ID	Chr	Latitude	Interaction	GAHypo	TLNSD	A	B	C	D
1	PHYC	5	0.0008	0.0053	0.0007	0.0464	1	4	1	12
2	MASC05657	2	0.0015	0.003	0.5509	0.0107	2	1	45	5
3	MASC09206	1	0.0018	0.008	0.0048	0.1806	3	5	4	22
4	MASC04123	4	0.002	0.6459	0.9914	0.0015	4	41	65	2
5	MASC01545	5	0.004	0.1401	0.3519	0.4806	5	17	27	43
6	MASC03470	5	0.0043	0.6222	0.0015	0.2799	6	40	2	28
7	MASC04925	3	0.0085	0.0542	0.0232	0.6168	7	12	9	49
8	MASC02841	3	0.0157	0.9957	0.0413	0.5713	8	60	11	47
9	MASC02668	4	0.0158	0.0425	0.2932	0.0898	9	10	26	15
10	MASC04608	3	0.0208	0.0839	0.7705	0.4357	10	14	55	40
11	MASC04199	4	0.0322	0.003	0.2083	0.012	11	2	20	6
12	FLC	5	0.065	0.6914	0.165	0.4469	12	43	17	42
13	MASC04642	4	0.0691	0.7149	0.4218	0.026	13	45	34	8
14	MASC09219	3	0.0763	0.2447	0.8056	0.5349	14	25	58	46
15	MASC03128	5	0.0982	0.0138	0.0098	0.6111	15	6	6	48
16	MASC04262	3	0.1074	0.9856	0.9817	0.2048	16	59	64	24
17	MASC09216	4	0.1219	0.0405	0.5819	0.7262	17	9	47	55
18	MASC04523	3	0.1243	0.216	0.2516	0.1393	18	22	23	19
19	MASC03898	3	0.1271	0.2063	0.285	0.0656	19	19	25	14
20	MASC09214	4	0.1306		0.9575		20	65	62	65
21	MASC03344	3	0.1359	0.0484	0.216	0.4093	21	11	21	38
22	MASC03263	4	0.1501	0.039	0.4177	0.6615	22	8	33	50
23	MASC03336	4	0.1545	0.3065	0.7493	0.0025	23	31	53	3
24	MASC09224	3	0.1908				24	64	66	64
25	MASC04275	5	0.1928	0.0326	0.7308	0.9608	25	7	51	61
26	MASC03218	3	0.1978	0.3064	0.9651	0.0075	26	30	63	4
27	MASC05434	2	0.2061	0.0031	0.1286	0.1418	27	3	16	20
28	MASC05803	2	0.223	0.4262	0.7569	0.1165	28	33	54	18
29	MASC02820	4	0.2553	0.9957	0.0758	0.1925	29	61	15	23
30	MASC02675	5	0.265	0.4309	0.3951		30	34	29	67
31	MASC04350	5	0.3068	0.3032	0.5487	0.4376	31	29	44	41
32	MASC03340	1	0.3377	0.9327	0.0016	0.9389	32	57	3	59
33	MASC01582	5	0.3984	0.1158	0.4567	0.0564	33	16	38	13
34	MASC09208	5	0.4068	0.0915	0.5423	0.2976	34	15	43	29
35	MASC09222	2	0.4078	0.2145	0.6152	0.8375	35	21	48	57
36	MASC09225	4	0.4341	0.189	0.3974	0.6978	36	18	30	52
37	MASC09204	1	0.4425		0.4427	0.3189	37	62	37	32
38	MASC05029	1	0.446	0.2473	0.9011		38	26	61	63
39	MASC09209	5	0.4692	0.2837	0.4333	0.018	39	28	35	7
40	MASC04170	1	0.4773	0.4648	0.3656	0.9573	40	35	28	60
41	MASC04516	3	0.4804	0.2292	0.5042	0.0014	41	23	40	1
42	MASC04531	5	0.507	0.7203	0.0459	0.1567	42	46	12	21
43	MASC01361	5	0.5156	0.9625	0.0065	0.2725	43	58	5	27
44	MASC04983	5	0.5687				44	67	67	66
45	MASC05258	4	0.5879	0.2078	0.4156	0.0929	45	20	32	16
46	MASC05360	2	0.6034	0.8711	0.8991	0.5288	46	51	60	45
47	MASC09223	1	0.6925	0.8852	0.6706	0.3146	47	52	50	31
48	MASC03911	1	0.7062	0.6533	0.6247	0.6995	48	42	49	53
49	MASC03631	1	0.708	0.7279	0.5422	0.3653	49	47	42	34
50	MASC03754	1	0.7213	0.9171	0.199	0.4061	50	55	19	37
51	MASC09203	1	0.7375	0.5523	0.7385	0.2307	51	38	52	25
52	MASC06808	2	0.749	0.0572	0.0107	0.8759	52	13	7	58
53	MASC05386	2	0.7646	0.4901	0.0741	0.705	53	36	14	54
54	MASC03001	3	0.7705	0.2707	0.178	0.418	54	27	18	39
55	MASC03447	1	0.7952	0.927	0.0237	0.3217	55	56	10	33
56	MASC01171	3	0.7967	0.9082	0.4587	0.7354	56	54	39	56
57	MASC05962	2	0.798		0.255	0.4043	57	63	24	36
58	MASC03612	5	0.8153		0.2268	0.3117	58	66	22	30
59	MASC07090	3	0.8166	0.8059	0.5729	0.4927	59	49	46	44
60	MASC05857	2	0.8183	0.2335	0.7916	0.0456	60	24	56	11

S.No	SNP_ID	Chr	Latitude	Interaction	GAHypo	TLNSD	A	B	C	D
61	MASC04591	5	0.831	0.8886	0.0133	0.9837	61	53	8	62
62	MASC02577	1	0.8586	0.7039	0.886	0.2659	62	44	59	26
63	MASC03658	1	0.8815	0.5312	0.0733	0.0407	63	37	13	10
64	MASC04209	1	0.9083	0.559	0.4395	0.3838	64	39	36	35
65	MASC09210	5	0.9267	0.852	0.7992	0.0972	65	50	57	17
66	MASC03952	5	0.9722	0.3312	0.5352	0.6953	66	32	41	51
67	MASC04819	3	0.975	0.7392	0.4083	0.0403	67	48	31	9

Supplementary Table 4

p-values obtained from for two different models testing for latitudinal association with a completely independent set of markers across 56 strains. (A) Model: Latitude ~ *FRI* functionality + SNP + SNP * *FRI* functionality. The *p*-values for the entire model, *FRI* functionality, SNP, and for interaction with *FRI* are tabulated. Ranks are given for the interaction with *FRI*. (B) Model: Logistic regression model of SNP ~ Latitude. SNP_ID refers to unique identifier for SNP assays (see <http://naturalvariation.org>). The table is sorted according to the rankings obtained for *FRI*-dependent interaction with latitude.

S.No	SNP_ID	Chr	Model	<i>FRI</i>	SNP	Interaction (A)	Latitude (B)	Rank A	Rank B
1	44607503	2	9E-05	9E-05	8E-04	0.00009	0.0669	1	15
2	44607971	1	9E-05	9E-05	3E-04	0.00009	0.0756	2	19
3	44607332	2	9E-05	9E-05	0.448	0.0021	0.8365	3	64
4	44606338	5	9E-05	0.0011	0.005	0.0027	0.1083	4	22
5	44606550	4	9E-05	0.0061	0.002	0.0032	0.0097	5	6
6	44606183	1	0.0001	0.0039	0.256	0.0036	0.7846	6	59
7	44607627	2	9E-05	9E-05	7E-04	0.0048	0.0675	7	16
8	44607727	2	9E-05	0.0853	0.031	0.0049	0.1595	8	28
9	44607751	4	0.0002	0.0001	0.355	0.0099	0.6177	9	48
10	PHYC	5	9E-05	0.0048	0.004	0.0137	0.0213	10	10
11	21607148	5	0.0002	0.5391	0.003	0.0255	0.0041	11	3
12	44607470	2	0.0007	0.0883	0.025	0.0336	0.0487	12	14
13	21607640	1	0.0001	0.008	0.003	0.0342	0.0221	13	11
14	44607250	5	9E-05	0.0021	0.009	0.0433	0.1002	14	21
15	44606460	5	0.0002	9E-05	0.018	0.0457	0.1407	15	26
16	44607372	1	0.0006	0.0063	0.135	0.048	0.3208	16	35
17	44606631	3	9E-05	0.0146	0.003	0.057	0.0044	17	4
18	44607685	1	9E-05	9E-05	0.001	0.0598	0.0126	18	8
19	44607364	1	0.001	0.0002	0.507	0.0639	0.1609	19	29
20	44606867	1	0.0028	0.0002	0.308	0.0902	0.762	20	58
21	44607792	4	0.0006	0.0002	0.277	0.1278	0.1403	21	25
22	21607556	2	0.0023	0.2412	0.05	0.1356	0.0403	22	12
23	44607389	3	0.0008	0.0005	0.575	0.1359	0.7544	23	57
24	44607528	1	0.0067	0.0084	0.402	0.1421	0.7198	24	56
25	44606134	1	0.0005	0.0086	0.036	0.1612	0.0713	25	18
26	44607545	4	0.0029	0.0002	0.321	0.1794	0.8851	26	66
27	21607250	3	0.0019	0.0001	0.599	0.1898	0.8422	27	65
28	44607014	3	9E-05	0.0009	6E-04	0.1948	0.0004	28	1
29	44607841	4	0.0003	0.0405	0.009	0.2167	0.0066	29	5
30	21607496	3	0.0044	0.1019	0.477	0.2335	0.3426	30	37
31	44608020	3	0.0134	0.0314	0.351	0.2398	0.2445	31	31
32	44606484	5	0.0007	0.0119	0.439	0.2566	0.0024	32	2
33	FLC	5	0.0022	0.0002	0.939	0.2993	0.4111	33	41
34	44606273	3	0.0077	0.0228	0.543	0.3061	0.6601	34	51
35	44607307	1	0.0127	0.0017	0.095	0.3287	0.6678	35	52
36	44606354	1	0.0007	0.0409	0.024	0.3377	0.0142	36	9
37	44606989	2	0.0045	0.0041	0.983	0.3435	0.8179	37	60
38	44606843	3	0.0018	0.0002	0.824	0.344	0.5148	38	45
39	44606199	2	0.0028	0.0002	0.82	0.4136	0.9311	39	68
40	44607561	4	0.001	0.0028	0.036	0.4194	0.0108	40	7
41	44607701	2	0.0015	0.0008	0.299	0.4318	0.0404	41	13
42	44606208	3	0.0087	0.0014	0.912	0.4388	0.9917	42	71
43	44607446	5	0.0037	0.0004	0.362	0.5047	0.9009	43	67
44	21607463	1	0.0087	0.0065	0.924	0.5338	0.9351	44	69
45	44607718	3	0.0035	0.0003	0.595	0.5496	0.8255	45	61
46	44607775	1	0.0043	0.0009	0.436	0.5526	0.3139	46	34
47	44607898	2	0.0035	0.0016	0.359	0.5668	0.4164	47	42
48	44606118	4	0.0106	0.0014	0.87	0.5742	0.6848	48	55
49	44607397	3	0.0021	0.0003	0.59	0.5852	0.3223	49	36
50	44608060	1	0.0039	0.0008	0.4	0.5973	0.2513	50	32
51	44607759	4	0.0041	0.001	0.565	0.618	0.6765	51	53
52	44606313	1	0.0049	0.0006	0.525	0.6253	0.56	52	46
53	44606940	2	0.0007	0.0002	0.233	0.6292	0.2321	53	30
54	44606981	1	0.0068	0.0012	0.421	0.6471	0.3441	54	38
55	21697327	4	0.0176	0.0555	0.681	0.6748	0.4299	55	44
56	44606753	1	0.0018	0.0004	0.095	0.6838	0.1227	56	23
57	44607553	4	0.0476	0.0652	0.765	0.6963	0.4169	57	43
58	44607479	2	0.0054	0.0007	0.791	0.7222	0.3566	58	39
59	44607193	5	0.0055	0.0026	0.926	0.7245	0.8339	59	63
60	44606387	3	0.0068	0.0007	0.885	0.7247	0.8307	60	62
61	44607873	1	0.004	0.0003	0.287	0.7387	0.5846	61	47
62	44606794	1	0.0054	0.0017	0.761	0.8111	0.1506	62	27
63	44606607	3	0.0014	0.0005	0.065	0.8348	0.0775	63	20
64	44606916	4	0.0055	0.0007	0.774	0.8535	0.6765	64	54

S.No	SNP_ID	Chr	Model	<i>FRI</i>	SNP	Interaction (A)	Latitude (B)	Rank A	Rank B
65	44607160	5	0.0037	0.0005	0.722	0.8843	0.6312	65	50
66	44607955	4	0.0047	0.0022	0.188	0.9104	0.0688	66	17
67	44606216	4	0.0072	0.0495	0.873	0.9307	0.276	67	33
68	44607299	1	0.0032	0.0064	0.355	0.9356	0.9446	68	70
69	21607631	4	0.0028	0.0003	0.348	0.9685	0.6289	69	49
70	44607405	4	0.0036	0.0047	0.214	0.9749	0.1342	70	24
71	44606102	3	0.0059	0.0587	0.98	0.9833	0.3713	71	40

Supplementary Table 5

Oligonucleotide primers.

Lab designation	Sequence
G-4861	CTC AGC TTC TCT CCC ACC AC
G-4862	CCC CAT AAG TGT CTG CCA GT
G-4863	CAA GTA TGG AGC AGC GTG AA
G-4864	GCA TAC CCC ATT TTC ATT GG
G-4865	TAC CGC AAG CTT CGA GAT TT
G-4866	TCG AGA GCC AAG GCT AAC AT
G-4867	CTG TGG TTT CTG GCT CCA AT
G-4868	TCC CTT TCT CAA AGG CTG AA
G-4873	GAT TGG CAG TTG AAC AAG CA
G-4874	GCA TAC CCC ATT TTC ATT GG
G-5349	CAA ATC GCA TAA ATGCAT GG
G-5350	AGT GGT GGG AGA GAA GCT GA
G-5351	CTT GTG CTC ATG AAC GGC TA
G-5352	CGT GAT GAC AAA CCA CCA AG
G-5353	CCA ATG AAA ATG GGG TAT GC
G-5354	CCT GAT GCG TCT TCT TCT CC
G-5355	GGA GAA GAA GAC GCA TCA GG
G-5356	TTC TTT CGG GAA TTT CAT CG
G-5357	GTT TGT GGC TCC CAT TTT GT
G-5358	GGA AAA GAC CGA AAC ACC AA
G-5359	GTG TCG TGA GTC GTG ACC AG
G-5360	TGG AAT CAA ACC CAA CAT CTC
G-5361	ACG CAA AGC TAC ACG GAA AC
G-5362	GAC GCC ACT GAT CCC ATA TT
G-5363	GGC TTC AGC AAA TCC TTT CA
G-5364	TCG AAC CCA GAT GAC ACA AA
G-5365	TTT TGT GTC ATC TGG GTT CG
G-5366	TGC CCG TTT AAT ACC TGC AT
G-5367	TCT CCA TCG ACG TTA AAC CA

PHYC region was amplified in four fragments using primer combinations G-5351 and G-5352; G-4863 and G-4864; G-4873 and G-4868, and G-5363 and G-5364. Other primers were used as sequencing primers and to fill in sequence gaps where necessary.

Supplementary Table 6Strains sequenced for *PHYC*.

Strain	Stock Number
Bay-0	N955
Bur-0	N1029
C24	Lab Stock
Col-0	N1093
Est-1	N1151
Fr-2	N1169
Ga-0	N1181
Gu-0	N1213
Gy-0	N1217
HR5	N22205
Jm-1	N1261
Kondara	N6175
Ler	Lab Stock
LI-2	N1343
Ms-0	N1377
Mz-0	N1383
Nd-1	N1680
Nok-3	N1405
Ra-0	N1481
REN1	N22253
Ri-0	N1493
RLD1	N913
Se-0	N1503
Sf-2	N1517
Te-0	N1551
Ts-1	N1553
Tsu-1	N6926
Van-0	CS6884
Wei-0	N6182
<i>A. lyrata</i>	Lab stock