Plant Physiology Preview. Published on November 4, 2009, as DOI:10.1104/pp.109.140772

Distinct patterns of genetic variation alter flowering responses of Arabidopsis accessions to different day lengths

Antonis Giakountis[1], Frederic Cremer[1], Sheina Sim[2], Matthieu Reymond[1], Johanna Schmitt[2] and George Coupland[1,3]

1. Max Planck Institute for Plant Breeding Research, Carl von Linne Weg 10, D-50829 Cologne, Germany
2. Department of Ecology and Evolutionary biology, Brown University, Providence, RI 02912, USA.
3. To whom correspondence should be addressed:
   Email: coupland@mpiz-koeln.mpg.de
   Fax: (0049) 221 5062 207

Abstract

Many plants flower in response to seasonal changes in day length. This response often varies between accessions of a single species. We studied the variation in photoperiod response found in the model species *Arabidopsis thaliana*. Seventy-two accessions were grown under six day lengths varying in 2 h intervals from 6 h to 16 h. The typical response was sigmoidal so that plants flowered early under days longer than 14 h, late under days shorter than 10 h and at intermediate times under 12 h days. However, many accessions diverged from this pattern and were clustered into groups showing related phenotypes. Thirty-one mutants and transgenic lines were also scored under the same conditions. Statistical comparisons demonstrated that some accessions show stronger responses to different day lengths than are found among the mutants. Genetic analysis of two such accessions demonstrated that different quantitative trait loci conferred an enhanced response to shortening the day length from 16 h to 14 h. Our data illustrate the spectrum of day-length response phenotypes present in accessions of Arabidopsis and demonstrate that similar phenotypic variation in photoperiodic response can be conferred by different combinations of loci.

Introduction

Growth and reproduction of many plant species are regulated by seasonal changes in day length. Specific traits controlled by day length include flowering, bud dormancy in trees and tuberisation of potato (Thomas and Vince-Prue, 1997). Within a species there is often quantitative variation for the precise length of day that induces a response, and the distribution of accessions that respond to different day lengths suggests that this trait is associated with adaptation to growth at particular latitudes. Examples of such distributions include induction of flowering by day length in cultivated populations of soybean (Borthwick and Parker, 1939) and natural populations of *Xanthium strumarium* (Ray and Alexander, 1966) or repression of bud growth in poplar (Bohlenius et al., 2006). The mechanisms controlling photoperiodic flowering are best understood in *Arabidopsis thaliana* (Kobayashi and Weigel, 2007; Turck et al., 2008) , but no comprehensive analysis of quantitative variation in photoperiod response within this species has been reported.

Arabidopsis is a quantitative long-day plant that flowers earlier under long days (LDs) of spring and early summer than during short days (SD) of winter. Commonly used laboratory accessions such as Columbia (Col) or Landsberg *erecta* (L*er*) show a marked flowering response to day length and were used to screen for mutations that impair photoperiodic flowering (Redei, 1962; Koornneef et al., 1991). The genes identified by these mutations defined a pathway that promotes flowering in response to LDs. *GIGANTEA* (*GI*), *CONSTANS* (*CO*) and *FLOWERING LOCUS T* (*FT*) are central to this pathway (Putterill et al., 1995; Fowler et al., 1999; Kardailsky et al., 1999; Kobayashi et al., 1999; Park et al., 1999). Transcription of each of these genes is regulated by the circadian clock (Fowler et al., 1999; Park et al., 1999; Suarez-Lopez et al., 2001), while CO activity is promoted by exposure to light both at the transcriptional and post-transcriptional levels (Valverde et al., 2004; Imaizumi et al., 2005). This complex regulation ensures that CO activates *FT* transcription only under LDs.

In addition to the mutational analysis performed in Arabidopsis accessions commonly used in the laboratory, natural-genetic variation has been studied by analyzing genetic differences between a wider range of accessions. The most dramatic variation in flowering is between accessions that show a strong requirement for vernalization (extended exposure to low temperatures) to induce flowering and those that flower rapidly without vernalization. These distinct types are often referred to as winter annuals or summer annuals, respectively. Detailed genetic analysis based on crossing both types identified the semi-dominant locus *FLOWERING LOCUS C* (*FLC*) and the dominant locus *FRIGIDA* (*FRI*), that are present in

winter annuals and are required for the vernalization response (Burn et al., 1993; Clarke and Dean, 1994; Koornneef et al., 1994; Lee et al., 1994). *FLC* encodes a MADS box transcription factor that represses flowering, and FRI promotes *FLC* transcription prior to vernalization (Michaels and Amasino, 1999; Sheldon et al., 1999). Exposure of plants to vernalization for several weeks causes *FLC* transcript levels to fall due to changes in chromatin structure at the *FLC* locus (Bastow et al., 2004; Sung and Amasino, 2004). Summer annuals carry alleles of *FLC* or *FRI* that reduce the activity of one or both genes. Mutations at *FRI* and *FLC* appear to have occurred independently many times conferring the summer annual habit (Johanson et al., 2000; Le Corre et al., 2002; Gazzani et al., 2003; Michaels et al., 2003; Lempe et al., 2005; Shindo et al., 2005).

Although genetic differences at loci involved in vernalization are responsible for much of the variation in flowering time among Arabidopsis accessions, allelic differences at genes contributing to the photoperiodic response can also have important effects on flowering time. This variation was mainly characterized by comparing flowering time under extreme LDs of 16 h and SDs of 8 or 10 h (Alonso-Blanco et al., 1998; Lempe et al., 2005; Werner et al., 2005b). However, in one set of experiments day length and tmperature were varied continuously through the experiment recreating the effect of the changing seasons, which allowed identification of QTL that regulate flowering time and interact with seasonal changes in environmental parameters (Li et al., 2006). Also, the flowering times of the L*er* and Ws accessions were recently characterized under a wide range of photoperiods describing a quantitative response to photoperiod that exhibited a sigmoidal shape (Pouteau et al., 2008; Wilczek et al., 2009). Most of the described natural-genetic variation in photoperiod response causes earlier flowering under SDs. For example, recessive alleles at *PHYTOCHROME C* (*PHYC*) and *FLOWERING LOCUS M* (*FLM*) were identified as causing earlier flowering under SDs and therefore reducing the difference in flowering time between LDs and SDs (Werner et al., 2005b; Balasubramanian et al., 2006; Li et al., 2006). Similarly, a single amino acid change in the CRYPTOCHROME 2 (CRY2) photoreceptor was shown to be present in the Cape Verde Islands (Cvi) accession and to promote early flowering under SDs (El-Assal et al., 2001). Sequence variation at *PHYC* was proposed to be significant in natural populations because both active and inactive alleles are common and these formed a latitudinal cline with the inactive allele more frequent at lower latitude while the active allele was more prevalent at higher latitude (Balasubramanian et al., 2006). Such a distribution may be consistent with the notion that a strong response to day length is more significant at higher latitudes where more extreme seasonal variation in day length occurs. Similarly, the dominant

CRY2 allele that reduces day-length sensitivity was identified in an accession from low latitude (El-Assal et al., 2001).

Across the latitudinal range of Arabidopsis day length varies continuously and the LDs or SDs most commonly used in laboratory experiments are experienced only rarely. To more thoroughly analyze the day-length response of Arabidopsis, we tested over 70 accessions under 6 different day lengths and the results were compared with those for many characterized mutants and transgenic lines. Accessions that show a greater delay in flowering time than L*er* as day length is reduced from 16 h to 14 h were selected and the genetic basis of this response was examined. Our results provide an extensive analysis of variation in photoperiodic flowering responses in Arabidopsis and indicate that the enhanced responses to day length shown by two accessions are caused by different combinations of quantitative trait loci.

Results

Variation in flowering time of Arabidopsis accessions under a wide range of day lengths

Quantitative responses to photoperiod were measured in Arabidopsis by scoring flowering time of 72 genetically divergent accessions under 6 different day lengths. The day lengths used varied in 2 h intervals from 6 h to 16 h. The accessions included L*er*, Col and Ws to act as points of reference with previous analyses of photoperiod response and with studies on mutants recovered in these backgrounds. Accessions showing extreme late flowering under standard 16 h day lengths were excluded from the analysis, because most of these exhibit a strong vernalization requirement. The accessions used are listed in Supplementary Table 1 and their genetic relatedness illustrated in Supplementary Figure 1 (Methods).

Flowering time was measured by counting total leaf number for each accession under all day lengths (Figure 1; Supplementary Table 1; Methods). In most cases the photoperiod response curve was sigmoidal (Figure 1A), as previously described for Ws and L*er* (Pouteau et al., 2008; Wilczek et al., 2009). Typically flowering time of these accessions occurred only slightly earlier when day length was lengthened from 6 h to 8 h, was broadly the same between day lengths of 8 h and 10 h, accelerated markedly as day length was lengthened from 10 h to 12 h and was little affected as day length was extended from 12h to 16 h.

Although the sigmoidal pattern observed in Figure 1A described the behaviour of most accessions, others deviated from the standard response. For example, one group showed earlier flowering than the mean under all day lengths (e.g. Dra-0; L*er*; Ws; Figure 1C), and

this group included CVI which exhibited a strongly diminished response to photoperiod. A second set of accessions showed an enhanced response to day length, flowering earlier than or similar to the mean under 16 h long days (LDs) but later than the mean under shorter days (e.g. Bs-1, Cen-0; Figure 1D).

Flowering times of Arabidopsis mutants and transgenic plants under a range of day lengths

To facilitate comparisons between accessions and mutants that impair photoperiodic responses, the flowering times of 31 previously described mutants and transgenic lines were scored under the same range of day lengths (Figure 1B; Supplementary Table 2). These lines showed a wide range of responses. Early and late-flowering lines showing little response to changing day length were analyzed and neither showed the sigmoidal pattern characteristic of wild-type plants (Supplementary Figure 2AB and Supplementary Table 2).

Mutants impaired in circadian-clock function were also tested (Supplementary Figure 2C). The photoperiodic responses of *lhy-11 cca1-1* double mutants and transgenic lines overexpressing both *LHY* and *CCA1* (called 35S:*CCA1 lhy-1*) were compared. Both of these genotypes are severely impaired in circadian clock function (Schaffer et al., 1998; Wang and Tobin, 1998; Mizoguchi et al., 2002), but 35S:*CCA1 lhy-1* is late flowering, whereas *lhy-11 cca1-1* is early flowering. The 35S:*CCA1 lhy-1* plants were almost entirely insensitive to day length. The *lhy-11 cca1-1* plants were similarly insensitive to changes in day length from 16 h to 8 h days so that in this interval flowering time only varied from 7 to 15 leaves. However, when day length was shortened to 6 h then flowering was strongly delayed to 42 leaves. These data indicate that photoperiodic responses are almost completely abolished across a wide range of day lengths by strong impairment of the circadian clock, and that *lhy-11 cca1-1* plants show a very different response being strongly delayed in flowering only when day length falls below 8 h.

Photoperiodic responses also require active photoreceptors, therefore the photoperiodic response curves of *phyA-201* and *phyB-1* mutants were compared with wild-type plants (Supplementary Figure 2D). The curves describing the flowering-time behaviour of these mutants were similar in shape to those of wild-type plants. However, *phyB-1* mutants were generally earlier flowering than wild-type plants, especially under SDs (10 h, 8 h and 6 h). In contrast the flowering time of *phyA-201* mutants tended to be slightly later than wild-type plants, particularly under 12 h and 8 h days.

Taken together these data define the photoperiodic responses of a wide range of flowering-time mutants, and provide a basis for comparison with the responses of the accessions.

Comparisons of day-length responses of mutants, transgenic plants and accessions

To classify the accessions according to their photoperiodic response, the flowering- time data were analyzed using the Cluster programme (Figure 2A; Methods). Accessions with a generally later flowering phenotype formed clusters located at the top of the figure, while mostly earlier flowering accessions are located towards the bottom. This analysis identified several groups each of which contained accessions showing broadly similar photoperiodic responses and the photoperiod response curves of these accessions were compared. Principal components (PC) analysis was used to compare the responses of individual accessions, and to contrast the accessions with the mutants (Figure 2B; Supplementary Figure 3 and 4). Components that most effectively separated the responses of accessions and mutants were identified (Figure 2B). These were PC5, which compared flowering time under 16 h with flowering time under 14 h, and PC4, which compared flowering time under 10 h and 6 h with flowering time under 8 h (see also Sup Fig. 4 for component loading). These components clearly identified accessions that showed stronger photoperiodic flowering responses than those present in the mutants and transgenic lines.

Based on the cluster and principal components analyses, four accessions that showed interesting differences in their photoperiodic responses compared to the laboratory accession L*er* were selected for further analysis. The photoperiod response curves of these accessions also confirm the differences inferred from the PC analyses. Cen-0 and Bs-1 both flowered markedly later under 14 h days than 16 h days, whereas L*er* flowered at the same time under both day lengths (Figure 2C). Therefore, Bs-1 and Cen-0 show increased photoperiod discrimination compared to L*er*, distinguishing between 14 h and 16 h day lengths whereas L*er* does not. A few other accessions, such as Ang-0, showed similar responses to Bs-1 and Cen-0 (Figure 2A; Supplementary Table 1). However, not all accessions that were later flowering than Ler at 14 h showed such a steep acceleration in flowering between 14h and 16 h days (Supplementary Figure 2E). Similarly, the photoperiod response curves of Dijon-G and Sha differed under SDs (Figure 2D). Dijon-G distinguished between 10 h and 8 h photoperiods, flowering later under 8 h than 10 h days. In contrast, Sha flowered at similar times under both day lengths. Thus the statistical analysis of the photoperiodic responses of the accessions defined genotypes exhibiting increased discrimination of photoperiod under LD (16 h vs 14 h) or SD (10 h vs 8 h) and provides a basis for subsequent genetic analysis.

Overall the analysis of flowering time under 6 different day lengths described considerable quantitative variation for photoperiodic response among the 72 accessions that were tested, placed these accessions in response groups and identified accessions exhibiting phenotypes distinct from those found among the tested mutants and transgenic lines.

Effect of vernalization on accessions showing enhanced response to changing day length

In many late-flowering Arabidopsis accessions the delay in flowering observed under inductive photoperiods can be overcome by exposure to low temperatures (vernalization). Therefore whether vernalization suppresses the late flowering of Bs-1 and Cen-0 under 14 h days was tested. The accessions were exposed to 4°C for 4 weeks under 8 h days, and then returned to 14 h days at normal growth temperatures (Figure 3; Methods). Cen-0 plants exposed to vernalization flowered at a similar time to those that were not vernalized, indicating that the delay in flowering of this accession under 14 h days was not caused by a requirement for vernalization (Fig. 3A). In contrast, Bs-1 flowered approximately 20 leaves earlier after vernalization, indicating that the later flowering of this accession under 14 h days was at least partially suppressed by vernalization (Figure 3A). The insensitivity of Cen-0 to vernalization was consistent with previous reports (Shindo et al., 2005), however Bs-1 was previously described as almost insensitive to vernalization (Shindo et al., 2005). The stronger response to vernalization that we observed may be due to the plants being returned after vernalization into 14 h days rather than longer days of 16 h. Flowering of non-vernalized plants is delayed under 14 h and therefore the difference between the flowering times of vernalized and non-vernalized plants may be more pronounced.

The vernalization requirement in Arabidopsis is mainly conferred by the floral repressor FLC, whose transcription is in turn repressed by vernalization. The abundance of *FLC* mRNA in Cen-0 and Bs-1 was measured before and after vernalization, and compared with other accessions. Prior to vernalization *FLC* mRNA accumulated to relatively high levels in the Cen-0 accession but to low levels in Bs-1 (Figure 3B). These results are broadly in agreement with previous data (Shindo et al., 2005). In Bs-1, the abundance of *FLC* mRNA was further reduced by vernalization, and the difference was mainly observed after return to normal growth temperatures. This reduction was consistent with the earlier flowering induced by the treatment. In contrast, the high level of *FLC* mRNA in Cen-0 was not stably reduced by vernalization. However, previous data indicated that Cen-0 *FLC* mRNA is improperly spliced (Lempe et al., 2005), suggesting that Cen-0 *FLC* may contribute little to flowering time of this accession despite the relatively high level of its mRNA.

Sequence variation at the *FRI* locus plays an important role in determining the level of *FLC* expression. To further study the contribution of the *FRI*/*FLC* system to the flowering time of the Bs-1 and Cen-0 accessions, the *FRI* allele of each genotype was tested for polymorphisms characterized in other accessions (Le Corre et al., 2002; Caicedo et al., 2004; Lempe et al., 2005; Shindo et al., 2005). The inactive *FRI* allele present in L*er* carries a 16 bp deletion within the protein coding sequence, and a similar deletion is present in the Bs-1 allele (Figure 3C). Therefore, the low level of *FLC* mRNA in Bs-1 is consistent with the presence of an inactive *FRI* allele (Lempe et al., 2005). In contrast the *FRI* allele of Cen-0 does not carry the deletions present in Columbia or L*er*, and was previously characterized as an active allele. This conclusion is consistent with the higher level of *FLC* mRNA detected in Cen-0. Taken together these results suggest that the relatively low level of *FLC* mRNA in Bs-1 may nevertheless contribute to its late-flowering phenotype under 14 h days creating the observed vernalization response. In contrast Cen-0 exhibits high levels of *FLC* mRNA that are improperly spliced, consistent with the observation that it did not show a response to vernalization.

Genetic basis of enhanced response to changing day length differs between accessions.

QTL mapping was carried out to identify loci contributing to the enhanced response to photoperiod of the Bs-1 and Cen-0 accessions. Each of these accessions was crossed to L*er*. The flowering times of F2 populations were scored under 14 h and 16 h days (Supp. Fig. 5). Under 14 h days approximately 17 % of F2 plants derived from the cross of Bs-1 to L*er* flowered later than any of the corresponding F2 plants grown under 16 h days (Supp. Figure 5A and B). These data demonstrate that a proportion of F2 plants derived from this cross can be identified as late flowering under 14 h days compared to 16 h days. Analysis of late-flowering F2 plants under 14 h days allows mapping of Bs-1 alleles that delay flowering under these conditions. Loci identified by this approach might also cause late flowering under 16 h days, and therefore not be responsible for generating a difference in flowering time between 14 h and 16 h days (see below).. The F2 population derived from the Cen-0 cross to L*er* was also scored under the same conditions and approximately 40 % of plants appeared to flower later under 14 h days than under 16 h days, although only around 5% were outside of the range of flowering times observed under 16 h days (Supplementary Figure 5E and F). The effect of vernalization on flowering time of the segregating F2 populations was also tested (Supplementary Figure 5 C and D). As observed in the parental Bs-1 line, the number of late-flowering plants under 14 h in the F2 population derived from the Bs-1 cross to Ler

was greatly reduced by vernalization. In contrast, vernalization had little effect on flowering time of the F2 derived from the cross of Cen-0 to Ler (Supplementary figure 5 G and H).
DNA was extracted from F2 plants derived from each cross grown under 14 h days to construct genetic maps and facilitate identification of QTL that delay flowering under these conditions. SNPs between L*er* and Bs-1 or Cen-0 were identified (Methods, Supplementary table 3), and used to construct genetic maps (Supplementary Figure 6 and 7). QTLs for flowering time under 14 h days identified in the crosses of Bs-1 or Cen-0 to L*er* (Figure 4A; Supp. Table 4). This analysis identified one major QTL, *ALTERED FLOWERING TIME 2* (*AFT2*) and a putative QTL, *AFT1*. *AFT2* is located on the upper arm of chromosome 5 with a high probability of contributing to flowering time and was responsible for approximately 35 % of the variation in flowering time under 14 h days. *AFT1* was located on the lower arm of chromosome 1 and was responsible for only 5 % of the variation in flowering time of the mapping population (Figure 4A; Supplementary Table 4).
Flowering time variation under 14 h days in the F2 population derived from crossing L*er* to Cen-0 was conferred by a different set of QTL than detected in the cross of Bs-1 to L*er* (Figure 4A). In this cross five high probability QTL (*AFT3*, *AFT4*, *AFT5*, *AFT6*, *AFT7*) were detected. These were distributed across the upper and lower arms of chromosomes 4 and 5. The QTL located on the upper arm of chromosome 5 (*AFT6*) was located at a different position from *AFT2*, detected in the cross of L*er* and Bs-1 (Supplementary Table 4). Overall the QTLs on chromosomes IV (*AFT3*, *AFT4* and *AFT5*) and V (*AFT6* and *AFT7*) in the Cen-0 cross to L*er* contributed 11 and 27 % of variation in flowering time, respectively (Supplementary Table 4).
These experiments identified regions in both populations that are likely to contain QTL that delay flowering under 14 h days, and showed that those present in the Bs-1 to L*er* cross differ from those present in the Cen-0 to L*er* cross.

Identification of QTL causing enhanced discrimination between 14 h and 16 h day lengths

To test the heritability of the QTL identified in the F2 mapping populations and to determine whether they had differing effects on flowering time under 14 h and 16 h days, the flowering times of F3 families made by self-fertilizing selected F2 plants were scored under both day lengths. From the Bs-1 cross to L*er* five F3 families were scored in total and the range of flowering times observed in one of these, family 72, is shown in Figure 5A. Each of these families was derived from an F2 plant that was predicted to be heterozygous for the region

containing the major effect QTL (*AFT2*) on chromosome 5. To allow comparison of the effects of QTL under both day lengths each F3 family was grown under 14 h and 16 h days and flowering time was scored. All of the F3 families grown under 14 h contained plants that flowered later than any of the plants grown under 16 h, confirming that the F2 plants contained QTL conferring late flowering under 14 h. Plants in populations grown under both day lengths were tested with a molecular marker linked to *AFT2* (chr5_7.79). Plants homozygous or heterozygous for the Bs-1 allele at this locus were later flowering under 14 h days with higher leaf numbers than L*er* plants, but did not show a significant difference in flowering time under 16 h days (Figure 5B). Analysis of variance confirmed that the difference under 14 h days but not under 16 h days was statistically significant ($p<0.05$) (Figure 5 B; Supplementary Table 5AB). However, the weaker effect QTL detected on chromosome 1 (*AFT1*) may also contribute, particularly because *AFT2* was predicted to explain only 35% of the variation in flowering time in the F2 population (Supplementary Table 4).

Flowering time was also scored in F3 families derived from the cross of Cen-0 to L*er* (Figure 5 C, D). More QTL were identified in the F2 of this cross (Figure 4A) making confirmation of their effect more complex. The F2 individuals selected for F3 progeny testing were fixed or heterozygous for different combinations of QTL. F2 plant 20 carried Cen-0 and L*er* alleles at *AFT3* (chromosome 4) and *AFT6* (chromosome 5) and only L*er* alleles at the other three loci (Figure 5D). The F3 progeny contained severely late-flowering plants under 14 h (Figure 5D), indicating that Cen-0 alleles at *AFT3* and *AFT6* interact to delay flowering under these conditions. However, the presence of Cen-0 alleles at all loci more uniformly delays flowering under 14 h (Plant 97; Figure 5C), although plants with this genotype do not exhibit a more extreme late-flowering phenotype than observed in the progeny of plant 20. Taken together these data indicate that interactions between the Cen-0 alleles at *AFT3* and *AFT6* cause the most severe delays in flowering in the Cen-0 cross to L*er*, but that the effect of these loci under 14 h is further enhanced by Cen-0 alleles at the other loci.

To further study the complex inheritance of flowering-time variation in the Cen-0 cross to L*er*, QTL were mapped in segregating F3 populations derived from plants 97 and 20 grown under 14 h and 16 h days. The mean flowering time of these families was significantly later under 14 h compared to 16 h (Figure 6A; Supplementary Table 5C,D). The F3 plants grown under both conditions were analyzed with 40 markers in regions of all five chromosomes that were segregating for Cen-0 and L*er* alleles. Interactions between flowering time QTL and day-length were then analyzed (Figure 6B; Supplementary Table 5G). Homozygosity for Cen-

0 alleles at *AFT3* (Chr4_2.81) delayed flowering, particularly under 16 h, and reduced the effect of the day-length difference on flowering time. In contrast, homozygous Cen-0 at *AFT6* (Chr5_3.60) delayed flowering under 14 h and enhanced the effect of day length. Finally, *AFT15*, a QTL on chromosome 5 that was not detected in the F2 (Chr5_7.27; see also below), delayed flowering under 14 h when homozygous for Cen-0 and enhanced the effect of day length on flowering time. Taken together these data demonstrated strong interactions between day length and the Cen-0 allele present at *AFT6* and *AFT15* that would be consistent with them causing a delay in flowering under 14 h compared to 16 h as observed in the parental accession. However, the Ler allele at *AFT3* delayed flowering under 14 h but not 16 h, and therefore did not show the effect observed in the parental L*er* accession.

The flowering time data under both day lengths were then combined and QTL were tested for their effect on flowering time independently of day length. Univariate ANOVA identified 4 regions for which the Cen-0 alleles were significantly correlated with late flowering independently of day length (Figure 6C; Supplementary Table 5E). The markers used for this analysis were located in regions previously shown to contain the *AFT3* (marker Chrom 4_2.81), *AFT4* (marker Chrom 4_9.58) and *AFT6* (marker Chrom 5_3.60) QTL. The region containing *AFT7* was not polymorphic in either population preventing its detection, while *AFT5* was not detected. Therefore, this analysis validated three of the four QTL identified in the F2 and segregating in the F3 material. In addition, a further QTL for flowering time (*AFT15*) was identified on chromosome 5 using markers at 7.27 Mb (Figure 6C) that was not detected in the F2 population, probably due to its smaller effect.

Finally interactions between loci on chromosomes 4 and 5 were found to have important effects on flowering time in these families (Figure 6D; Supplementary Table 5F). The Cen-0 allele at *AFT3* (Chr4_2.81) interacts with the Cen-0 allele near *AFT6* (Chr5_3.6) to delay flowering in all genotypic combinations except when *AFT3* is Cen-0 heterozygous and *AFT6* Cen-0 homozygous. Interaction was also observed between Cen-0 alleles near *AFT3* (Chr4_0.28) and *AFT15*, although in this case *AFT15* alone caused a severe delay in flowering (Fig. 6D).

Taken together analysis of F3 families confirmed most of the QTL proposed from the F2 data. These analyses also detected a strong interaction between day length and *AFT2* in delaying flowering in the Bs-1 cross to L*er*, and between day length and *AFT6* and *AFT15* in delaying flowering in the Cen-0 cross to L*er*.

Accessions showing enhanced photoperiodic flowering response under short days

Accessions showing differential sensitivity to SDs of less than 10 h light were also identified (Figure 2D). Flowering time of the Sha accession did not change as day length was shortened from 10 h to 6 h, while Dijon-G flowered much later under 6 h than 10 h. To identify the loci responsible for this difference in day-length sensitivity the two accessions were crossed and an F2 population was made. F2 plants were grown under 8 h or 10 h days and flowering time was scored. In parallel, DNA was extracted from both populations and used to construct a genetic map (Supplementary Figure 6 and 7). The flowering-time data and mapping information were used to identify QTL that influence flowering time under 8 h or 10 h days (Figure 4B). Under 8 h days QTL were identified on chromosomes 1 (*AFT8*), 4 (*AFT11*, *AFT12*) and 5 (*AFT13*, *AFT14*) (Supplementary Table 4). Under 10 h days the same QTL were detected as under 8 h days, but in addition QTL were found on chromosome 3 (*AFT9*, *AFT10*) (Supplementary Table 4). The detection of QTL under 10 h but not 8 h days suggests the involvement of different genes in controlling flowering time under 10 h compared to 8 h days. These genes may act together with or independent of the QTL that influence flowering time under both day lengths.

Detection of conditional epistatic interactions that influence flowering under short days in the cross of Dijon-G to Sha

A genome-wide screen was performed to identify epistatic interactions influencing flowering time in the F2 population derived from crossing Dijon-G to Sha (Fig 7; Methods). The results are illustrated by heat maps (Figures 7A,B), in which the strongest interactions appear in red, as represented in the scale diagrams (Methods). This analysis revealed one highly significant epistatic interaction between two loci influencing day-length perception. This interaction was between a locus at the top of chromosome 5 (1.2 to 2.1 Mb) and a second one in the middle of the same chromosome (13.6 to 17.1 Mb) (Fig 7A). The interaction was conditional on day length because it appears only under SD of 8 h but not under SD 10 h (Fig 7 A B). The phenotypic effect of this epistatic interaction is to delay or accelerates flowering by approximately ten leaves, and it is statistically significant based on ANOVA (Figure 7C). However, no significant difference was observed in plants grown under 10 h (Fig 7 D). These data indicate that genetic interactions with significant phenotypic effects on flowering time can be environmentally dependent and thereby contribute to day-length perception.

Discussion

We identified extensive quantitative variation in the photoperiodic responses of Arabidopsis accessions. The response curves of most accessions were sigmoidal, as previously described for Ws and L *er* (Pouteau et al., 2008; Wilczek et al., 2009). These curves are in agreement with the classical description of *A. thaliana* as a facultative long-day species (Laibach, 1951), flowering earlier under LDs than SDs. The sigmoidal curve was previously used to define two key determinants of the photoperiod response, the critical day length and the ceiling photoperiod (Pouteau et al., 2008). The ceiling photoperiod is the longest day length under which the accession reached the plateau in flowering time characteristic of SDs. In contrast the critical photoperiod is the shortest photoperiod under which the accession shows the full LD response. In our data variation in both critical photoperiod and ceiling photoperiod was observed in many accessions, however these parameters were often difficult to score precisely because the flowering times under SDs frequently did not form a perfectly horizontal plateau, and under LDs a progressive delay in flowering time was sometimes observed as day length was shortened. Therefore, rather than comparing these two defined positions on the photoperiod response curves we used more general statistical approaches such as principal components analysis or clustering to evaluate the general shape of the response and thereby compare the photoperiod response curves of different accessions. These approaches allowed the accessions to be placed in broad phenotypic groups that were defined relative to the mean flowering response. These groups provide a basis for genetic and molecular analysis as well as comparison with existing mutants. One group was early flowering under all photoperiods, and this included Cvi, which showed a strongly diminished response to photoperiod (Alonso-Blanco et al., 1998; El-Assal et al., 2001). This early flowering group also included Ws, which was previously used for mutational analysis of photoperiodic response (Pouteau et al., 2008). However, the early flowering of this accession under all photoperiods reduces the absolute difference in flowering time between LD and SD, and may make it more difficult to use for mutational studies. Future genetic analysis of those accessions showing enhanced responses to day length may be particularly useful, because as well as showing an enhanced response at extreme day lengths some of them demonstrated increased capacity to distinguish between similar day lengths. For example, Cen-0 and Bs-1 discriminated between 16 h and 14 h days whereas L*er* and Col flowered at the same time under both day lengths. One interpretation of this result is that Cen-0 and Bs-1 have a longer critical day length than L*er* or Col. In addition to the three broad categories, extensive quantitative variation for photoperiodic response was detected consistent with previous studies demonstrating

tremendous variation for flowering time in Arabidopsis (Laibach, 1951; Alonso-Blanco et al., 1998; Lempe et al., 2005; Shindo et al., 2005; Werner et al., 2005a).

Altered photoperiodic responses shown by mutants or transgenic plants were compared with those of the accessions. Photoperiod-insensitive genotypes included late-flowering mutants under LDs that were impaired in the photoperiodic flowering pathway or transgenic plants overexpressing genes in this pathway that flowered early under SDs. These results were broadly consistent with previous publications and data obtained under a narrower range of day lengths (Redei, 1962; Koornneef et al., 1991; Kardailsky et al., 1999; Kobayashi et al., 1999; Onouchi et al., 2000; Mizoguchi et al., 2005). The circadian clock provides the time-keeping mechanism required to measure day length, and genetic variation in clock function influences flowering time. Plants overexpressing the clock components LHY and CCA1 are strongly impaired in circadian clock regulation of different processes (Schaffer et al., 1998; Wang and Tobin, 1998) and in our data these plants were almost day-length insensitive, flowering much later than wild-type plants under LDs. This result is consistent with the previous observation that the mRNA of the long-day flowering pathway component CO is reduced in abundance in *LHY* overexpressing plants (Suarez-Lopez et al., 2001). Similarly, *lhy-11 cca1-1* double loss of function mutants were early flowering under all conditions tested, except in extreme SDs of 6 h light, where flowering time was severely delayed. The early flowering of *lhy-11 cca1-1* double mutants under SDs was previously proposed to be due to the earlier phase of expression of clock-regulated genes such as *CO* (Mizoguchi et al., 2002; Mizoguchi et al., 2005), so that they are expressed in the light in the double mutant under SDs but not in wild-type plants. The abrupt delay in flowering of the double mutant under 6 h SDs might be due to the expression of *CO* occurring in darkness under such extreme SD conditions. Alternatively, in *lhy-11 cca1-1* double mutants increased expression of repressors of flowering may be activated only under certain environmental conditions (Fujiwara et al., 2008) or metabolic defects caused by impaired clock function (Dodd et al., 2005) may indirectly delay flowering under extreme SDs.

Statistical approaches were used to compare the photoperiodic responses of the mutants and transgenic lines with those of the accessions. The extensive variation found in the mutant and transgenic lines occupied much of the phenotypic space occupied by the accessions. However, some accessions clearly showed photoperiodic responses that were not found among the mutants. The responses of some of the accessions may be caused by allelic variation at several genes creating complex interactions that cannot be induced with single mutations in the widely used laboratory accessions Col-0, L*er* and Ws. The presence of response types in the

accessions that were not observed in any of the mutants emphasises the value of using natural-genetic variation to study photoperiodic responses.

The ecological significance of the genetic variation in day-length responses that we detected is not immediately clear. Correlations between photoperiodic responses and latitude at which accessions were collected have been demonstrated for other species (Ray and Alexander, 1966). Also the expansion of the geographical range of crop plants that occurred after domestication involved selection for photoperiodic insensitive varieties (Yano et al., 2000; Turner et al., 2005; Purugganan and Fuller, 2009). In Arabidopsis accessions relationships have been detected between the presence of particular alleles at *FRI* or *PHYC* and the latitude at which the accessions were collected (Stinchcombe et al., 2004; Balasubramanian et al., 2006). We did not observe a strong relationship between photoperiodic response and the latitude at which the accessions were collected. In particular, the Cen-0 and Bs-1 accessions that showed a longer critical day length than the mean response and enhanced discrimination between similar LDs were collected from France and Switzerland, at similar latitudes to many of the other accessions. Recently the ecological significance of different flowering pathways was explored in field experiments by planting a wide range of mutant genotypes in 5 sites across the European range of Arabidopsis (Wilczek et al., 2009). These experiments indicated that the significance of different flowering pathways is highly dependent on germination time and that flowering of most genotypes is suppressed in winter if they germinate at an appropriate time during late summer or autumn. Therefore the relationship between variation in photoperiodic flowering regulation and latitude may be difficult to assess in isolation but may depend on variation in the regulation of other traits including germination. The significance of variation in Arabidopsis photoperiodic response to adaptation at different latitudes may therefore emerge when analyzed together with a more thorough description of the phenotypic variation in other traits in the same accessions.

In the three mapping populations a total of 15 QTL were detected and named *ALTERED FLOWERING TIME* (*AFT*). None of these QTL were mapped to high enough resolution to identify the underlying genes with certainty, but some are located in regions previously shown to contain one or more genes regulating flowering time, while others are located in regions in which no genes regulating flowering time were previously identified. Those QTL that interacted with day length and contribute to the increased discrimination in day length response between 14 h and 16 h or between 10 h and 8 h were studied. In the Bs-1 cross to L*er*, *AFT2* and putatively *AFT1* were detected as causing late flowering under 14 h in the F2. Furthermore, phenotypic and genotypic testing of F3 progeny strongly indicated that *AFT2*

interacts with day length conferring a delay in flowering between 14 h and 16 h days. The location of *AFT2* on chromosome 5 is close to the flowering-time genes *FRL1*, *HUA2*, *PRR5* and *FPF1*, which were previously described (Kania et al., 1997; Chen and Meyerowitz, 1999; Michaels et al., 2004; Doyle et al., 2005; Nakamichi et al., 2007; Wang et al., 2007). In addition, *AFT1* had a small effect on flowering time in this cross and is located on chromosome 1 in a broad region containing two genes that promote flowering within the photoperiod pathway (*FKF1*, *FT*) (Kardailsky et al., 1999; Kobayashi et al., 1999; Nelson et al., 2000) and a general repressor of flowering (*FLM*) (Ratcliffe et al., 2001; Scortecci et al., 2001). Strong loss of function alleles in the photoperiod pathway similar to *PpdH-1* of barley (Turner et al., 2005) or *Hd-1* of rice (Yano et al., 2000) were not expected to be detected because accessions showing a severe late-flowering phenotype under 16 h LDs were excluded from the analysis. Nevertheless, *AFT1* may represent in Bs-1 a weak loss of function allele of *FKF1* or *FT* or a strong allele of *FLM*.

The inheritance of flowering-time variation was more complex in the Cen-0 cross to L*er*, and a total of 6 QTL were detected in the F2 and F3 generations. Of these, *AFT6* and *AFT15* showed strong interactions with day length in F3 families, delaying flowering more strongly under 14 h than 16 h. *AFT6* is located on chromosome 5 in a region containing *FLC*. Furthermore, *AFT6* delayed flowering more strongly when combined with *AFT3*, a QTL located on chromosome 4 close to the position of *FRI*. Therefore, *AFT6* and *AFT3* may represent *FLC* and *FRI*, two loci previously shown to contribute much of the variation for flowering time among Arabidopsis accessions (Lempe et al., 2005, Shindo et al., 2005). Nevertheless, *FLC* is not expected to show such a strong interaction with day length, because it is a central component of the vernalization pathway, and both parental accessions, Cen-0 and L*er*, harbour weak alleles of *FLC*. *AFT15*, the other locus that showed a strong interaction with day length in this cross, is located on chromosome 5 close to *AFT2*, and therefore to *FRL1*, *HUA2*, *PRR5* and *FPF1* (Kania et al., 1997; Chen and Meyerowitz, 1999; Michaels et al., 2004; Doyle et al., 2005; Nakamichi et al., 2007)

The cross between Dijon-G and Sha identified seven QTL influencing flowering time under SDs of 10 h or 8 h. Most of these did not show an interaction with day length and were detected in mapping populations grown under 10 h and 8 h days. However, *AFT9* and *AFT10* delayed flowering only under 10 h days and were not detected under 8 h days. These two QTL are located in a region of chromosome 3 not previously shown to contain genes that affect flowering time.

The experiments reported here demonstrated that Arabidopsis accessions are a rich source of quantitative phenotypic and genetic variation in photoperiodic response. Furthermore, the QTL analysis showed that most of this variation is genetically tractable and in some cases allowed candidate genes to be proposed. Construction of near isogenic lines as well as more detailed genetic mapping and molecular analysis should allow identification of some of the genes underlying these QTL and examination of the mechanisms by which they contribute to photoperiodic response.

Methods

Flowering time analysis

For the flowering time analysis 72 accessions from the Altmann and the Nordborg collection and 31 mutants and transgenic lines were selected (Supplementary Tables 1 and 2). The accessions were taken through one generation of single seed selection to further reduce variation. The flowering time was scored in six different day lengths three of which are short days (SD) of 6, 8 and 10 hours of light and the remaining ones are long days (LD) of 12, 14, 16 hours of light respectively. The analysis was performed in controlled environment growth chambers at 22 °C, after stratification for 3 days at 4 °C. A population of 18 individuals represented each accession. Flowering time parameters such as a) number of total leaves b) days until bolting at the stage of 1 cm c) days until anthesis were monitored. The experimental design was a Randomized Complete Block (RCB). For the vernalization treatment, 10 day old seedlings, grown under SD of 8 h at 22 ºC, were exposed to 4 ºC for one month under the same photoperiod and then moved to 22 ºC under the desired day length for flowering time scoring. For the genetic analysis, 100 to 150 individuals were used for each F2 population and populations of 50 individuals were used per F3 family and condition.

Statistical analysis

For the statistical analysis SigmaStat v.3 was used. A two way ANOVA was performed with accessions and day lengths as the two factors and for parameters such as TLN and BT for flowering. Demonstration of the results was performed with SigmaPlot v. 10.

The hierarchical clustering shown in Figure 2A and the PCA were performed using Cluster v. 3. The raw data were mean-centered across the different day lengths and accessions in order to provide an internal control for comparisons. Self-Organising Maps (SOMs) were calculated for both the accessions (100.000 iterations) and the day length (20.000 iterations). Both factors were then clustered according to the complete linkage clustering method using

Euclidean distance as similarity metric. TreeView v. 1.6 was used to demonstrate the results. PCA was performed using Cluster.

QTL mapping was performed using the MapQTL program. Linkage maps were created using JoinMap. A permutation test defined the LOD threshold for each population. First an interval mapping was performed and subsequently MQM mapping with automatically selected cofactors was used. SPSS v13 was used for the univariate ANOVA in the F3 families of Bs-1 x Ler and Cen-0 x Ler crosses.

The genetic relationship between the accessions (Supplementary Figure 1) was calculated with the MEGA software using 149 polymorphic SNPs (www.naturalvariation.org). Genome wide genetic interactions were performed with the J/qtl software.

Analysis of genome wide genetic interactions (Figure 7A) was performed in R (http://www.R-project.org/). Interactions were calculated with the function "scantwo" using 4000 permutations. The interactions were visualised with J/qtl software.

DNA isolation and genotyping

DNA isolation was performed with 100 mg of fresh tissue using either the CTAB method (for small number of samples) or the semi-automated method for DNA extraction using the Biosprint robot (QIAGEN) for the mapping populations. Genotyping in the F2 populations was performed in collaboration with Sequenom inc (San Diego, USA). Polymorphic markers were selected for each cross after parental screening of a pool of 360 markers (Supplementary Table 3; for further information see www.naturalvariation.org). For validation and further genotyping a standard PCR protocol was used after which the PCR fragments were analysed in 3 % agarose gels after electrophoresis in 100 V for 40 min approximately. The chr5_7.79 marker used for the validation in the F3 of *AFT2* the Bs-1 x Ler cross and shown in Figure 5 was a dCAPS marker cleaved with HinfI. The marker utilized the primers 5'-TCCACCGCCTTCACAATCATTAACAACTCGAC and 5'-GACAATTTGATCACCCTGCAC

Markers used for validation in the F3 of the QTL in the Cen-0 x Ler cross and shown in Figure 6 were as follows. Chr4_2.81 is a dCAPS marker cleaved with HindIII and the primers used were 5'-GGCTGCTTTCTTAGCATCAGATGATTCTTCTTACATCACTGGAGAAGC and 5'-AAGTATCCAATGGCCTCGTG. The other 3 markers used in Figure 6 are described at www.naturalvariation.org. Chr4_9.58 is AtMSQT_NW_173. Chr5_3.60 is AtMSQT_NW_208. Chr5_7.27 is AtMSQT_NW_216.

Expression analysis

Total RNA isolation was performed in 100 mg of fresh tissue using the RNAeasy kit (Qiagen). 3 mg of RNA were tested in formaldehyde gel in order to evaluate the quality of the RNA. 5 mg of total RNA were DNase treated with the turbo DNase kit (Ambion). The following primer pairs were used in order to perform Real-time PCR after evaluation of the correct PCR condition in a gradient reaction. Actin : Forward primer GGTAACATTGTGCTCAGTGGTGG, reverse primer : AACGACCTTAATCTTCATGCTGC ; FLC forward primer : ACGCATCCGTCGCTCTTCT; reverse primer : GCATGCTGTTTCCCATATCGA

Acknowledgements

We are grateful to Thomas Altmann (IPK Gatersleben) for providing the seeds of the accessions and to Detlef Weigel (MPI for Developmental Biology, Tübingen) for suggesting we use Sequenom for the mapping as well as for providing Sequenom with the marker primers. We are also indebted to Maarten Koornneef for his advice in designing these experiments and for discussions. Amaury de Montaigu and Maria von Korff made valuable comments on the text. The laboratory of G.C. is partly funded by a core grant from the Max Planck Society.

Figure Legends

Figure 1. Characterization of flowering responses of Arabidopsis accessions, mutants and transgenic lines under a range of day lengths.

A. Flowering times of 72 accessions under six day lengths. Day length is plotted in hours along the horizontal axis. Flowering time, measured as total leaf number (TLN), is plotted on the vertical axis. The flowering time data are also presented in Supplementary Table 1. The photoperiodic response curve of each accession is represented. The grey region highlights those accessions showing a sigmoidal photoperiodic response curve as determined by the correlation coefficients between the theoretical sigmoidal pattern and the actual data for the accessions.

B. Flowering times of 31 mutants and transgenic lines under six different day lengths. Day length is plotted in hours along the horizontal axis. Flowering time, measured as total leaf number (TLN), is plotted on the vertical axis. The flowering time data are also presented in Supplementary Table 1.

C. Flowering times of a set of early-flowering accessions under all day lengths. Closed circles, Cvi; open circles, Sha; closed triangles, Dra-0; open triangles, Ws-0; closed squares, Ler; orange squares, average response.

D. Flowering times of a set of accessions showing a greater than average difference in flowering time between 6 h and 16 h days and similar or earlier flowering than average at 16 h. Closed circles, Cen-0; open circles, Col-2; closed triangles, Col-0; open triangles, Col-3; closed squares, Fr-4; open squares, Enkheim-T; closed diamond, Bs-1; orange circles, average response.

Figure 2. Characterization of flowering responses of Arabidopsis accessions under a range of day lengths.

A. Hierarchical clustering analysis of day-length responses of 72 accessions. The flowering time responses of each genotype under six different day lengths were analyzed using Cluster. The colours represent: green, earlier flowering than the mean; red, later flowering than the mean; grey, missing values; black, equal to mean.

B. Principal components analysis comparing photoperiod discrimination of mutants and accessions. The Y axis is principal component 5, which is flowering time under 16 h versus flowering time under 14 h. The X axis is PC4, which is flowering time under 10 h and 6 h versus flowering time under 8 h. PC5 explains 8% of the phenotypic variation, while PC4 explains 9% of the variation. The large rectangle represents the space defined by the photoperiodic responses of mutants. The green squares represent mutants and the light blue diamond shape symbols indicate accessions. Black circle : Ler, dark blue circle : Bs-1 red circle : Cen-0.

C. Flowering times of accessions Cen-0 and Bs-1 compared to L*er*. The bracket illustrates that flowering of Bs-1 and Cen-0 is delayed by shortening day length from 16 h to 14 h, but flowering of Ler is not.

D. Flowering times of accessions Dijon-G and Sha that show different responses to decreasing day length below 10 h. The bracket illustrates the difference between 10 h and 8 h days which delays flowering in Dijon-G but not Sha.

Figure 3. Vernalization response of Bs-1 and Cen-0 accessions under 14 h days and its relationship to *FLC* expression levels.

A. Flowering times of Bs-1 and Cen-0 accessions with or without vernalization. Flowering time is plotted as total leaf number (TLN) on the Y axis. The accessions with or without vernalization are arranged on the X-axis.
B. *FLC* mRNA abundance before, during and after vernalization in Bs-1 and Cen-0 accessions. *FLC* mRNA level relative to actin mRNA level is plotted on the Y axis. Each accession without vernalization (NV), exposed to vernalization for 1 or 4 weeks (1wV and 4wV, respectively), or exposed to vernalization for 4 weeks and then returned to normal growth temperatures for 15 days (15 dav) are arranged on the X-axis.
C. Analysis of *FRI* alleles present in selected accessions. Polymorphisms associated with a 16 bp deletion in the coding sequence (top) or a deletion in the promoter (pFRI; bottom) were tested. L*er* (L*er*) and Columbia (Col-0) act as controls. L*er* carries the 16 bp deletion in the *FRI* coding sequence, whereas Col-0 carries the deletion in the *FRI* promoter.

Figure 4. Positions of QTL that influence flowering time under various photoperiods.

A.QTL identified in the F2 of the cross between Bs-1 and Ler (BL) or between Cen-0 and L*er* (CL) grown under 14 h days. The left hand panel represents QTL located on chromosome 1, the central panel illustrates those located on chromosome 4 whereas the right hand panel represents those located on chromosome 5. Arrows between the vertical dotted lines represent QTL identified in each cross. Arrows that point upwards indicate QTL for which the L*er* allele causes early flowering. The three solid red lines in each panel represent the positions of markers located in the genomic sequence of Columbia (left), mapped in the BL cross (centre) and mapped in the CL cross (right). Dotted black lines connect markers in the sequence to their position in each genetic map. Long vertical black lines separate the data for each chromosome.
B.QTL identified in the F2 of the cross between Dijon and Sha grown under 10 h (DS10) or 8 h (DS8) days. Structure of each panel as for A, except data are shown for chromosomes 1, 3, 4 and 5. Arrows pointing upwards represent those QTL for which Sha alleles cause early flowering.

Figure 5. Analysis of flowering time in F3 populations used to validate QTL and test their interaction with day length.

The flowering times of plants in three F3 families grown under 14 h and 16 h days. In each histogram the horizontal axis represents flowering time as leaf number. The vertical axis illustrates number of plants. Blue indicates plants grown under 16 h days, whereas cream illustrates plants grown under 14 h days. The diagram below each histogram illustrates the genotype of the parental F2 plant. White illustrates L*er* homozygous alleles, black illustrates Bs-1 or Cen-0 homozygous alleles and hatched illustrates heterozygous alleles. The positions of the QTL mapped in the F2 are indicated by the rectangles above each diagram and the names of each QTL are indicated. The number to the left of the genotype indicates the number of the F2 plant whose genotype is illustrated, the number on the right indicates the leaf number at flowering of this F2 plant.
A. F3 family derived from F2 plant 72 in the Bs-1 cross to L*er*.

B. ANOVA analysis of the relationship between *ERD2* alleles and flowering time under 14 h or 16 h days in the F3 family shown in panel A. Left hand panel, analysis of data for plants grown under 14 h days. Right hand panel, analysis of data for plants grown under 16 h days. Y axis, flowering time represented as total leaf number. X axis, genotype at *ERD2*, marker used chr5_7.79 (Methods). Asterisk illustrates significantly different from Ler ($P<0.05$).
C. F3 family derived from F2 plant 97 in the Cen-0 cross to L*er*.
D. F3 family derived from F2 plant 20 in the Cen-0 cross to L*er*.

Figure 6. Validation of the QTL in the Cen-0 to L*er* cross using F3 familes 20 and 97, and analysis of interactions between QTL and day length

A. Mean flowering times of each family and parental controls under 14 h and 16 h days. Y axis flowering time represented as total leaf number. Error bars indicate S.D. X axis genotypes and day length. CL20, F3 family derived from F2 plant 20 of the Cen-0 x Ler cross. CL97, same nomenclature for F2 plant 97.
B. Univariate ANOVA detection of two-way interactions between markers and changing day length in the control of flowering time. Loci on top of chromosome 4 (4_0.28) and chromosome 5 (5_3.6 and 5_7.27) interact differently with the two day lengths. The Cen-0 allele at the chromosome 4 locus delays flowering under 16 h reducing the difference in flowering time between 14 h and 16 h days. The Cen-0 alleles at the chromosome 5 loci more strongly delay flowering under 14 h and enhance the difference in flowering time between the two day lengths. Data for families 20 and 97 were combined. Y axis flowering time represented by total leaf number. X axis genotype and day length. Upper symbols correspond to allelic variation at the corresponding loci (L: L*er* homozygous, C: Cen-0 homozygous H: heterozygous alleles) while lower numbers represent day length. The position of the markers used are indicated together with the name of the QTL mapped in the region. All markers are described in Methods.
C. Univariate ANOVA detection of flowering time effects independently of daylength. Markers on chromosome 4 (4_0.28 and 4_9.58) and 5 (5_3.6) detect loci that delay flowering when Cen-0 alleles are homozygous or heterozygous, whereas the Cen-0 allele at Chr5_7.27 delays flowering only when homozygous. Markers are described in Methods. Flowering time was scored under 14 h and 16 h for both families and all data combined for analysis. The genotype at each marker is shown L: L*er* homozygous, C: Cen-0 homozygous H: heterozygous alleles. The position of each marker is shown together with the name of the QTL mapped in the region.
D. Univariate ANOVA detection of two-way interactions between markers in determining flowering time. Allelic variation at a locus at the top of chromosome 4 (linked to marker 4_0.28) interacts with two other loci on chromosome 5 (linked to markers 5_3.6 and 5_7.27). Flowering time was scored under 14 h and 16 h for both families and all data combined for analysis. Upper symbols of x-axis correspond to alleles of the chromosome 4 locus while bottom symbols correspond to alleles of the chromosome 5 loci. L: L*er* homozygous, C: Cen-0 homozygous H: heterozygous alleles.

Figure 7. Genome wide detection of epistatic interactions in the mapping population created by crossing Dijon-G to Sha

A. Heat map of two-dimensional genome scan for interactions under 8 h SD
B. Heat map of two dimensional genome scan for interactions under 10 h SD

In A and B the numerals on the horizontal and vertical axes illustrate the five chromosomes. The upper left triangle shows the epistasis LOD scores. The lower right triangle illustrates the joint LOD scores. The colour scale on the right of each panel indicates separate scales for the epistasis and joint LOD scores (on the left and right respectively).
The white arrow in A indicates the presence of an epistatic interaction, which is conditional as it occurs only under 8 h, between a locus on the top of Chrom 5 (horizontal axis) and one in the middle of the same chromosome (vertical axis).

C. Effect plot of the epistatic interaction under 8 h days marked in Figure 10A. Vertical axis represents flowering time as total leaf number. Horizontal axis represents genotype. Homozygous Dijon.G is indicated by D and homozygous Shakdara is indicated with S. Upper symbols represent the locus at the top of chromosome 5, while lower symbols represent the locus in the middle of chromosome 5. The presence of Shakdara alleles at the top of chromosome 5 makes a difference in flowering time of around 20 leaves if the locus in the middle of chromosome 5 is also Shakdara. Asterix indicates a significant difference ($P<0.05$).

D. The same analysis with the same loci as in C, but for plants grown under 10 h days. No significant effect of genotype on flowering time was observed.

References

Alonso-Blanco, C., El-Assal, S.E.-D., Coupland, G., and Koornneef, M. (1998). Analysis of natural allelic variation at flowering time loci in the Landsberg erecta and Cape Verde Islands ecotypes of Arabidopsis thaliana. Genetics 149, 749-764.

Balasubramanian, S., Sureshkumar, S., Agrawal, M., Michael, T.P., Wessinger, C., Maloof, J.N., Clark, R., Warthmann, N., Chory, J., and Weigel, D. (2006). The PHYTOCHROME C photoreceptor gene mediates natural variation in flowering and growth responses of Arabidopsis thaliana. Nature Genetics 38, 711-715.

Bastow, R., Mylne, J.S., Lister, C., Lippman, Z., Martienssen, R.A., and Dean, C. (2004). Vernalization requires epigenetic silencing of FLC by histone methylation. Nature 427, 164-167.

Bohlenius, H., Huang, T., Charbonnel-Campaa, L., Brunner, A.M., Jansson, S., Strauss, S.H., and Nilsson, O. (2006). CO/FT regulatory module controls timing of flowering and seasonal growth cessation in trees. Science 312, 1040-1043.

Borthwick, H.A., and Parker, M.W. (1939). Photoperiodic responses of several varieties of soybean. Botanical Gazette 101, 341-365.

Burn, J.E., Smyth, D.R., Peacock, W.J., and Dennis, E.S. (1993). Genes conferring late flowering in Arabidopsis thaliana. Genetica 90, 147-155.

Caicedo, A.L., Stinchcombe, J.R., Olsen, K.M., Schmitt, J., and Purugganan, M.D. (2004). Epistatic interaction between Arabidopsis FRI and FLC flowering time genes generates a latitudinal cline in a life history trait. P Natl Acad Sci USA 101, 15670-15675.

Chen, X.M., and Meyerowitz, E.M. (1999). HUA1 and HUA2 are two members of the floral homeotic AGAMOUS pathway. Mol Cell 3, 349-360.

Clarke, J.H., and Dean, C. (1994). Mapping FRI, a locus controlling flowering time and vernalization response in Arabidopsis thaliana. Molecular and General Genetics 242, 81-89.

Dodd, A.N., Salathia, N., Hall, A., Kevei, E., Toth, R., Nagy, F., Hibberd, J.M., Millar, A.J., and Webb, A.A.R. (2005). Plant circadian clocks increase photosynthesis, growth, survival, and competitive advantage. Science 309, 630-633.

Doyle, M.R., Bizzell, C.M., Keller, M.R., Michaels, S.D., Song, J.D., Noh, Y.S., and Amasino, R.M. (2005). HUA2 is required for the expression of floral repressors in Arabidopsis thaliana. Plant J 41, 376-385.

El-Assal, S.E.D., Alonso-Blanco, C., Peeters, A.J.M., Raz, V., and Koornneef, M. (2001). A QTL for flowering time in *Arabidopsis* reveals a novel allele of *CRY2*. Nature Genetics 29, 435-440.

Fowler, S., Lee, K., Onouchi, H., Samach, A., Richardson, K., Coupland, G., and Putterill, J. (1999). *GIGANTEA*: A circadian clock-controlled gene that regulates photoperiodic flowering in *Arabidopsis* and encodes a protein with several possible membrane-spanning domains. Embo J 18, 4679-4688.

Fujiwara, S., Oda, A., Yoshida, R., Niinuma, K., Miyata, K., Tomozoe, Y., Tajima, T., Nakagawa, M., Hayashi, K., Coupland, G., and Mizoguchi, T. (2008). Circadian Clock Proteins LHY and CCA1 Regulate SVP Protein Accumulation to Control Flowering in Arabidopsis. Plant Cell 20, 2960-2971.

Gazzani, S., Gendall, A.R., Lister, C., and Dean, C. (2003). Analysis of the molecular basis of flowering time variation in Arabidopsis accessions. Plant Physiology 132, 1107-1114.

Imaizumi, T., Schultz, T.F., Harmon, F.G., Ho, L.A., and Kay, S.A. (2005). FKF1F-BOX protein mediates cyclic degradation of a repressor of CONSTANS in Arabidopsis. Science 309, 293-297.

Johanson, U., West, J., Lister, C., Michaels, S., Amasino, R., and Dean, C. (2000). Molecular analysis of FRIGIDA, a major determinant of natural variation in Arabidopsis flowering time. Science 290, 344-347.

Kania, T., Russenberger, D., Peng, S., Apel, K., and Melzer, S. (1997). FPF1 promotes flowering in Arabidopsis. Plant Cell 9, 1327-1338.

Kardailsky, I., Shukla, V.K., Ahn, J.H., Dagenais, N., Christensen, S.K., Nguyen, J.T., Chory, J., Harrison, M.J., and Weigel, D. (1999). Activation tagging of the floral inducer *FT*. Science 286, 1962-1965.

Kobayashi, Y., and Weigel, D. (2007). Move on up, it's time for change - mobile signals controlling photoperiod-dependent flowering. Gene Dev 21, 2371-2384.

Kobayashi, Y., Kaya, H., Goto, K., Iwabuchi, M., and Araki, T. (1999). A pair of related genes with antagonistic roles in mediating flowering signals. Science 286, 1960-1962.

Koornneef, M., Hanhart, C.J., and van der Veen, J.H. (1991). A genetic and physiological analysis of late flowering mutants in Arabidopsis thaliana. Mol Gen Genet 229, 57-66.

Koornneef, M., Blankestijn-De vries, H., Hanhart, C., Soppe, W., and Peeters, T. (1994). The phenotype of some late-flowering mutants is enhanced by a locus on chromosome 5 that is not effective in the Landsberg erecta wild-type. Plant J 6, 911-919.

Laibach, F. (1951). Uber sommer und winterannuelle Rasse von *Arabidopsis thaliana* (L.) Heynh. Ein Beitrag zur Atiologie der Blutenbildung. Beitr. Biol. Pflanz. 28, 173-210.

Le Corre, V., Roux, F., and Reboud, X. (2002). DNA polymorphism at the FRIGIDA gene in Arabidopsis thaliana: Extensive nonsynonymous variation is consistent with local selection for flowering time. Mol Biol Evol 19, 1261-1271.

Lee, I., Michaels, S.D., Masshardt, A.S., and Amasino, R.M. (1994). The late-flowering phenotype of FRIGIDA and mutations in LUMINIDEPENDENS is suppressed in the Landsberg erecta strain of Arabidopsis. Plant J 6, 903-909.

Lempe, J., Balasubramanian, S., Sureshkumar, S., Singh, A., Schmid, M., and Weigel, D. (2005). Diversity of flowering responses in wild Arabidopsis thaliana strains. Plos Genet 1, 109-118.

Li, Y., Roycewicz, P., Smith, E., and Borevitz, J.O. (2006). Genetics of Local Adaptation in the Laboratory: Flowering Time Quantitative Trait Loci under Geographic and Seasonal Conditions in <italic>Arabidopsis</italic>. PLoS ONE 1, e105.

Michaels, S.D., and Amasino, R.M. (1999). *FLOWERING LOCUS C* encodes a novel MADS domain protein that acts as a repressor of flowering. Plant Cell 11, 949-956.

Michaels, S.D., Bezerra, I.C., and Amasino, R.M. (2004). FRIGIDA-related genes are required for the winter-annual habit in Arabidopsis. P Natl Acad Sci USA 101, 3281-3285.

Michaels, S.D., He, Y.H., Scortecci, K.C., and Amasino, R.M. (2003). Attenuation of FLOWERING LOCUS C activity as a mechanism for the evolution of summer-annual flowering behavior in Arabidopsis. P Natl Acad Sci USA 100, 10102-10107.

Mizoguchi, T., Wheatley, K., Hanzawa, Y., Wright, L., Mizoguchi, M., Song, H.R., Carre, I.A., and Coupland, G. (2002). *LHY* and *CCA1* are partially redundant genes required to maintain circadian rhythms in Arabidopsis. Developmental Cell 2, 629-641.

Mizoguchi, T., Wright, L., Fujiwara, S., Cremer, F., Lee, K., Onouchi, H., Mouradov, A., Fowler, S., Kamada, H., Putterill, J., and Coupland, G. (2005). Distinct roles of GIGANTEA in promoting flowering and regulating circadian rhythms in Arabidopsis. Plant Cell 17, 2255-2270.

Nakamichi, N., Kita, M., Niinuma, K., Ito, S., Yamashino, T., Mizoguchi, T., and Mizuno, T. (2007). Arabidopsis clock-associated pseudo-response regulators PRR9, PRR7 and PRR5 coordinately and positively regulate flowering time through the canonical CONSTANS-dependent photoperiodic pathway. Plant and Cell Physiology 48, 822-832.

Nelson, D.C., Lasswell, J., Rogg, L.E., Cohen, M.A., and Bartel, B. (2000). *FKF1*, a clock-controlled gene that regulates the transition to flowering in *Arabidopsis*. Cell 101, 331-340.

Onouchi, H., Igeno, M.I., Perilleux, C., Graves, K., and Coupland, G. (2000). Mutagenesis of plants overexpressing *CONSTANS* demonstrates novel interactions among *Arabidopsis* flowering-time genes. Plant Cell 12, 885-900.

Park, D.H., Somers, D.E., Kim, Y.S., Choy, Y.H., Lim, H.K., Soh, M.S., Kim, H.J., Kay, S.A., and Nam, H.G. (1999). Control of circadian rhythms and photoperiodic flowering by the *GIGANTEA* gene. Science 285, 1579-1582.

Pouteau, S., Carre, I., Gaudin, V., Ferret, V., Lefebvre, D., and Wilson, M. (2008). Diversification of Photoperiodic Response Patterns in a Collection of Early-Flowering Mutants of Arabidopsis. Plant Physiology 148, 1465-1473.

Purugganan, M.D., and Fuller, D.Q. (2009). The nature of selection during plant domestication. Nature 457, 843-848.

Putterill, J., Robson, F., Lee, K., Simon, R., and Coupland, G. (1995). The *CONSTANS* gene of *Arabidopsis* promotes flowering and encodes a protein showing similarities to zinc finger transcription factors. Cell 80, 847-857.

Ratcliffe, O.J., Nadzan, G.C., Reuber, T.L., and Riechmann, J.L. (2001). Regulation of flowering in Arabidopsis by an FLC homologue. Plant Physiology 126, 122-132.

Ray, P.M., and Alexander, W.E. (1966). Photoperiodic adaptation to latitude in *Xanthium strumarium*. American Journal of Botany 53, 806.

Redei, G.P. (1962). Supervital mutants of Arabidopsis. Genetics 47, 443-460.

Schaffer, R., Ramsay, N., Samach, A., Corden, S., Putterill, J., Carre, I.A., and Coupland, G. (1998). The *late elongated hypocotyl* mutation of *Arabidopsis* disrupts circadian rhythms and the photoperiodic control of flowering. Cell 93, 1219-1229.

Scortecci, K.C., Michaels, S.D., and Amasino, R.M. (2001). Identification of a MADS-box gene, FLOWERING LOCUS M, that represses flowering. Plant J 26, 229-236.

Sheldon, C.C., Burn, J.E., Perez, P.P., Metzger, J., Edwards, J.A., Peacock, W.J., and Dennis, E.S. (1999). The FLF MADS box gene: A repressor of flowering in Arabidopsis regulated by vernalization and methylation. Plant Cell 11, 445-458.

Shindo, C., Aranzana, M.J., Lister, C., Baxter, C., Nicholls, C., Nordborg, M., and Dean, C. (2005). Role of FRIGIDA and FLOWERING LOCUS C in determining variation in flowering time of Arabidopsis. Plant Physiology 138, 1163-1173.

Stinchcombe, J.R., Weinig, C., Ungerer, M., Olsen, K.M., Mays, C., Halldorsdottir, S.S., Purugganan, M.D., and Schmitt, J. (2004). A latitudinal cline in flowering time in Arabidopsis thaliana modulated by the flowering time gene *FRIGIDA*. P Natl Acad Sci USA 101, 4712-4717.

Suarez-Lopez, P., Wheatley, K., Robson, F., Onouchi, H., Valverde, F., and Coupland, G. (2001). *CONSTANS* mediates between the circadian clock and the control of flowering in *Arabidopsis*. Nature 410, 1116-1120.

Sung, S.B., and Amasino, R.M. (2004). Vernalization in Arabidopsis thaliana is mediated by the PHD finger protein VIN3. Nature 427, 159-164.

Thomas, B., and Vince-Prue, B. (1997). Photoperiodism in Plants, 2nd ed. (San Diego, CA: Academic Press).

Turck, F., Fornara, F., and Coupland, G. (2008). Regulation and identity of florigen: FLOWERING LOCUS T moves center stage. Annual Review of Plant Biology 59, 573-594.

Turner, A., Beales, J., Faure, S., Dunford, R.P., and Laurie, D.A. (2005). The pseudo-response regulator Ppd-H1 provides adaptation to photoperiod in barley. Science 310, 1031-1034.

Valverde, F., Mouradov, A., Soppe, W., Ravenscroft, D., Samach, A., and Coupland, G. (2004). Photoreceptor regulation of CONSTANS protein and the mechanism of photoperiodic flowering. Science 303, 1003-1006.

Wang, Q., Sajja, U., Rosloski, S., Humphrey, T., Kim, M.C., Bomblies, K., Weigel, D., and Grbic, V. (2007). HUA2 caused natural variation in shoot morphology of A-thaliana. Curr Biol 17, 1513-1519.

Wang, Z.-Y., and Tobin, E.M. (1998). Constitutive expression of the CIRCADIAN CLOCK ASSOCIATED 1 (CCA1) gene disrupts circadian rhythms and suppresses its own expression. Cell 93, 1207-1217.

Werner, J.D., Borevitz, J.O., Uhlenhaut, N.H., Ecker, J.R., Chory, J., and Weigel, D. (2005a). FRIGIDA-independent variation in flowering time of natural Arabidopsis thaliana accessions. Genetics 170, 1197-1207.

Werner, J.D., Borevitz, J.O., Warthmann, N., Trainer, G.T., Ecker, J.R., Chory, J., and Weigel, D. (2005b). Quantitative trait locus mapping and DNA array hybridization identify an FLM deletion as a cause for natural flowering-time variation. P Natl Acad Sci USA 102, 2460-2465.

Wilczek, A.M., Roe, J.L., Knapp, M.C., Cooper, M.D., Lopez-Gallego, C., Martin, L.J., Muir, C.D., Sim, S., Walker, A., Anderson, J., Egan, J.F., Moyers, B.T., Petipas,

R., Giakountis, A., Charbit, E., Coupland, G., Welch, S.M., and Schmitt, J. (2009). Effects of Genetic Perturbation on Seasonal Life History Plasticity. Science 323, 930-934.

Yano, M., Katayose, Y., Ashikari, M., Yamanouchi, U., Monna, L., Fuse, T., Baba, T., Yamamoto, K., Umehara, Y., Nagamura, Y., and Sasaki, T. (2000). Hd1, a major photoperiod sensitivity quantitative trait locus in rice, is closely related to the Arabidopsis flowering time gene CONSTANS. Plant Cell 12, 2473-2484.

Figure 1

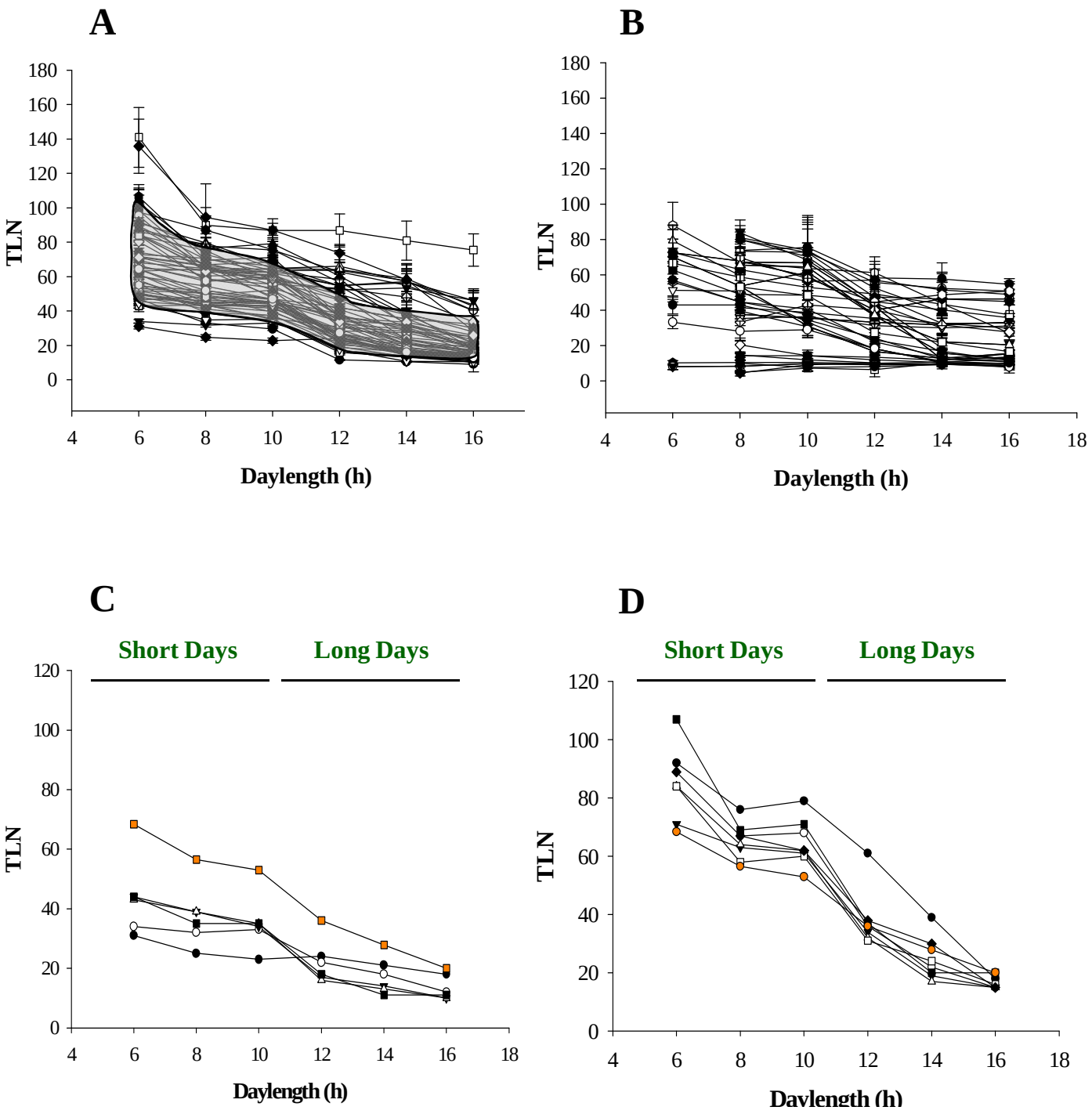

Figure 1. Characterization of flowering responses of Arabidopsis accessions, mutants and transgenic lines under a range of day lengths.

A. Flowering times of 72 accessions under six day lengths. Day length is plotted in hours along the horizontal axis. Flowering time, measured as total leaf number (TLN), is plotted on the vertical axis. The flowering time data are also presented in Supplementary Table 1. The photoperiodic response curve of each accession is represented. The grey region highlights those accessions showing a sigmoidal photoperiodic response curve as determined by the correlation coefficients between the theoretical sigmoidal pattern and the actual data for the accessions.

B. Flowering times of 31 mutants and transgenic lines under six different day lengths. Day length is plotted in hours along the horizontal axis. Flowering time, measured as total leaf number (TLN), is plotted on the vertical axis. The flowering time data are also presented in Supplementary Table 1.

C. Flowering times of a set of early-flowering accessions under all day lengths. Closed circles, Cvi; open circles, Sha; closed triangles, Dra-0; open triangles, Ws-0; closed squares, Ler; orange squares, average response.

D. Flowering times of a set of accessions showing a greater than average difference in flowering time between 6 h and 16 h days and similar or earlier flowering than average at 16 h. Closed circles, Cen-0; open circles, Col-2; closed triangles, Col-0; open triangles, Col-3; closed squares, Fr-4; open squares, Enkheim-T; closed diamond, Bs-1; orange circles, average response.

Figure 2

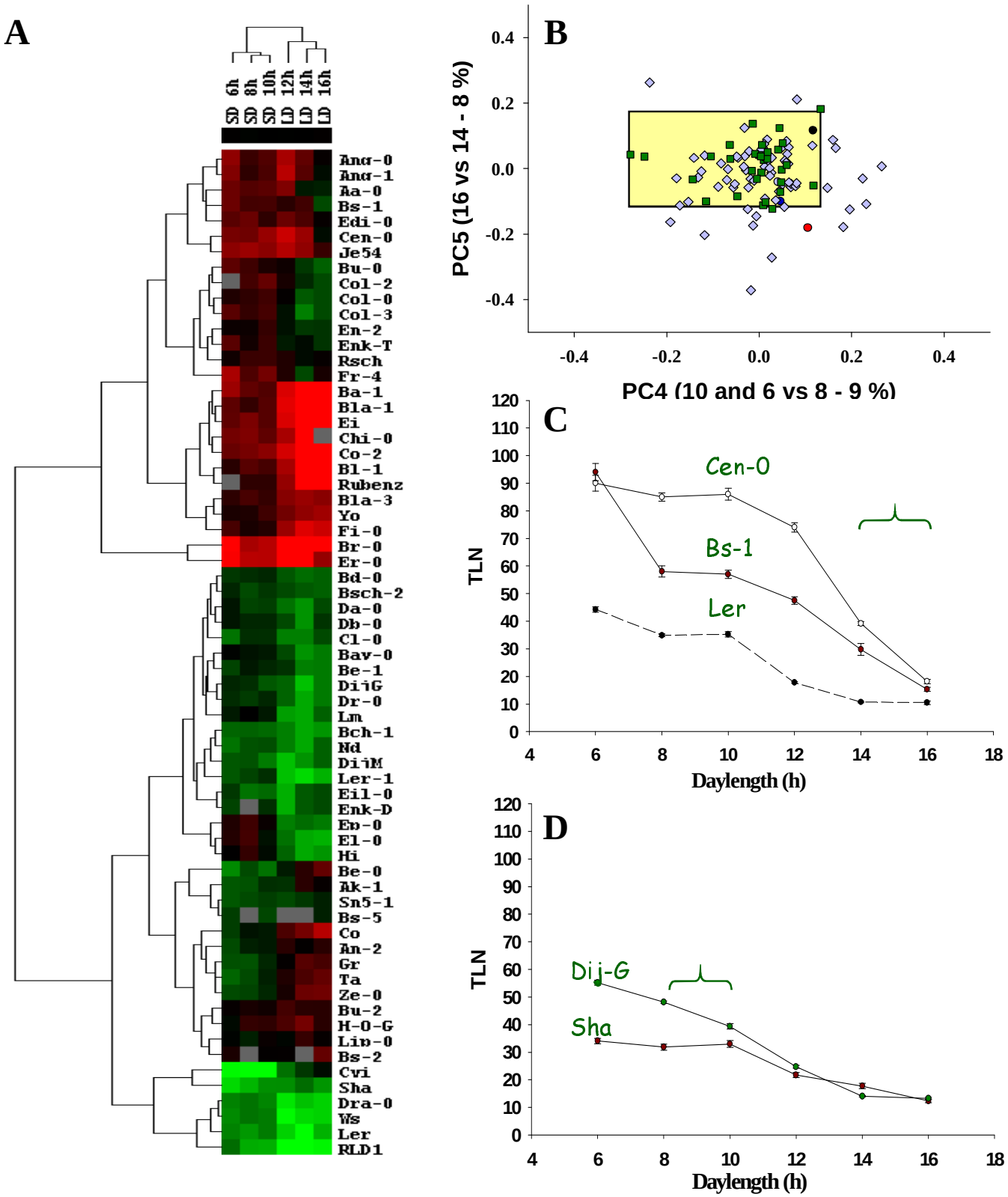
A
SD 6h
SD 8h
SD 10h
LD 12h
LD 14h
LD 16h
Ana-0
Ana-1
Aa-0
Bs-1
Edi-0
Cen-0
Je54
Bu-0
Col-2
Col-0
Col-3
En-2
Enk-T
Rsch
Fr-4
Ba-1
Bla-1
Ei
Chi-0
Co-2
Bl-1
Rubenz
Bla-3
Yo
Fi-0
Br-0
Er-0
Bd-0
Bsch-2
Da-0
Db-0
Cl-0
Bav-0
Be-1
DijG
Dr-0
Lm
Bch-1
Nd
DijM
Ler-1
Eil-0
Enk-D
Ep-0
El-0
Hi
Be-0
Ak-1
Sn5-1
Bs-5
Co
An-2
Gr
Ta
Ze-0
Bu-2
H-O-G
Lip-0
Bs-2
Cvi
Sha
Dra-0
Ws
Ler
RLD1
B
PC5 (16 vs 14 - 8 %)
PC4 (10 and 6 vs 8 - 9 %)
C
Cen-0
Bs-1
Ler
TLN
Daylength (h)
D
Dij-G
Sha
TLN
Daylength (h)

Figure 2. Characterization of flowering responses of Arabidopsis accessions under a range of day lengths.

A. Hierarchical clustering analysis of day-length responses of 72 accessions. The flowering time responses of each genotype under six different day lengths were analyzed using Cluster. The colours represent: green, earlier flowering than the mean; red, later flowering than the mean; grey, missing values; black, equal to mean.
B. Principal components analysis comparing photoperiod discrimination of mutants and accessions. The Y axis is principal component 5, which is flowering time under 16 h versus flowering time under 14 h. The X axis is PC4, which is flowering time under 10 h and 6 h versus flowering time under 8 h. PC5 explains 8% of the phenotypic variation, while PC4 explains 9% of the variation. The large rectangle represents the space defined by the photoperiodic responses of mutants. The green squares represent mutants and the light blue diamond shape symbols indicate accessions. Black circle : Ler, dark blue circle : Bs-1 red circle : Cen-0.
C. Flowering times of accessions Cen-0 and Bs-1 compared to L*er*. The bracket illustrates that flowering of Bs-1 and Cen-0 is delayed by shortening day length from 16 h to 14 h, but flowering of Ler is not.
D. Flowering times of accessions Dijon-G and Sha that show different responses to decreasing day length below 10 h. The bracket illustrates the difference between 10 h and 8 h days which delays flowering in Dijon-G but not Sha.

Figure 3

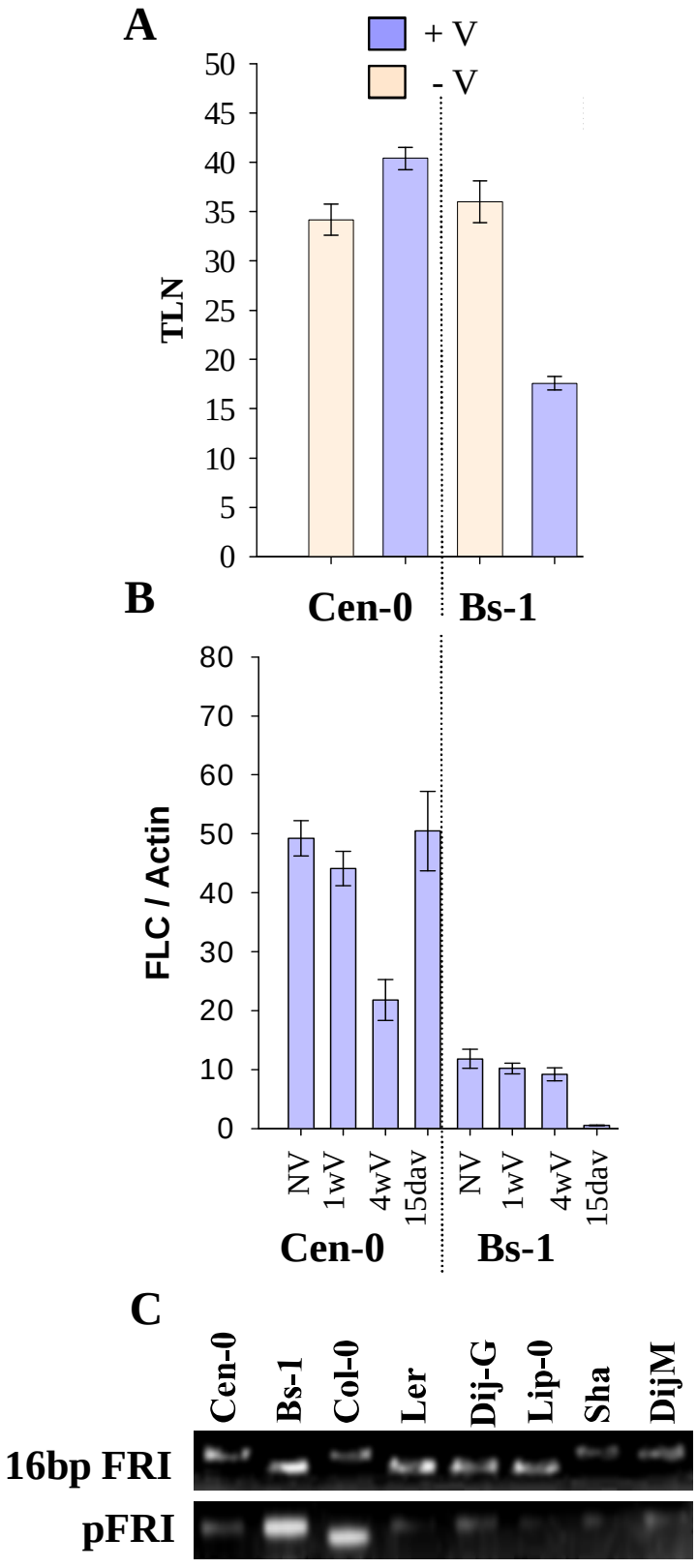
A
+ V
- V
TLN
50
45
40
35
30
25
20
15
10
5
0
Cen-0
Bs-1
B
FLC / Actin
80
70
60
50
40
30
20
10
0
NV
1wV
4wV
15dav
NV
1wV
4wV
15dav
Cen-0
Bs-1
C
Cen-0
Bs-1
Col-0
Ler
Dij-G
Lip-0
Sha
DijM
16bp FRI
pFRI

Figure 3. Vernalization response of Bs-1 and Cen-0 accessions under 14 h days and its relationship to *FLC* expression levels.

A. Flowering times of Bs-1 and Cen-0 accessions with or without vernalization. Flowering time is plotted as total leaf number (TLN) on the Y axis. The accessions with or without vernalization are arranged on the X-axis.
B. *FLC* mRNA abundance before, during and after vernalization in Bs-1 and Cen-0 accessions. *FLC* mRNA level relative to actin mRNA level is plotted on the Y axis. Each accession without vernalization (NV), exposed to vernalization for 1 or 4 weeks (1wV and 4wV, respectively), or exposed to vernalization for 4 weeks and then returned to normal growth temperatures for 15 days (15 dav) are arranged on the X-axis.
C. Analysis of *FRI* alleles present in selected accessions. Polymorphisms associated with a 16 bp deletion in the coding sequence (top) or a deletion in the promoter (pFRI; bottom) were tested. L*er* (L*er*) and Columbia (Col-0) act as controls. L*er* carries the 16 bp deletion in the *FRI* coding sequence, whereas Col-0 carries the deletion in the *FRI* promoter.

Figure 4

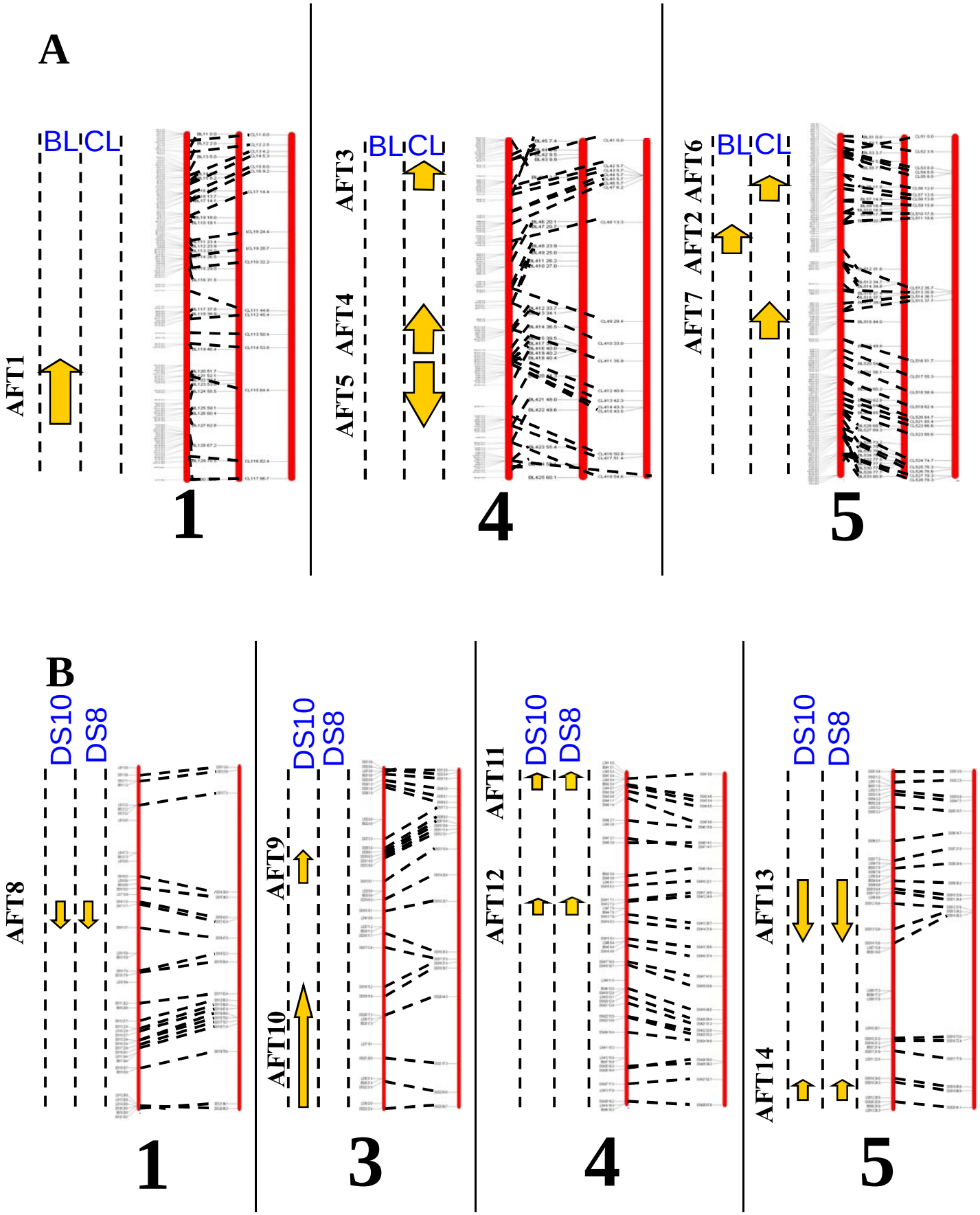
A
BL
CL
AFT1
1
AFT3
AFT4
AFT5
4
AFT2
AFT6
AFT7
5
B
DS10
DS8
AFT8
1
AFT9
AFT10
3
AFT11
AFT12
4
AFT13
AFT14
5

Figure 4. Positions of QTL that influence flowering time under various photoperiods.

A.QTL identified in the F2 of the cross between Bs-1 and Ler (BL) or between Cen-0 and L*er* (CL) grown under 14 h days. The left hand panel represents QTL located on chromosome 1, the central panel illustrates those located on chromosome 4 whereas the right hand panel represents those located on chromosome 5. Arrows between the vertical dotted lines represent QTL identified in each cross. Arrows that point upwards indicate QTL for which the L*er* allele causes early flowering. The three solid red lines in each panel represent the positions of markers located in the genomic sequence of Columbia (left), mapped in the BL cross (centre) and mapped in the CL cross (right). Dotted black lines connect markers in the sequence to their position in each genetic map. Long vertical black lines separate the data for each chromosome.
B.QTL identified in the F2 of the cross between Dijon and Sha grown under 10 h (DS10) or 8 h (DS8) days. Structure of each panel as for A, except data are shown for chromosomes 1, 3, 4 and 5. Arrows pointing upwards represent those QTL for which Sha alleles cause early flowering.

Figure 5

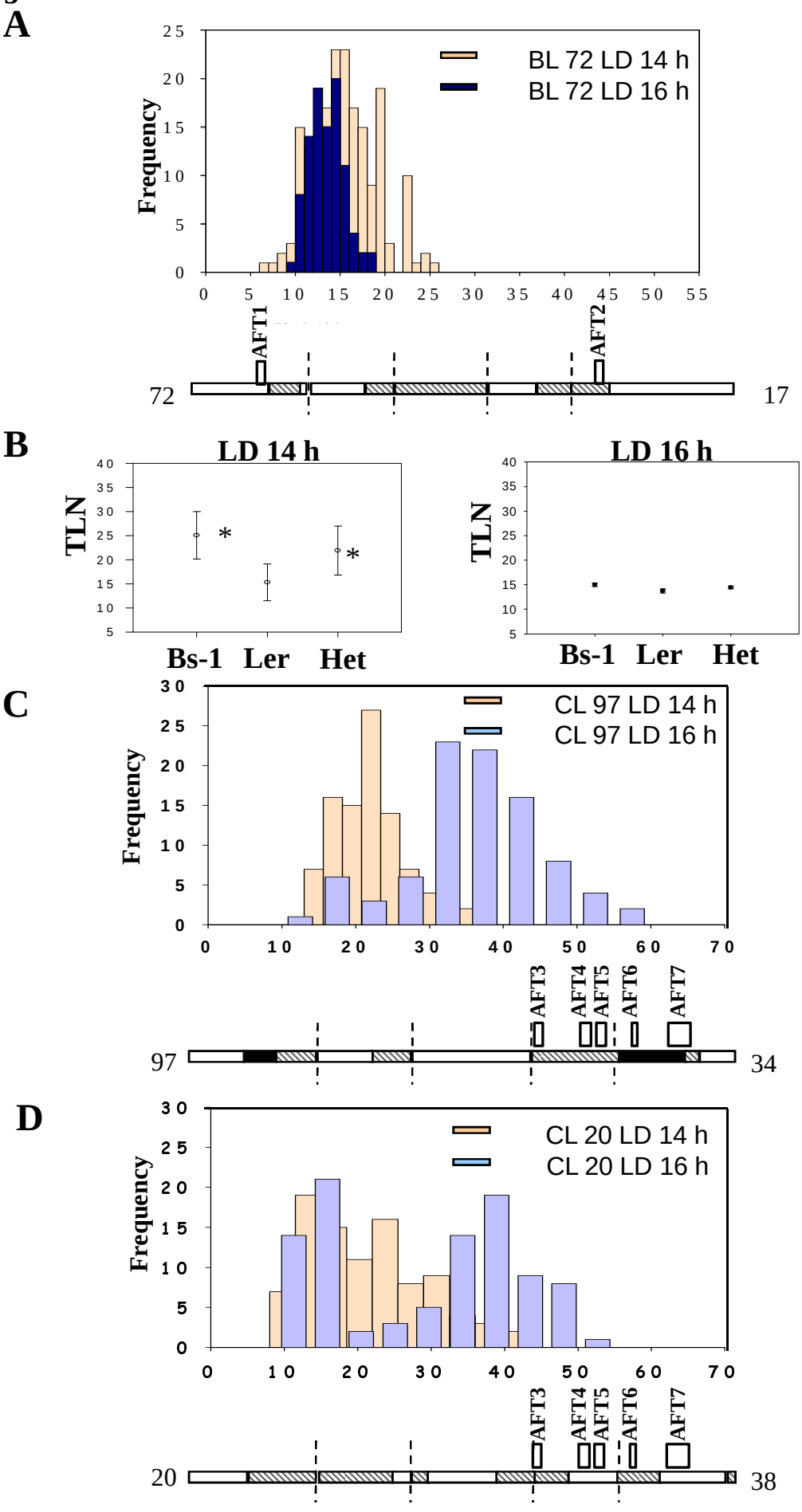
A
Frequency
BL 72 LD 14 h
BL 72 LD 16 h
AFT1
AFT2
72
17
B
LD 14 h
LD 16 h
TLN
Bs-1 Ler Het
C
CL 97 LD 14 h
CL 97 LD 16 h
AFT3
AFT4
AFT5
AFT6
AFT7
97
34
D
CL 20 LD 14 h
CL 20 LD 16 h
20
38

Figure 5. Analysis of flowering time in F3 populations used to validate QTL and test their interaction with day length.

The flowering times of plants in three F3 families grown under 14 h and 16 h days. In each histogram the horizontal axis represents flowering time as leaf number. The vertical axis illustrates number of plants. Blue indicates plants grown under 16 h days, whereas cream illustrates plants grown under 14 h days. The diagram below each histogram illustrates the genotype of the parental F2 plant. White illustrates L*er* homozygous alleles, black illustrates Bs-1 or Cen-0 homozygous alleles and hatched illustrates heterozygous alleles. The positions of the QTL mapped in the F2 are indicated by the rectangles above each diagram and the names of each QTL are indicated. The number to the left of the genotype indicates the number of the F2 plant whose genotype is illustrated, the number on the right indicates the leaf number at flowering of this F2 plant.

A. F3 family derived from F2 plant 72 in the Bs-1 cross to L*er*.

B. ANOVA analysis of the relationship between *ERD2* alleles and flowering time under 14 h or 16 h days in the F3 family shown in panel A. Left hand panel, analysis of data for plants grown under 14 h days. Right hand panel, analysis of data for plants grown under 16 h days. Y axis, flowering time represented as total leaf number. X axis, genotype at *ERD2*, marker used chr5_7.79 (Methods). Asterisk illustrates significantly different from Ler ($P<0.05$).

C. F3 family derived from F2 plant 97 in the Cen-0 cross to L*er*.

D. F3 family derived from F2 plant 20 in the Cen-0 cross to L*er*.

Figure 6

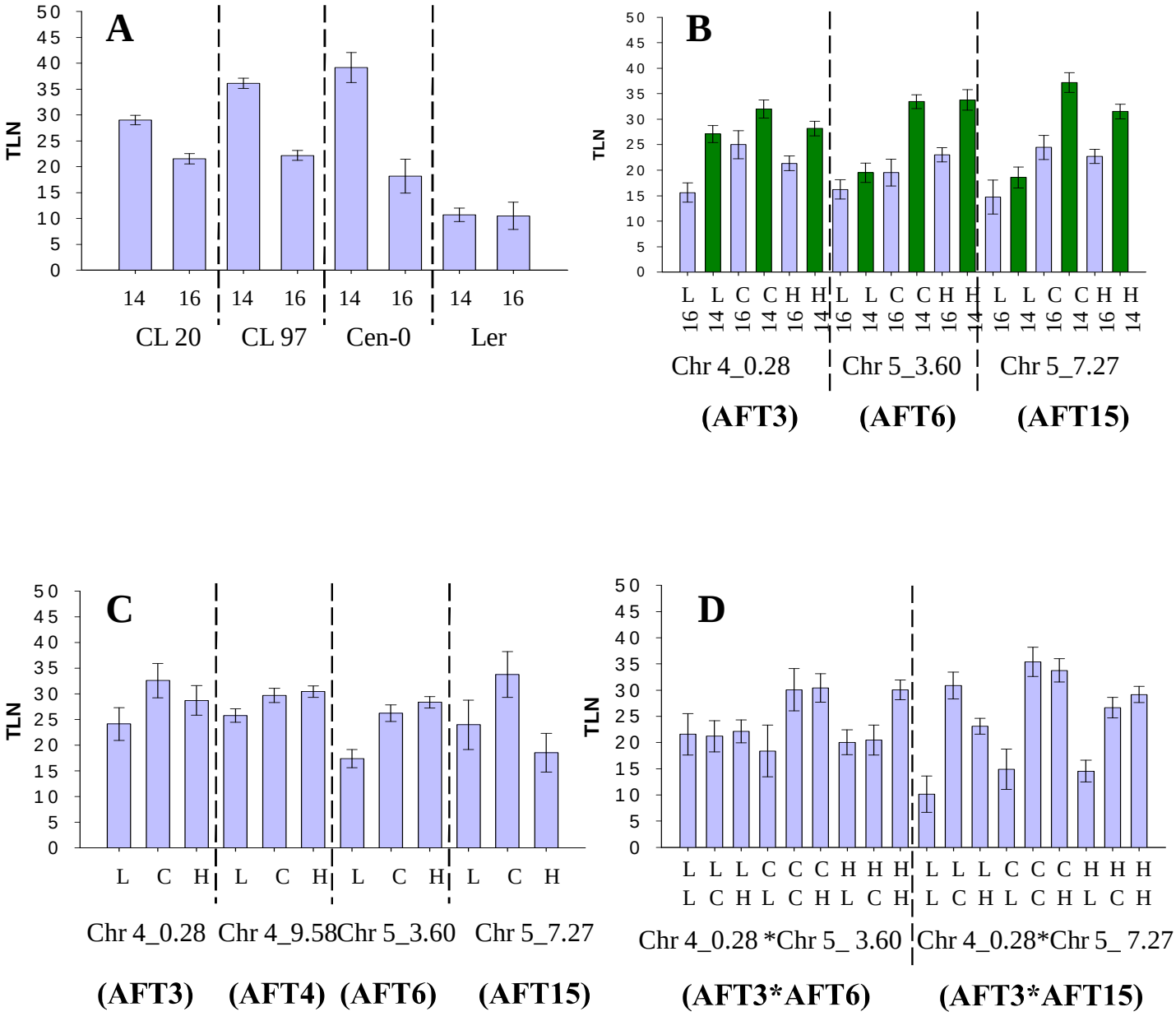
A
TLN
14 16 14 16 14 16 14 16
CL 20
CL 97
Cen-0
Ler
B
TLN
L L C C H H L L C C H H L L C C H H
16 14 16 14 16 14 16 14 16 14 16 14 16 14 16 14 16 14
Chr 4_0.28
Chr 5_3.60
Chr 5_7.27
(AFT3)
(AFT6)
(AFT15)
C
TLN
L C H L C H L C H L C H
Chr 4_0.28
Chr 4_9.58
Chr 5_3.60
Chr 5_7.27
(AFT3)
(AFT4)
(AFT6)
(AFT15)
D
TLN
L L L C C C H H H L L L C C C H H H
L C H L C H L C H L C H L C H L C H
Chr 4_0.28 *Chr 5_ 3.60
Chr 4_0.28*Chr 5_ 7.27
(AFT3*AFT6)
(AFT3*AFT15)

Figure 6. Validation of the QTL in the Cen-0 to L*er* cross using F3 familes 20 and 97, and analysis of interactions between QTL and day length

A. Mean flowering times of each family and parental controls under 14 h and 16 h days. Y axis flowering time represented as total leaf number. Error bars indicate S.D. X axis genotypes and day length. CL20, F3 family derived from F2 plant 20 of the Cen-0 x Ler cross. CL97, same nomenclature for F2 plant 97.

B. Univariate ANOVA detection of two-way interactions between markers and changing day length in the control of flowering time. Loci on top of chromosome 4 (4_0.28) and chromosome 5 (5_3.6 and 5_7.27) interact differently with the two day lengths. The Cen-0 allele at the chromosome 4 locus delays flowering under 16 h reducing the difference in flowering time between 14 h and 16 h days. The Cen-0 alleles at the chromosome 5 loci more strongly delay flowering under 14 h and enhance the difference in flowering time between the two day lengths. Data for families 20 and 97 were combined. Y axis flowering time represented by total leaf number. X axis genotype and day length. Upper symbols correspond to allelic variation at the corresponding loci (L: L*er* homozygous, C: Cen-0 homozygous H: heterozygous alleles) while lower numbers represent day length. The position of the markers used are indicated together with the name of the QTL mapped in the region. All markers are described in Methods.

C. Univariate ANOVA detection of flowering time effects independently of daylength. Markers on chromosome 4 (4_0.28 and 4_9.58) and 5 (5_3.6) detect loci that delay flowering when Cen-0 alleles are homozygous or heterozygous, whereas the Cen-0 allele at Chr5_7.27 delays flowering only when homozygous. Markers are described in Methods. Flowering time was scored under 14 h and 16 h for both families and all data combined for analysis. The genotype at each marker is shown L: L*er* homozygous, C: Cen-0 homozygous H: heterozygous alleles. The position of each marker is shown together with the name of the QTL mapped in the region.

D. Univariate ANOVA detection of two-way interactions between markers in determining flowering time. Allelic variation at a locus at the top of chromosome 4 (linked to marker 4_0.28) interacts with two other loci on chromosome 5 (linked to markers 5_3.6 and 5_7.27). Flowering time was scored under 14 h and 16 h for both families and all data combined for analysis. Upper symbols of x-axis correspond to alleles of the chromosome 4 locus while bottom symbols correspond to alleles of the chromosome 5 loci. L: L*er* homozygous, C: Cen-0 homozygous H: heterozygous alleles.

Figure 7

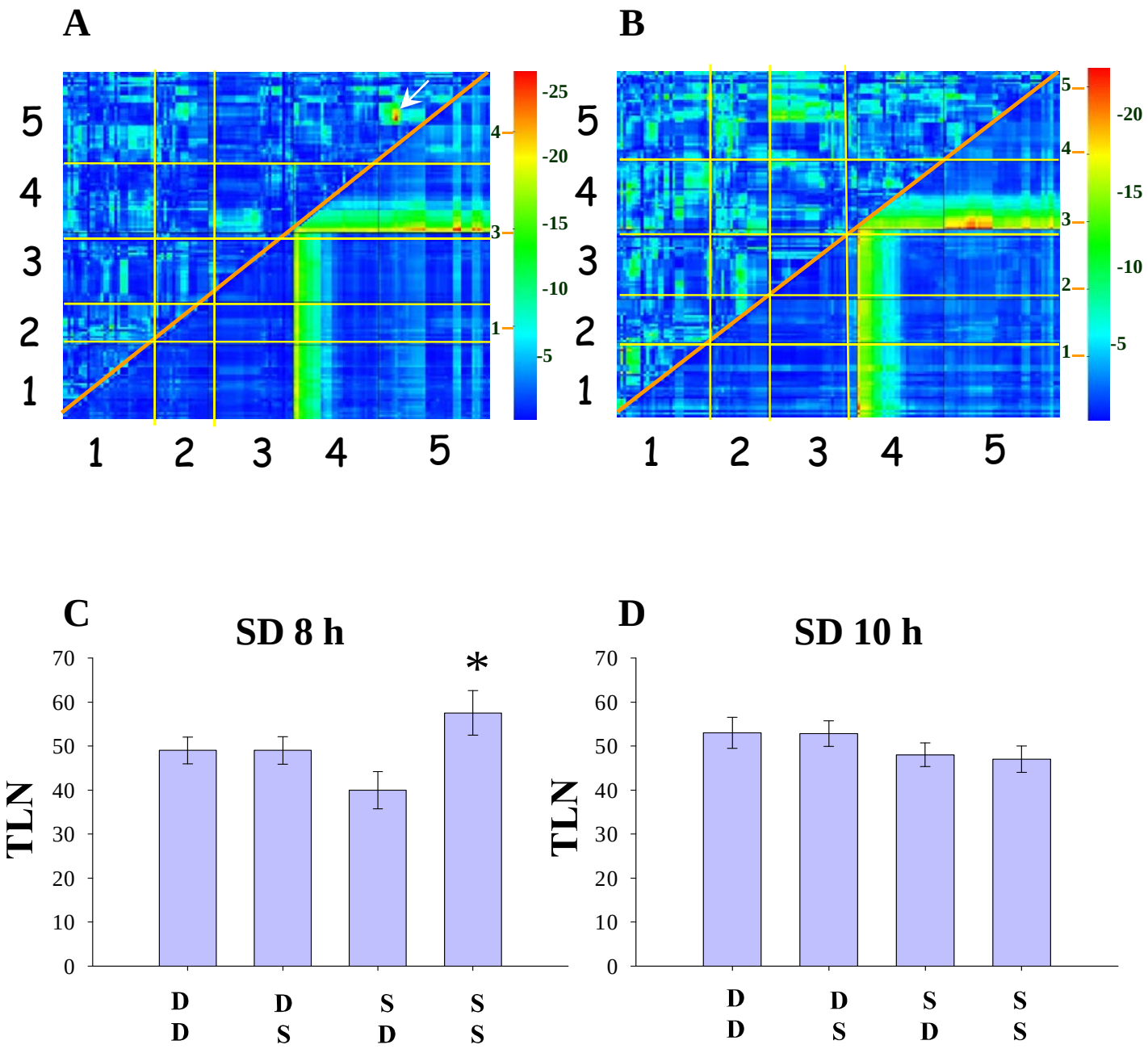
A
B
5
4
3
2
1
1 2 3 4 5
-25
-20
-15
-10
-5
C
SD 8 h
D
SD 10 h
TLN
70
60
50
40
30
20
10
0
*
D D
D S
S D
S S

Figure 7. Genome wide detection of epistatic interactions in the mapping population created by crossing Dijon-G to Sha

A. Heat map of two-dimensional genome scan for interactions under 8 h SD
B. Heat map of two dimensional genome scan for interactions under 10 h SD

In A and B the numerals on the horizontal and vertical axes illustrate the five chromosomes. The upper left triangle shows the epistasis LOD scores. The lower right triangle illustrates the joint LOD scores. The colour scale on the right of each panel indicates separate scales for the epistasis and joint LOD scores (on the left and right respectively).
The white arrow in A indicates the presence of an epistatic interaction, which is conditional as it occurs only under 8 h, between a locus on the top of Chrom 5 (horizontal axis) and one in the middle of the same chromosome (vertical axis).

C. Effect plot of the epistatic interaction under 8 h days marked in Figure 10A. Vertical axis represents flowering time as total leaf number. Horizontal axis represents genotype. Homozygous Dijon.G is indicated by D and homozygous Shakdara is indicated with S. Upper symbols represent the locus at the top of chromosome 5, while lower symbols represent the locus in the middle of chromosome 5. The presence of Shakdara alleles at the top of chromosome 5 makes a difference in flowering time of around 20 leaves if the locus in the middle of chromosome 5 is also Shakdara. Asterix indicates a significant difference ($P<0.05$).

D. The same analysis with the same loci as in C, but for plants grown under 10 h days. No significant effect of genotype on flowering time was observed.