Analysis of Natural Allelic Variation at Flowering Time Loci in the Landsberg *erecta* and Cape Verde Islands Ecotypes of *Arabidopsis thaliana*

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ABSTRACT

We have analyzed the flowering behavior of two Arabidopsis ecotypes: the laboratory strain Landsberg *erecta* (L*er*) and an ecotype from the tropical Cape Verde Islands (Cvi). They differ little in their flowering phenotypes and in their responses to photoperiod length changes and to vernalization treatment. However, segregating populations derived from crosses between them showed a much larger variation. An approach of *q*uantitative *t*rait *l*ocus (QTL) mapping in recombinant inbred lines (RILs) grown under three environments differing in day-length and/or vernalization treatment has been used to detect and locate flowering loci. Four main QTLs were identified, designated *e*arly *d*ay-length *i*nsensitive (*EDI*), *f*lowering *F*, *G*, and *H* (*FLF*, *FLG*, and *FLH*, respectively), to which most of the flowering behavior differences could be attributed. To further characterize the individual loci, near isogenic lines were constructed by introgressing Cvi early alleles of *EDI* and *FLH* into the L*er* genetic background. *EDI*-Cvi alleles produce earliness under both long- and short-day photoperiods, rendering L*er* plants almost day-length neutral. In addition, RILs were selected to analyze *FLF* and *FLG*. These loci interact epistatically and RILs carrying late alleles at *FLF* and *FLG* were very responsive to vernalization and showed an increased response to photoperiod length changes. The possible role of these loci for the control of flowering is discussed in the context of the current Arabidopsis model.

TO reproduce successfully, plants must flower under favorable environmental conditions, and therefore the time of flowering is likely to have an important adaptative significance (Murfet 1977). The transition from the vegetative to the reproductive phase is influenced by environmental factors such as photoperiod length and temperature, indicating that plants detect fluctuations in these parameters. The model plant *Arabidopsis thaliana* is being extensively used to dissect this developmental process genetically (reviewed in Martinez-Zapater *et al.* 1994; Coupland 1995; Amasino 1996; Koornneef *et al.* 1998b). A large number of mutations affecting flowering initiation, mostly in a quantitative manner, have been artificially generated. The genetical and physiological characterization of these mutations has shown that the regulation of this developmental switch in meristem fuction is complex. Several elements controlling the perception and transduction of light quality and day-length, such as the phytochromes A and B (Goto *et al.* 1991; Whitelam and Harberd 1997), the cryptochromes (Bagnall *et al.* 1996; Guo *et al.* 1998), and components of the circadian clock, like the *ELF3* and *LHY* genes (Hicks *et al.* 1996; Carré *et al.* 1997), have been identified. Other genes, like the *VRN* loci, seem to control the cold signaling involved in the flowering response to vernalization (Chandler *et al.* 1996). The environmental factors are thought to modulate the action of several endogenous signaling components such as gibberellins (Bagnall 1992; Wilson *et al.* 1992) and sucrose (Roldan *et al.* 1997). Furthermore, several loci that might be involved in the signal transduction pathways to flowering have been identified. Some of these have already been cloned and encode putative transcription factors such as *LD* (Lee *et al.* 1994b) and *CO* (Putterill *et al.* 1995) or an RNA binding protein like *FCA* (MacKnight *et al.* 1997), indicating that the regulation of flowering involves the sequential activation of genes.

In addition to induced mutations, genetic variation for flowering time has been found among natural populations (ecotypes) of Arabidopsis (Laibach 1951; reviewed in Napp-Zinn 1969,1987). Arabidopsis has a wide distribution throughout the Northern hemisphere (Rédei 1970) and differences found among ecotypes grown under the same environmental conditions are considered to reflect adaptations to different natural environments. Karlsson *et al.* (1993) analyzed 32 ecotypes in several environments with different photoperiod length and vernalization treatments, and they have shown that genotype by environment (G × E) interactions are very significant, which illustrates the diversity of responses found in nature. The identification of the loci responsible for this natural variation has been at-

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tempted for over 40 years (Napp-Zinn 1957; Van der Veen 1965). The advent of molecular markers and the development of genetic maps has facilitated the localization and characterization of some of the large effect alleles. Thus, the flowering behavior difference between very late ecotypes that respond to vernalization and the early ecotypes [classified under long-day (LD) light conditions] has been shown to involve two epistatic loci: the *FRI* locus mapped on the top of chromosome *4* (Clarke and Dean 1994; Lee *et al.* 1993; Burn *et al.* 1993) and *FLC* located on chromosome *5* (Koornneef *et al.* 1994; Lee *et al.* 1994a). Dominant alleles at both loci confer the lateness and vernalization requirement of late ecotypes. Moreover, these late alleles respond strongly to photoperiod changes, causing facultative LD strains to behave as "obligate" LD when they are not vernalized (Lee and Amasino 1995).

The identification of natural allelic variation of smaller effect has required the combination of genetic maps with statistical methods to locate quantitative trait loci (QTLs). Flowering QTL analyses have been performed in crosses between late and early ecotypes (Clarke *et al.* 1995; Kuittinen *et al.* 1997) as well as between early ones (Kowalski *et al.* 1994; Jansen *et al.* 1995; Mitchell-Olds 1996). The distinct number of QTLs detected in different crosses, varying between 2 and 12, does not fairly reflect the different number of segregating loci, but rather differences in the QTL detection power through the coverage of the corresponding molecular maps, the type and size of mapping population and the statistical approach. The combination of recombinant inbred line (RIL) populations and statistical methods that take into account the effect of multiple QTLs is particularly powerful [*m*ultiple *Q*TL *m*odel (MQM) mapping, Jansen and Stam 1994; or *c*omposite *i*nterval *m*apping (CIM), Zeng 1994], and allows the separation of linked flowering loci (Jansen *et al.* 1995; Kuittinen *et al.* 1997).

The analysis of QTL by environment (QTL × E) interactions in these populations enables the detection of loci causing the G × E interactions (Clarke *et al.* 1995; Jansen *et al.* 1995). Furthermore, epistasis has been detected among some QTLs (Clarke *et al.* 1995; Kuittinen *et al.* 1997). All of these studies have shown the wealth and complexity of the natural genetic variation that is available, but most of them were restricted to determine the number and approximate location of segregating loci. With the exception of the *FRI* and *FLC* loci no further analysis of this allelic variation has been reported. The genetical and physiological characterization of QTLs requires the introgression of the new alleles in a genetic background similar to the laboratory strains used to generate artificial mutations. By constructing near isogenic lines (NILs) comparisons of allele effects, allelism tests and fine mapping can be performed. Consequently, the loci at which the natural variation occurs might be determined, and eventually their characterization at the molecular level will be achieved.

In the present study we have analyzed the allelic variation affecting flowering time in two early ecotypes: the laboratory strain Landsberg *erecta* (L*er*) and an ecotype originating from the Cape Verde Islands (Cvi). A QTL mapping approach in RILs has been used to identify and locate the loci responsible for the flowering variation in three environments differing in photoperiod length and/or vernalization treatment. The four largest effect QTLs have been further characterized genetically and physiologically in relation to the flowering responses to day-length and vernalization. For that, NILs containing Cvi early alleles in a L*er* genetic background and several selected RILs carrying Cvi late alleles have been analyzed. The possible role of these loci for the control of flowering is discussed in the context of the current Arabidopsis model.

MATERIALS AND METHODS

Plant material: A set of 162 recombinant inbred lines (RILs) derived from crosses between the laboratory strain Landsberg *erecta* (L*er*) originating from Northern Europe (Rédei 1992) and the ecotype Cvi, from the tropical Cape Verde Islands (Lobin 1983) was used to identify flowering QTLs. These lines have been previously characterized for amplified fragment polymorphism (AFLP) and cleaved amplified polymorphic sequence (CAPS) markers (Alonso-Blanco *et al.* 1998).

Selected RILs were crossed with the following late flowering genotypes, in a predominantly L*er* genetic background: (i) the *FRI*-M73 introgression line containing the *FRI* locus from the genotype M73 (Koornneef *et al.* 1994) and (ii) the *ld* introgression line with the *ld-1* mutation originally generated in Columbia (Col) background (Koornneef *et al.* 1994). All crosses were performed using the L*er* background plants as female parents.

Construction of NILs: As a first step to constructing near isogenic lines (NILs), early flowering Cvi alleles were introgressed into L*er* genetic background by phenotypic selection under LD light conditions. Selection was basically performed to introgress nonrecessive Cvi alleles with relatively large effect. Three early flowering inbred lines were obtained with four backcross generations, and three final selfing generations. These lines were genotyped using 370 AFLP and CAPS markers. One line, referred to as S10, appeared to be completely L*er* for chromosomes *2*, *3*, and *4*, and contained Cvi introgressions at three genomic regions: top and bottom of chromosome *1* (genetic segments of ~25 and 20 cM, respectively), and bottom of chromosome *5* (~10 cM). This line was backcrossed to L*er* and an F_2 was genotyped for CAPS markers in the segregating regions. Two different F_2 plants for each of the three different homozygous introgression genotypes were selected as the final NILs. These lines are designated *EDI*-Cvi, *FLH*-Cvi, and *EDI*-Cvi,*FLH*-Cvi, because they contain Cvi alleles at the loci EDI and/or FLH, respectively. Lines containing Cvi alleles at the bottom of chromosome *1* were constructed but they were removed from the analysis because no significant effect on flowering could be detected.

Growth conditions: In experiments without vernalization treatment, seeds were sown in petri dishes on water-soaked filter paper and incubated for 3 days in a growth chamber at 24° with 16 hr light (for LD light conditions) or 8 hr light per day [for short-day (SD) light conditions]. The vernalization

treatment was given as described in Koornneef *et al.* (1994). For that, seeds were sown on Murashige-Skoog medium supplemented with 1% sucrose (MS-10). Subsequently, petri dishes were incubated in a cold room at 4° for 3 wk and then transferred to a climate chamber (24°, with 8 or 16 hr light per day) for 2 days before planting. LD experiments were performed in an air-conditioned green house supplemented with additional light from middle September until the beginning of April, providing a day-length of at least 14 hr. SD experiments were carried out in a single climate chamber with 8 hr light as described by Koornneef *et al.* (1995).

RIL evaluations: The complete set of RILs, parental lines, and reciprocal F_1 hybrids were evaluated for flowering under three different environmental conditions: LDs with and without vernalization treatment, and SD photoperiod conditions without vernalization. RILs were grown under both LD conditions, with and without vernalization treatment, in the same experiment and therefore the nonvernalized seeds were also sown on MS-10 medium. Twelve plants for each RIL and 24 for the parental lines and F_1 hybrids, were grown per treatment in a two-block design. Blocks were divided in rows of 12 plants, and the 6 plants of each genotype per block were grown in half a row, lines being completely randomized. For the SD experiment, 12 plants per line were grown in two pots sorted in a two-block design. Lines were completely randomized within the blocks.

NIL evaluations: The early flowering near isogenic lines, parents, and F_1 hybrids were evaluated under four different environments, namely LD and SD photoperiod conditions either with or without vernalization treatment. The vernalized and nonvernalized treated lines were grown together and therefore all seeds were sown on MS-10 medium. The design was basically similar to that described above for the RIL experiments, but 24 plants per genotype and treatment were grown.

Evaluations of F_1 hybrids and F_2 populations involving selected RILs, FRI-M73, and ld: The F_1 hybrids and F_2 populations involving the L*er*/Cvi RILs 40, 104, and 130, the parental lines, and the introgression lines *FRI*-M73 and *ld* were grown under LD condition experiments. For the F_1 hybrids, 24 plants per genotype were grown in a two-block design as described above for the RIL evaluations. This experiment was repeated and similar flowering data were collected on both occasions. Only data from the most complete experiment are presented. The six different derived F_2 populations were grown together in a single LD experiment. Each population consisted of 100–120 plants. Twenty-four plants of each parental line were grown in every experiment.

Measurement of flowering: The flowering phenotype was measured following two criteria: flowering time (FT) and total leaf number (TLN). FT was recorded as the number of days from the date of planting until the opening of the first flower. TLN was scored as the number of rosette leaves (RLN) plus the number of cauline leaves (CLN).

Statistical and QTL analyses: To map QTLs using the RIL population, a set of 99 markers covering most of the Arabidopsis genetic map was selected from the RIL L*er*/Cvi map (Alonso-Blanco *et al.* 1998). These markers spanned 482 cM, with an average distance between consecutive markers of 5 cM and the largest genetic distance being 12 cM. The phenotypic values recorded were transformed ($\log_{10}$) to improve the normality of the distributions and the values of 10 plants per RIL were used to calculate the line means for each of the four traits (FT, TLN, RLN and CLN) and the three environments (LD, SD, and LD with vernalization). The line means were used to perform the QTL analyses. Every trait was analyzed separately for each environment. All the statistical comparisons shown were based on the transformed data, but none of the conclusions was changed when using the original data. Therefore, results are presented in figures with the original scale. The computer program MapQTL (tm) version 3.0 (van Ooijen and Maliepaard 1996) was used to identify and locate QTLs linked to the molecular markers using both interval mapping and MQM mapping methods. In a first step, putative QTLs were identified using interval mapping. Thereafter, 1 marker at each putative QTL (between 3 and 9 depending on trait and environment) was selected as a cofactor and the selected markers were used as genetic background controls in the approximate MQM of MapQTL. To refine the mapping and to identify linked QTLs, different cofactor markers were tested around the putative QTL positions (van Ooijen and Maliepaard 1996), selecting as final cofactors the closest marker to each QTL, *i.e.*, those maximizing the LOD score. A LOD score of 2.4 was used as the significance threshold to declare the presence of a QTL, in both interval and MQM mapping, on the basis of thresholds previously obtained by simulation with Arabidopsis mapping data of another RIL population (Jansen *et al.* 1995; Van der Schaar *et al.* 1997). In the final MQM model the additive genetic effect was estimated at each QTL and 2-LOD support intervals were established as an ~95% confidence level (van Ooijen 1992).

For every trait and environment the contribution of each QTL to the phenotypic variance was estimated by analysis of variance components. For each analysis, the closest linked markers to the corresponding detected QTLs were used as random factors in ANOVA (the same markers used as cofactors in the MQM mapping with MapQTL). Because for all traits and environments the two markers corresponding to the QTLs located in the upper arm of chromosome *5* showed a highly significant interaction, and none of the remaining two-way interactions among the QTL markers was significant ($P > 0.005$), the interaction term between these two factors was included in the linear models. Thus, the contribution of this interaction was also estimated.

For FT and TLN a search for interactions between QTLs was performed using the computer program EPISTAT (Chase *et al.* 1997). Two-way interactions were searched among all pairwise combinations of the 99 markers using as significance threshold a log-likelihood ratio equivalent to $P < 0.005$. Ten thousand trials were used in the Monte Carlo simulations performed with EPISTAT to establish the statistical significance of the log-likelihood ratios of the interactions detected (Chase *et al.* 1997).

The overall G × E interaction was tested for each trait by a two-factor ANOVA using genotypes (RILs) and environments as classifying factors. For each trait and for each putative QTL, QTL × E interaction was tested by repeated measures ANOVA using the corresponding marker and the environment (repeated measurements of the RILs) as classifying factors ($P < 0.005$). The General Linear Model module of the statistical package SPSS version 7.5 was used for the ANOVAs and for the variance component analyses from the Type III sum of squares ANOVA.

Molecular markers: The introgresion lines containing early flowering Cvi alleles were genotyped using AFLP marker analysis, which was performed according to Vos *et al.* (1995). About 350 polymorphic bands amplified with the 14 primer combinations used previously to build the L*er*/Cvi molecular map (Alonso-Blanco *et al.* 1998) were scored for absence and presence. The genetic location of AFLP bands was therefore known previously and covered most of the genetic map.

CAPS and microsatellite markers previously mapped in the L*er*/Cvi RILs and/or the L*er*/Col RILs (Alonso-Blanco *et al.* 1998; *At*DB) were used to genotype genomic regions containing flowering loci, in the introgression lines and in the backcross-like and F_2 populations. CAPS markers were ana-

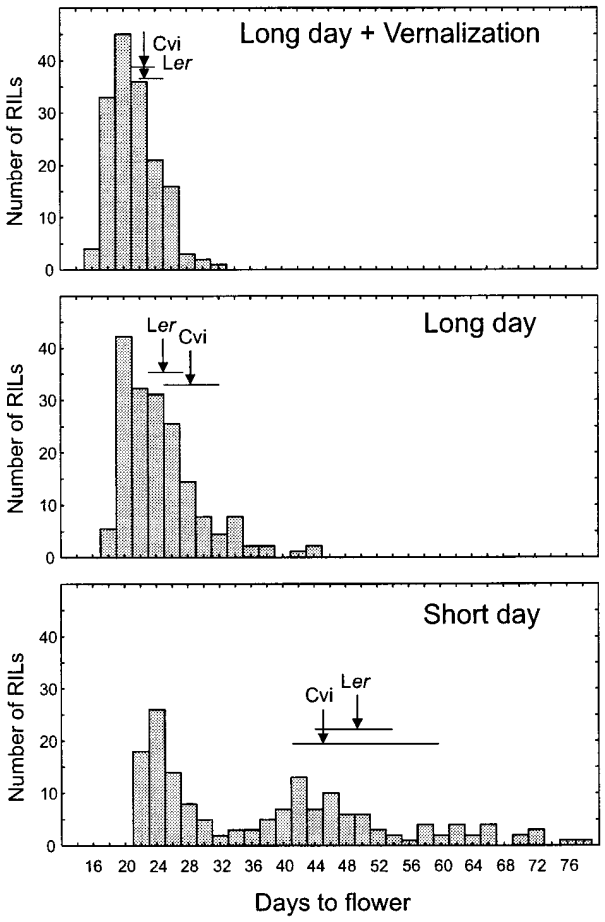

Figure 1.—Frequency distributions of flowering time means of the L*er*/Cvi RILs grown under three environments with different photoperiod length and/or vernalization treatment. Arrows correspond to the parental line means (20 plants per parent) and the horizontal bars represent their ranges of variation.

lyzed according to Konieczny and Ausubel (1993) and microsatellite markers according to Bell and Ecker (1994). The following PCR markers were used: PVV4, AXR1, PhyA and g2395 for the *e*arly *d*ay-length *i*nsensitive locus (*EDI*; this study); ANL2 and GA1 for the loci *FRI* and *ld* (Clarke and Dean 1994; Lee *et al.* 1994b), the two CAPS markers being linked at a genetic distance of 12 cM and flanking both flowering loci; nga158 and nga151 for the flowering *F* locus (*FLF*; this study); nga139 for the flowering *G* locus (*FLG*; this study); and g2368 for the flowering *H* locus (*FLH*; this study).

RESULTS

Flowering behavior of L*er*, Cvi, and the RI lines: The flowering phenotype of the parental ecotypes L*er* and Cvi, the reciprocal F_1 hybrids, and a set of 162 L*er*/Cvi RILs was evaluated under three different environmental conditions: LD photoperiod, with and without vernalization treatment, and SDs (Figure 1 and Table 1). Comparison of the flowering phenotypes between the SD and LD environments provided an estimate of the response to photoperiod length, and comparison of LD conditions with and without vernalization treatment provided an estimate of the vernalization response. Both ecotypes flower at rather similar times under LD conditions and can be considered as early flowering. The later flowering time of L*er* under SD indicates that Cvi responds less than L*er* to photoperiod length changes. In contrast, Cvi shows a more pronounced response to the vernalization treatment. The F_1 hybrids flower earlier or similar to the earliest parent (Table 1), although the FT means of the nonvernalized reciprocal F_1s grown under LD conditions were significantly different ($P < 0.001$; which was observed consistently and was even more pronounced in two other experiments not shown). Reciprocal differences have been observed previously in crosses between other Arabidopsis ecotypes suggesting a certain influence of maternal factors on flowering (Westerman 1970; Clarke and Dean 1994), but they have not been further analyzed.

Although the flowering differences between L*er* and Cvi are small, transgressive variation in both directions was observed in the RIL population under the three environments, indicating the presence in the two parental lines of alleles increasing and reducing flowering time (Figure 1; Table 1). A large amplification of the flowering range was observed in the RIL population when grown under SDs, and three major classes of flowering time appeared. In contrast, a reduction in the flowering range occurs when vernalizing the RILs (Figure 1; Table 1). The G × E interactions were highly significant ($P < 0.001$) when the flowering responses to vernalization or to photoperiod length were compared in the RIL population. This indicates the presence of allelic variation, whose effect is expressed differentially with the environments to control the different responses of the RILs to photoperiod length changes and to vernalization treatment.

The flowering phenotype was measured as FT and as TLN. As shown in Figure 2, both traits are tightly correlated in the RIL population and therefore both are expected to be mostly under the same genetic control as that observed previously with mutant genotypes (Koornneef *et al.* 1991).

Mapping loci that control the flowering behavior differences between L*er*, Cvi, and the RILs: To identify and locate the loci controlling the flowering behavior differences between L*er* and Cvi, the phenotypic values of the 162 RILs collected under the three environments were used for QTL analysis. Four flowering-related traits (FT, TLN, RLN and CLN) were analyzed separately for each environment (LD with and without vernalization treatment, and SD) using the MQM method of MapQTL (see materials and methods). The use of cofactors strongly improved the mapping accuracy of linked QTLs, which could not be separated with interval mapping. Figure 3 shows the QTL likelihood maps obtained for TLN under the three environmental conditions, indicating the genetic intervals where the putative QTLs

TABLE 1

Phenotypic values for flowering traits of the parental lines, reciprocal F_1 hybrids, and the RIL population grown in three different environments (10 plants were used per RIL; 20 plants for the rest of lines)

	Long day	Long day + vernalization	Short day
Flowering time (days)			
L*er*	25.0 ± 1.0	21.6 ± 1.0	49.4 ± 2.9
Cvi	28.0 ± 1.8	21.6 ± 0.9	45.4 ± 6.6
F1 L*er* × Cvi	25.1 ± 0.9	21.1 ± 0.9	—
F1 Cvi × L*er*	22.2 ± 1.9	19.8 ± 0.7	—
RIL mean	24.8 ± 5.1	21.4 ± 2.9	38.4 ± 14.7
Min.–max. RIL mean	18.1–44.8	16.3–32.0	21.1–78.0
RIL LSD	1.7	1.7	4.4
Total leaf number			
L*er*	10.4 ± 1.0	9.1 ± 1.0	32.5 ± 2.4
Cvi	11.6 ± 1.3	8.1 ± 1.0	27.0 ± 8.9
F1 L*er* × Cvi	10.9 ± 0.8	8.7 ± 0.6	—
F1 Cvi × L*er*	10.2 ± 1.7	9.5 ± 0.8	—
RIL mean	10.5 ± 5.2	8.6 ± 2.3	20.8 ± 14.0
Min.–max. RIL mean	5.7–32.1	5.4 ± 18.1	5.4–55.6
RIL LSD	1.9	0.6	4.1

Values are means ± SD. RIL mean, minimum and maximum, and least significant differences at $P \leq 0.01$ (LSD) for mean RIL comparisons are also shown.

were mapped. A total of 11 QTLs were detected along the five linkage groups. However, a clear distinction can be made between large effect (major) and small effect (minor) loci (Table 2). Allelic variation at four loci mapping, respectively, on top of chromosome *1*, and on top, middle and bottom of chromosome *5*, had a large effect on both TLN and FT (15% of the phenotypic variance could be attributed in at least one environment). We have named them *EDI*, and *FLF*, *FLG* and *FLH*, respectively. Cvi alleles produce earliness at *EDI* and *FLH* and lateness at *FLF* and *FLG*, this allelic variation accounting for nearly all the RIL phenotypic variance in the three environments and for the parental phenotypes (see Figure 4 in which *FLH* has not been included but its effect is in agreement with the phenotypes of L*er* and Cvi). The remaining seven QTLs had small additive effects (in general less than 5% of the variance could be attributed to each one) and were detected under only the LD with vernalization environment.

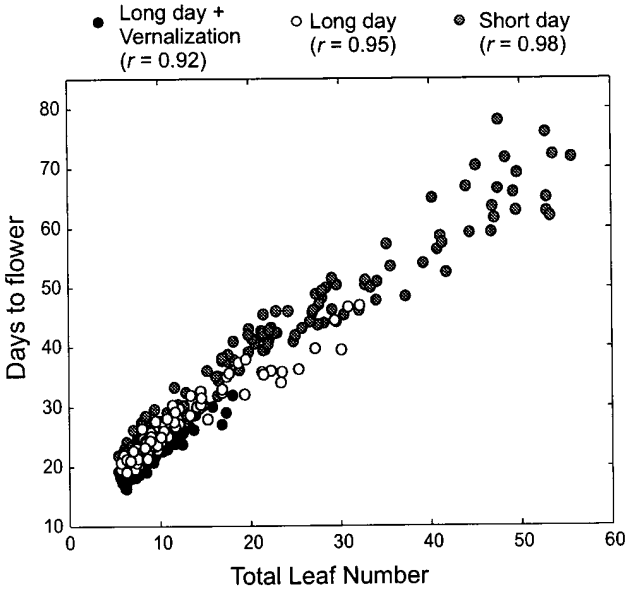

Figure 2.—Relationship between flowering time means and total leaf number means in the RIL population. *r*, Pearson correlation coefficient.

The QTLs detected for FT and TLN were in most cases mapped in the same intervals, indicating pleiotropy at these loci. The four main QTLs showed comparable contributions to the phenotypic variance of both traits (Table 2). However, two small effect QTLs on chromosome *2* appeared as significantly affecting FT but not TLN (markers FD.81 and DF.140C) and two others as significant for TLN but not for FT (BF.325L and HH.171C-Col). These putative QTLs were considered either significant or not on the basis of the 2.4 LOD threshold, but the likelihood values for both traits always increased around the corresponding positions (see, for instance, chromosome *2* in Figure 3). In agreement with this, one of the small QTLs affecting FT but not TLN (DF.140C) was significant for RLN. Only the QTL located at the bottom of chromosome *4* (around DHS1) appeared to affect CLN but not RLN and FT in the LD conditions. Therefore, most of the QTLs identified affected FT and TLN, although small differ-

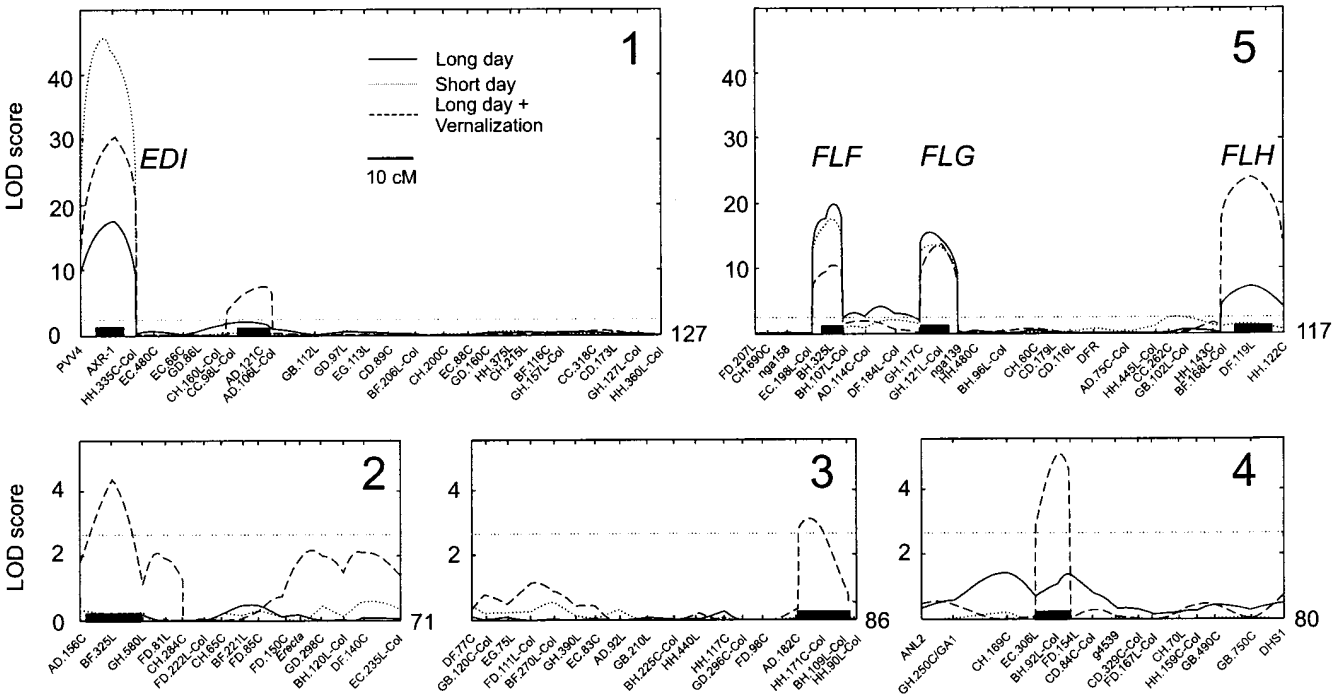

Figure 3.—QTL likelihood maps for total leaf number in the three different environments. The abscissas correspond to the genetic maps in cM, the linkage group number being indicated in the right upper corner of each map. Horizontal dashed line corresponds to the LOD score threshold of 2.4. Two LOD support intervals for the significant QTLs are shown as solid bars along abscissas. The largest effect QTLs have been named *EDI*, *FLF*, *FLG*, and *FLH*.

ences might exist in their relative effect on both traits, or in their relative contribution to RLN or CLN.

Epistasis between QTLs was analyzed by performing a genome-wide search for two-way interactions. The two major QTLs located on the top and middle of chromosome *5* (*FLF* and *FLG*) show very significant synergistic interaction for all traits and all environments ($P < 0.0001$; see Table 2 and Figure 4). These loci have relatively small additive effects individually (*FLF* shows practically no effect while *FLG* has small effect), and lateness in the three environmental conditions is mainly observed when both Cvi alleles are present. Interactions were also detected between these regions and markers at the bottom of chromosome *1*. However, because pseudolinkage is observed in the RIL population between markers at the bottom of chromosome *1* and the top of chromosome *5* (22% recombination frequency due to the lack of RILs of one of the recombinant genotypes) these interactions were rejected as not true epistasis. Another significant epistatic interaction was detected between the QTL linked to BF.325L on chromosome *2*, and the marker HH.440L on chromosome *3*, which had not been associated previous to flowering.

The significant interaction of the three environments with *EDI*, *FLF*, and *FLG* (Table 2) indicates that these are the loci responsible for the different flowering responses in the RILs. The QTL on chromosome *1* around AD.121C also showed significant QTL × E interaction but it was due to its genetic linkage with EDI, since it was not significant when analyzing the interaction of both QTLs simultaneously. The remaining QTLs did not show significant interactions with the environments and therefore were not considered as environment specific. The overall effect of the three major loci on the flowering responses was examined. The responses of each RIL were quantified as the difference in TLN between the LD and SD conditions (photoperiod length response) and between the LD and the LD with vernalization treatment (vernalization response). Figure 4 shows the TLN frequency distributions of the RILs classified according to these three loci under the three environments. Several conclusions can be summarized as follows:

(1) *EDI*, *FLF*, and *FLG* are the loci controlling the differences in photoperiod length response. RILs carrying late alleles at *EDI*, or at *FLF* and *FLG*, not only flower later but responded more to photoperiod length than the RILs carrying early alleles at these loci. An extremely low response was shown by the genotypes *EDI*-Cvi,*FLF*-L*er*,*FLG*-L*er*, which led to the naming of this locus as *e*arly, *d*ay-lenth *i*nsensitive (*EDI*). Therefore, to "abolish" the photoperiod response in the L*er*/Cvi RILs required early alleles at the three loci.

(2) *FLF* and *FLG* are the main loci controlling the differences in vernalization response. The *FLF* and *FLG* effects are much smaller under vernalization conditions than in normal LDs. In other words, the lateness observed under LDs in RILs carrying *FLF*-Cvi,*FLG*-Cvi alleles, is very much diminished by a 3-wk vernalization treatment. It is expected that a longer vernalization

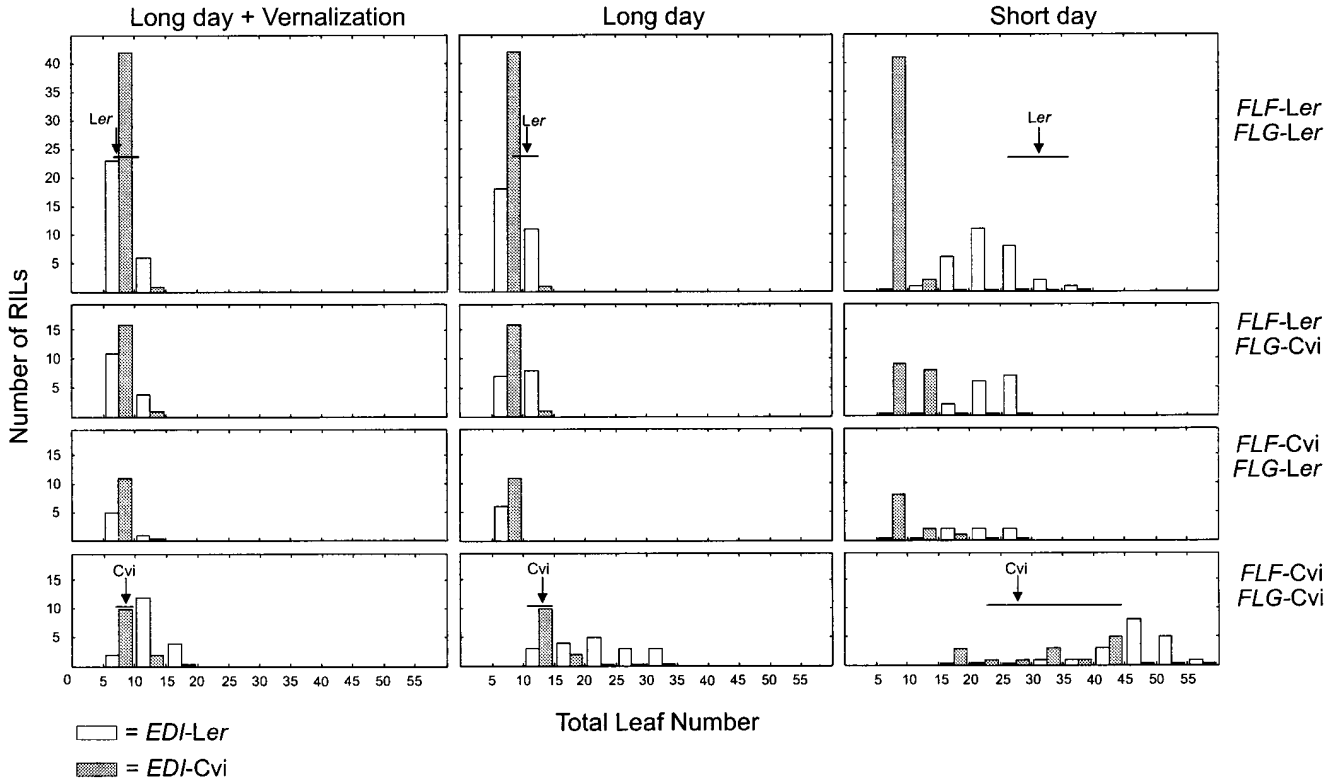

Figure 4.—Frequency distributions of TLN means of the RILs grown in three environments with different photoperiod length and/or vernalization treatment. The RILs have been classified according to their genotype at the closest markers to the loci *EDI*, *FLF* and *FLG*. The four distributions within each environment (vertical) correspond to the distributions of the four RIL classes obtained according to their genotypes at *FLF* and *FLG* (legend in the right part of the figure). Within each graph, the RILs are classified in relation to the genotype at *EDI* and the two distributions are overlaid. Arrows indicate the parental line means (20 plants per parent) and the horizontal bars represent their ranges of variation.

treatment would have reduced even more the effect of these loci, since saturation of the vernalization response in late-flowering responsive genotypes requires longer treatments (Lee and Amasino 1995).

Characterization of Cvi early alleles: the loci *EDI* and *FLH*: Near isogenic lines containing Cvi alleles at *EDI*, and/or *FLH* in a L*er* genetic background were constructed by phenotypic and genotypic selection (see Figure 5 and material and methods). The introgression line containing Cvi alleles only in the *EDI* region was used for further genetic mapping, analyzing an F_2 population under SD conditions where the flowering segregation could be classified qualitatively and behaved as monogenic. The location of *EDI* was narrowed to a segment smaller than 10 cM comparable to the 2 LOD support interval established in the QTL analysis (data not shown). The genetic length of the introgression segment in the monogenic *FLH*-Cvi NIL (10 cM approximately) confirmed the *FLH* position obtained in the MQM analysis of the RILs.

The NILs and the line S10, from which they were derived, were analyzed under LD and SD photoperiod conditions, with and without vernalization treatment (Figure 5). The Cvi allele of *EDI* was largely dominant, which was particularly manifest under SD conditions where L*er* plants flowered on average with 18.9 more leaves than the *EDI*-Cvi plants. *EDI*-Cvi plants flowered with almost the same TLN under both photoperiod length conditions, thus behaving as an almost day-length neutral genotype. These plants responded little to vernalization, showing a comparable response to L*er*. At the *FLH* locus, the slight earliness produced by the Cvi allele behaved on average codominantly. However, its effect was almost absent under LD conditions without vernalization, differing from the effect estimate obtained in the RIL population. This suggests *FLH* might be involved in some undetected epistatic interaction, or that some introgressed fragment not detected in the extensively genotyped lines affected flowering time. In contrast, under SDs, *FLH*-Cvi plants flower on average with 3.4 fewer leaves than L*er* plants, an effect not detected in the QTL analysis. These plants responded to photoperiod length in a comparable way to L*er*. However, it is remarkable that they responded more than L*er* to vernalization, an effect that was mainly observed under SD conditions. The allelic effects at *EDI* and *FLH* were basically additive because plants of the *EDI*-Cvi,*FLH*-Cvi line flowered earlier than the monogenic introgression lines in all environments.

Characterization of Cvi late alleles: the loci *FLF* and *FLG*: Three RILs were selected on the basis of their genotype as being L*er* at *EDI* and *FLH* (and as much as

TABLE 2

QTLs detected for four flowering related traits in three environments differing in photoperiod length and/or vernalization treatment

			Long-day + vernalization		Long-day		Short-day		
Trait	QTL	Map position	% of variance	Additive allele effect	% of variance	Additive allele effect	% of variance	Additive allele effect	QTL × E interaction
Flowering time			89.3		90.5		93.2		
	AXR-1 (*EDI*)	1–7.5	43.9	−3.5	27.5	−4.6	56.2	−18.1	*
	AD.121C	1–40.5	3.8	1.1	NS		NS		*
	BF.325L	2–7.2	NS		NS		NS		
	FD.81L	2–18.7	1.5	1.0	NS		NS		NS
	DF.140C	2–62.3	3.0	1.0	NS		NS		NS
	HH.171C-Col	3–78.4	NS		NS		NS		
	BH.92L-Col	4–30.2	0.8	−0.7	NS		NS		NS
	DHS1	4–80.2	NS		NS		NS		
	BH.325L (*FLF*)	5–15.7	2.9		5.1		3.9		*
	GH.121L-Col (*FLG*)	5–41.5	14.8		23.2		10.6		*
		FLF × *FLG*	4.0	3.2	31.4	8.6	22.5	21.7	
	DF.119L (*FLH*)	5–110	14.5	−2.0	3.3	−1.7	NS		NS
Total leaf number			89.5		90.7		91.9		
	AXR-1 (*EDI*)		33.6	−2.4	20.1	−3.8	54.7	−15.8	*
	AD.121C		4.9	1.1	NS		NS		*
	BF.325L		1.5	−1.0	NS		NS		NS
	FD.81L		NS		NS		NS		
	DF.140C		NS		NS		NS		
	HH.171C-Col		2.1	−0.6	NS		NS		NS
	BH.92L-Col		2.5	−0.8	NS		NS		NS
	DHS1		NS		NS		NS		
	BH.325L (*FLF*)		4.6		6.8		6.5		*
	GH.121L-Col (*FLG*)		13.8		18.2		11.0		*
		FLF × *FLG*	5.3	2.8	38.7	8.7	19.7	22.2	
	DF.119L (*FLH*)		21.2	−1.9	6.9	−2.4	NS		NS

(*continued*)

TABLE 2

Continued

Trait	QTL	Map position	Long-day + vernalization		Long-day		Short-day		QTL × E interaction
			% of variance	Additive allele effect	% of variance	Additive allele effect	% of variance	Additive allele effect	
Rosette leaf number			90.7		91.1		91.8		
	AXR-1 (*EDI*)		33.3	−1.8	19.4	−3.1	54.4	−12.6	*
	AD.121C		7.6	0.9	1.9	1.0	NS		*
	BF.325L		1.5	−0.7	NS		NS		NS
	FD.81L		NS		NS		NS		
	DF.140C		2.3	0.4	NS		NS		NS
	HH.171C-Col		NS		NS		NS		
	BH.92L-Col		3.8	−0.8	NS		NS		NS
	DHS1		NS		NS		NS		
	BH.325L (*FLF*)		3.5		4.9		4.9		*
	GH.121L-Col (*FLG*)		13.9		22.8		13.0		*
		FLF × *FLG*	3.6	2.0	36.7	7.1	19.5	18.3	
	DF.119L (*FLH*)		21.4	−1.5	5.4	−1.7	NS		NS
Cauline leaf number			72.1		79.7		86.6		
	AXR-1 (*EDI*)		26.7	−0.5	23.7	−0.9	53.4	−3.2	*
	AD.121C		NS		NS		NS		
	BF.325L		3.0	−0.2	NS		NS		NS
	FD.81L		NS		NS		NS		
	DF.140C		NS		NS		NS		
	HH.171C-Col		NS		NS		NS		
	BH.92L-Col		NS		NS		NS		
	DHS1		5.6	−0.2	3.0	−0.4	NS		NS
	BH.325L (*FLF*)		8		13.5		12.0		*
	GH.121L-Col (*FLG*)		4.7		11.9		3.7		*
		FLF × *FLG*	5.6	0.5	20.6	1.8	17.6	3.9	
	DF.119L (*FLH*)		18.5	−0.4	7.0	−0.5	NS		NS

The closest marker to each QTL is shown and its location is indicated by the linkage group number followed with its map position. Only QTLs with LOD score > 2.4 are reported. The QTLs with the largest effects have been designated as *EDI*, *FLF*, *FLG*, and *FLH* and are indicated between parentheses. For each trait and environment, the relative contribution of each QTL was estimated by analysis of variance components. Because of the epistatic interaction between *FLF* and *FLG* (see text) the relative contribution of their interaction was included in the model and it is shown (*FLF* × *FLG*). The additive allele effects of *FLF* and *FLG* have been added together. The additive allele effects are estimated as the mean differences between the two RIL genotypic groups carrying the Cvi and L*er* alleles (a positive value implies Cvi allele increasing the corresponding phenotypic value; a negative value, Cvi allele decreasing). Allele effects are shown in the original scale of measurement (days for flowering time and number of leafs for leaf numbers). For each trait, the QTLs interacting with the environments are indicated by * ($P < 0.005$). NS, not significant.

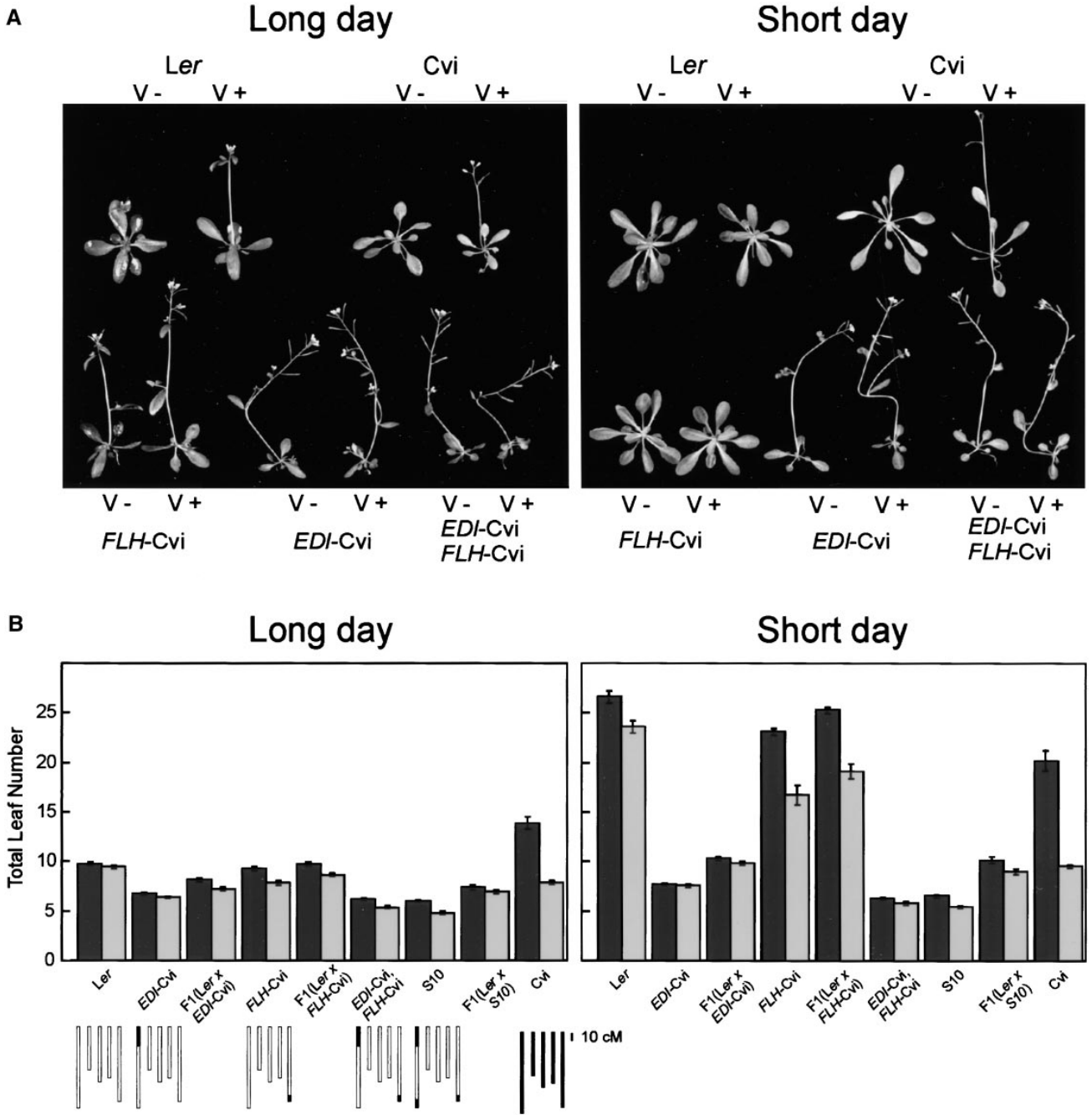

Figure 5.—(A) Phenotype of the parental lines L*er* and Cvi, and the introgression lines in L*er* genetic background containing Cvi alleles at *EDI* and/or *FLH*. Plants were grown in four different environmental conditions: LD photoperiod (left side); SD photoperiod (right side); V−, without vernalization treatment; V+, with vernalization treatment. Plants were photographed 25 days after planting. (B) Total leaf number of the parental lines L*er* and Cvi, the introgression lines in L*er* genetic background containing Cvi alleles at *EDI* and/or *FLH*, and the F_1 hybrids with L*er*. The line S10 from which the introgression lines were derived is also included (see materials and methods). Plants were grown in four different environmental conditions: LD photoperiod (left side); SD photoperiod (right side); without vernalization treatment (dark columns); with vernalization treatment (light columns). Graphical genotypes of the lines are shown in the left lower side, each of the five bars corresponding to one linkage group. Total leaf numbers are the mean of 20–24 plants and the standard errors are represented by error bars.

possible in the rest of the genome), but carrying Cvi alleles at *FLF* and/or *FLG*. RIL 130 was selected as genotype *FLF*-Cvi,*FLG*-Cvi, RIL 104 as *FLF*-Cvi,*FLG*-L*er*, and RIL 40 as *FLF*-L*er*,*FLG*-Cvi (the chromosome 5 regions of RILs 40 and 104 are not overlapping). To confirm the presence of two linked flowering loci we performed a reconstruction experiment, under LD conditions, to obtain the expected late flowering genotype when the homozygotes *FLF*-Cvi and *FLG*-Cvi are combined. For that, an F_1 hybrid between the genotypes *FLF*-Cvi (RIL

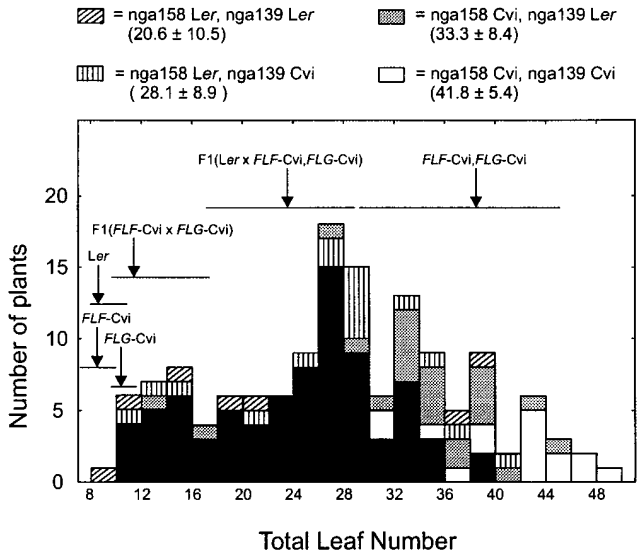

Figure 6.—Frequency distribution of TLN in the backcross-like population derived from the cross RIL *FLF*-Cvi,*FLG*-Cvi × F_1(RIL *FLF*-Cvi × RIL *FLG*-Cvi). Plants were grown under LD light conditions. The genotypes at markers closely linked to *FLF* (nga158) and *FLG* (nga139) were determined for 64 out of the 142 plants of the population (not filled columns). The symbols for genotypes at these markers in the segregating gametes are indicated in the upper part. The TLN mean ± standard deviation of the four genotypic classes are also shown. Arrows indicate the line means of the parents and some hybrids; the horizontal bars represent their ranges of variation.

104) and *FLG*-Cvi (RIL 40) (heterozygote in repulsion for both loci) was crossed with the line *FLF*-Cvi,*FLG*-Cvi (RIL 130) (Figure 6). This population was partially genotyped for the microsatellite markers nga158 and nga139, closely linked to the support intervals established, respectively, for *FLF* and *FLG* in the QTL analyses (Figure 3). Indeed, 10 out of the 13 latest plants of this population originated from recombinant gametes between both markers, thus confirming the presence of two flowering linked loci at a genetic distance of at least 15 cM. The flowering phenotypes of the different genotypic classes of this population and of the F_1 hybrids between these RILs and L*er* (Figures 6 and 7), indicate that late Cvi alleles at both *FLF* and *FLG* behave additively (codominantly); *i.e.*, their allelic effects are dosage dependent.

Another locus, *FLC*, at which natural allelic variation has been reported previously, maps in the region of *FLF* (Koornneef *et al.* 1994; Lee *et al.* 1994a). *FLC*-L*er* alleles are known to be early in relation to most other tested ecotypes, and late *FLC* alleles interact synergistically with late *FRI* alleles and with mutant alleles at the *LD* locus. The *FRI* locus maps on top of chromosome *4*, where no QTL was identified in the L*er*/Cvi material, and it is very closely linked to *LD* (Clarke and Dean 1994; Lee *et al.* 1994b). To determine whether *FLF* might be *FLC*, we studied the genetic interactions between *FLF* and *FLG* and the loci *FRI* and *ld*. We analyzed the flowering phenotype of F_1 hybrids and derived F_2 populations between the three selected RILs and the late flowering introgression lines in L*er* genetic background, *FRI*-M73 and *ld* (Figures 7 and 8). F_1 and F_2 populations were grown under LD conditions in different experiments and therefore they are comparable only indirectly, through the corresponding common controls. Transgression over the latest parent was observed in all F_2 populations indicating the effect of Cvi late alleles. The latest flowering plants of each F_2 population were genotyped for molecular markers closely linked to *FLF*, *FLG*, *FRI*, and *ld* (Figure 8; see material and methods). Thus, it was confirmed that the late flowering phenotype was due to the effects of *FLF*-Cvi and/or *FLG*-Cvi and not to interactions of *FRI*-M73 or *ld* with Cvi alleles in other genomic regions (either detected in the QTL analysis or not) that might be segregating. Taking together the flowering phenotype of the F_1 hybrids and of the latest F_2 plants, and the proportion of toward-lateness transgression in these populations, several conclusions can be summarized as follows:

(1) *FLG*-Cvi behaves additively with *ld* to produce lateness and shows a weak synergistic interaction with *FRI*-M73. The phenotypes of the corresponding F_1 hybrids and F_2 populations were in agreement, confirming that both *FLG*-Cvi and *FRI*-M73 are partly dominant and *ld* is recessive.

(2) *FLF*-Cvi behaves as a late allele of *FLC* in its synergistic interaction with *FRI*-M73, and with *ld*, although it must be a weaker allele than *FLC*-Sf2 or *FLC*-Col when compared with TLNs reported previously (Koornneef *et al.* 1994; Lee *et al.* 1994a). The phenotypes of the corresponding F_1 hybrids and F_2 populations were again in agreement with *FLF*-Cvi and *FRI*-M73 being partly dominant and *ld* recessive. Therefore, it is likely that *FLF* and *FLC* are the same locus.

DISCUSSION

In this article we have analyzed the flowering behavior of two early Arabidopsis ecotypes: the laboratory strain L*er* originating from Northern Europe (Rédei 1992) and the ecotype Cvi (Lobin 1983). They hardly differ in their flowering phenotype (measured as both TLN and FT) and in their responses to photoperiod length and vernalization treatment. However, segregating populations derived from crosses between these ecotypes show a much larger variation than that observed in other crosses between early ecotypes such as L*er* and Col (Jansen *et al.* 1995). The flowering behavior differences between the L*er*/Cvi lines can be mainly attributed to four loci referred to as *EDI*, *FLF*, *FLG*, and *FLH*. Cvi alleles at *EDI* and *FLH* produce earliness while at *FLF* and *FLG* Cvi alleles produce lateness, thus explaining the similar behavior of the parental lines and the transgression in the RILs. Another seven putative minor QTLs might contribute secondarily to these differences,

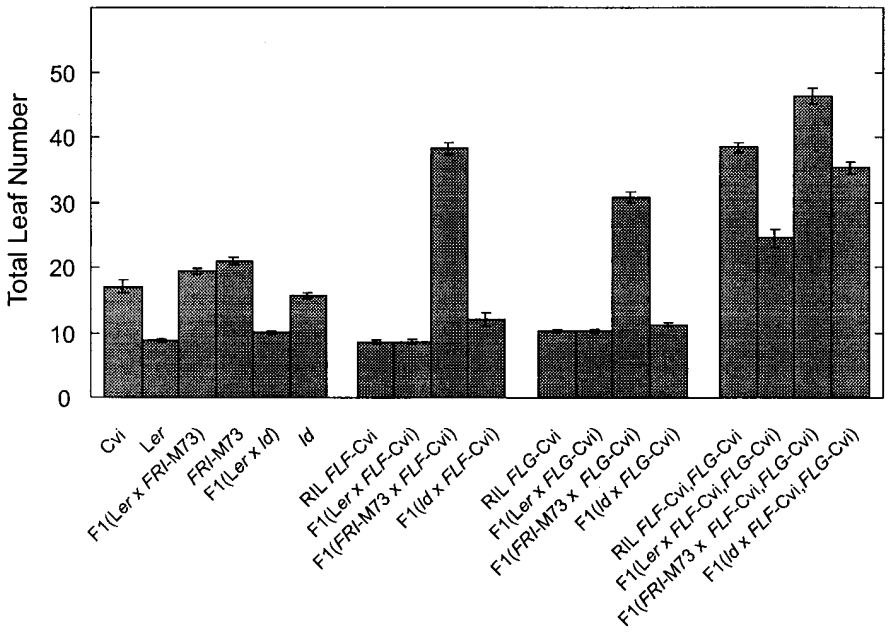

Figure 7.—TLN means and standard errors of F_1 hybrids involving L*er*, the three RILs selected as RIL *FLG*-Cvi, RIL *FLF*-Cvi, and RIL *FLF*-Cvi,*FLG*-Cvi, and the late flowering introgression lines *FRI*-M73 and *ld*. Plants were grown under LD light conditions.

but they were found only in the environment with the lowest phenotypic variation and further confirmation is necessary. This is, at least partly, due to the limitations for detecting minor QTLs in small populations where several QTLs with large effects are segregating, as seen, for instance, with the effect of *FLH*, which was not detected under SD conditions in the RIL population but was present in the L*er* genetic background NILs. Alleles with major effect at the loci *FRI* and *FLC* have appeared responsible previously for most flowering differences between several very late, vernalization-responsive ecotypes and early ones (classified according to their flowering behavior under long-day light conditions; Napp-Zinn 1969; Burn *et al.* 1993; Koornneef *et al.* 1994; Lee *et al.* 1994a; Clarke *et al.* 1995; Kuittinen *et al.* 1997; Sanda *et al.* 1997). It was shown before that large allelic effects can also be present in crosses between some early ecotypes (Van Der Veen 1965; Kowalski *et al.* 1994; this study). Furthermore, it can be predicted that strong effect alleles will probably segregate in crosses between late ecotypes, since some of them carry large effect late alleles with genetic behavior different than the allelic variation at *FRI* and *FLC* (Burn *et al.* 1993; C. Alonso-Blanco and M. Koornneef, unpublished results). Therefore, major effect mutations seem to contribute frequently to the natural flowering variation observed among Arabidopsis ecotypes, although how many loci are involved is still unknown.

Late alleles at two of the major loci identified in the L*er*/Cvi population, *FLF* and *FLG*, interact synergistically. A similar type of interaction has been previously shown to occur between natural late alleles at *FRI* and *FLC* (Koornneef *et al.* 1994; Lee *et al.* 1994a) and in addition, *FLF*-Cvi and *FLG*-Cvi interact synergistically with late alleles at *FRI*. Epistasis has also been detected in two previous crosses where it has been analyzed (Clarke *et al.* 1995; Kuittinen *et al.* 1997) and therefore, epistasis among natural alleles might account for an important proportion of the phenotypic variation, as shown among alleles of mutant loci (Koornneef *et al.* 1998a), and among induced and natural alleles (Sanda and Amasino 1996a,b).

The Cvi ecotype shows a slightly reduced response to photoperiod length changes and a more pronounced vernalization response than the L*er* ecotype. The three major loci, *EDI*, *FLF*, and *FLG*, control most of the response differences to photoperiod and vernalization, as shown by their strong QTL × E interactions. Early alleles at these loci not only reduced flowering time but also diminished the response to photoperiod length. In fact, as shown with the near isogenic line *EDI*-Cvi in L*er* genetic background, the combination of *EDI*-Cvi alleles with *FLF*-L*er*,*FLG*-L*er* is able to render Arabidopsis practically day-length neutral in its flowering behavior. On the other hand, *FLF*,*FLG* accounted for much of the vernalization response, the late-flowering effect of Cvi alleles being eliminated by a 3-wk vernalization treatment. In agreement with these results, the Cvi ecotype flowered at almost similar times under LD and SD conditions when vernalized; *i.e.*, Cvi eventually behaved as almost day-length neutral when the effect of *FLF*-Cvi,*FLG*-Cvi was physiologically removed by the vernalization treatment. In other Arabidopsis populations where QTL × E interactions have been analyzed, the largest effect QTLs also showed significant interaction (Clarke *et al.* 1995; Jansen *et al.* 1995). In addition, allelic variation at *FLC* and *FRI* is differentially expressed depending on the vernalization treatment (Koornneef *et al.* 1994; Lee *et al.* 1994a; Lee and Amasino 1995). Therefore, major effect loci controlling the flowering

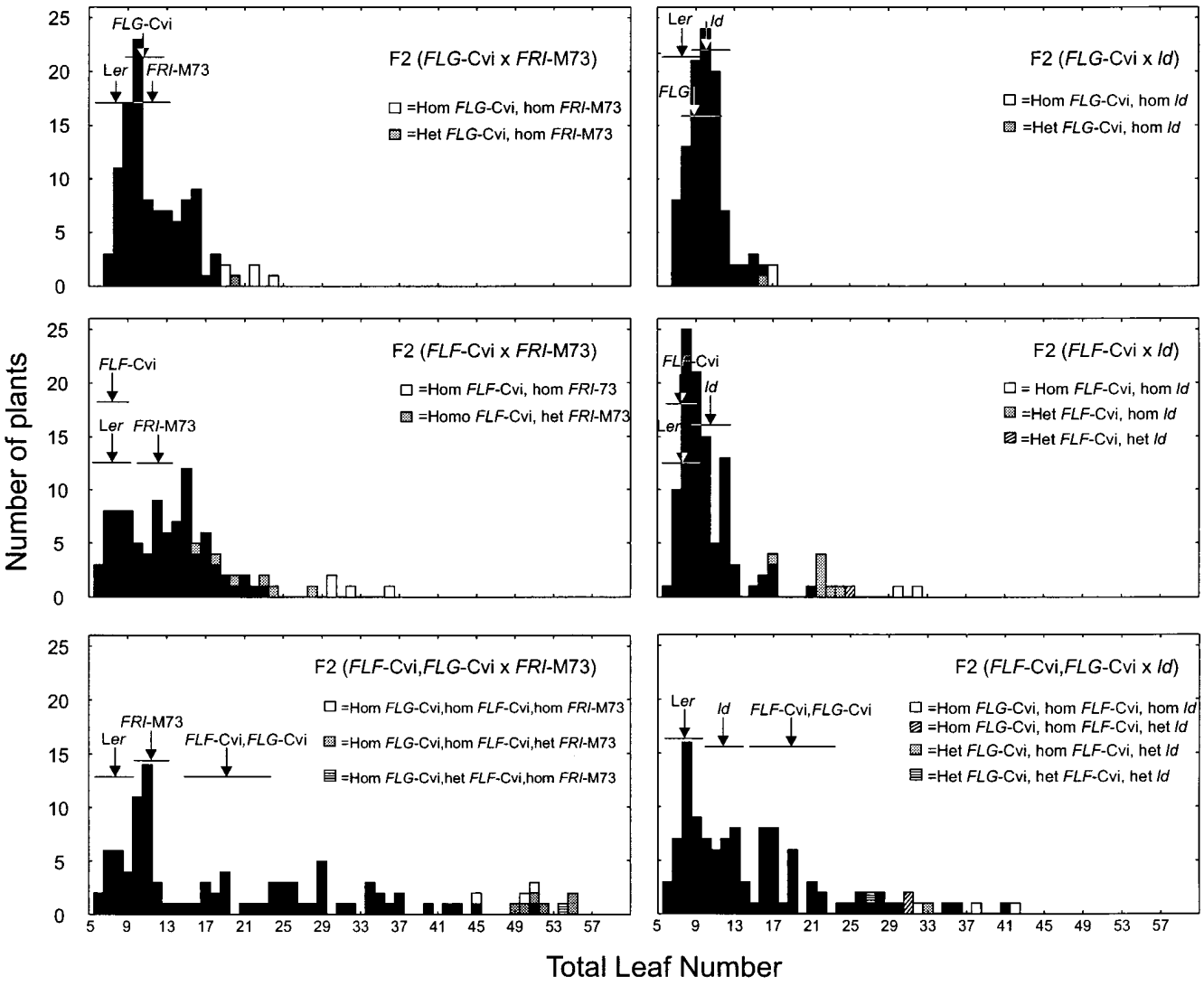

Figure 8.—Frequency distributions of TLN in F_2 populations derived from crosses between the three RILs selected as genotypes *FLG*-Cvi (upper part), *FLF*-Cvi (middle part) and *FLF*-Cvi,*FLG*-Cvi (lower part) and the two late flowering introgression lines *FRI*-M73 (left side) and *ld* (right side). Plants were grown under LD light conditions. The genotypes at molecular markers closely linked to *FLF*, *FLG*, *FRI*, and *ld* were determined for the latest flowering plants of each F_2 (not filled columns). In each graph, the cross involved and the symbols for the deduced genotypes at the corresponding flowering loci are indicated in the right upper corner. Arrows indicate the parental line means and the horizontal bars represent their ranges of variation. Hom, homozygote; het, heterozygote.

differences among Arabidopsis populations seem to interact with the environment, which might be an important factor for maintaining natural genetic variation (Mitchell-Olds 1995).

Many of the Arabidopsis flowering loci have been characterized genetically and physiologically in relation to the vernalization and photoperiod responses and a model for the control of the transition from the vegetative to the reproductive phase is being developed (reviewed in Martinez-Zapater *et al.* 1994; Coupland 1995; Amasino 1996; Koornneef *et al.* 1998b). Three major flowering promotion pathways with partly additive and partly redundant functions have been defined, namely, the autonomous (also called constitutive or endogenous), the photoperiod (or long-day), and the vernalization pathways. The vernalization flowering promotion is thought to act on certain targets common to the autonomous pathway, and it has been suggested they might involve gibberellin metabolism or sensitivity. Mutants of loci involved in the autonomous flowering promotion pathway (*fca*, *fld*, *fpa*, *fve*, *fy*, and *ld*) are more responsive to day-length and vernalization than the L*er* wild type, whereas mutations in the photoperiod pathway (*co*, *fd*, *fe*, *fha*, *ft*, *fwa*, and *gi*) are less responsive to day-length changes. The analysis of *EDI* suggests it might be involved in the photoperiod flowering promotion pathway given the lack of photoperiod response observed in the *EDI*-Cvi NIL in L*er* genetic background.

The dominance associated with the flowering behavior of the *EDI*-Cvi allele indicates that its product might promote flowering (or repress the vegetative phase) and this function would be reduced in the *EDI*-L*er* allele. The *EDI*-Cvi line flowers earlier than L*er* under both LD and SD conditions and somehow resembles the phenotype of transgenic lines carrying the *CO* gene under control of a 35S-promoter (Simon *et al.* 1996), suggesting that the photoperiod promotion pathway is overfunctioning under both day-length conditions, leading to the earliness and day-length insensitivity observed. In other words, *EDI* function could be controlled by photoperiod length when encoded by the L*er* allele but might be expressed independently of day-length when encoded by the Cvi allele.

Late alleles at the *FLF* and *FLG* loci are very responsive to vernalization and confer a more pronounced response to photoperiod length, as seen from the behavior of the *EDI*-L*er*,*FLF*-Cvi,*FLG*-Cvi RILs, features also shared with the late alleles at *FRI* and *FLC* (Lee and Amasino 1995). The similar physiological behavior of the *FLF*-Cvi and *FLG*-Cvi alleles and the late mutant alleles of the autonomous flowering promotion pathway suggest that they act in the same pathway. Given the codominance of these Cvi late alleles it is not possible to speculate whether they might promote or repress the flowering process. However, recessive early alleles at the *FLC* locus have been obtained by mutagenesis (Sanda and Amasino 1995) and candidate mutant alleles at the positions of *FRI* and *FLG* are not known, which might indicate that their gene products play a role in inhibiting the flowering process. The similar physiological and genetic behavior of late alleles at the *FLF* and *FLC* loci, together with their matching map positions, suggests they are probably the same locus. In addition, the similar genetic and physiological characteristics of *FLF*-Cvi and *FLG*-Cvi and the late alleles at *FRI*, and the fact that they are partly interchangeable in their genetic interactions, suggest they have certain redundant functions repressing flowering within the autonomous promotion pathway. As proposed by Lee *et al.* (1994a) and Sanda and Amasino (1996a), the effect of *FLC*/*FLF* would be counteracted by the autonomous pathway mutant genes, such as *LD*, given their epistatic interaction. Since *FLG*-Cvi does not interact with *ld*, *LD* might act directly on *FRI*, *FLC*/*FLF* but probably not on *FLG*.

Considering together the behavior of the three loci *EDI*, *FLF*, and *FLG*, it is worth noting that RILs *EDI*-Cvi,*FLF*-Cvi,*FLG*-Cvi respond to photoperiod length, in contrast to the *EDI*-Cvi NILs. Under the discussed model, in such genotypes the photoperiod pathway would be promoting flowering at the same level in both day-lengths. This photoperiod response would therefore imply that under SDs there is also an inhibition (or lack of promotion) of the autonomous flowering pathway, which would operate through *FLF*,*FLG*. In agreement with this, similar genetic behavior has been observed in double mutants between nonresponsive and responsive loci, which show mostly an intermediate, additive, day-length response (Koornneef *et al.* 1998a).

The allelic variation at the *FLH* locus has a rather mild effect on flowering, Cvi alleles responding like L*er* to day-length changes. The additive behavior of *EDI* and *FLH* together with the more pronounced response of *FLH*-Cvi alleles to a vernalization treatment, suggest that *FLH* might be involved in the autonomous flowering promotion pathway. However, opposite to *FLF*,*FLG* and to other vernalization responsive loci, at *FLH* it is the early allele which increases the response; *i.e.*, *FLH*-Cvi early alleles make L*er* more vernalization responsive. This might suggest its role in the control of the vernalization response.

Figure 9 shows a scheme of the current general model for the control of flowering initiation (Koornneef *et al.* 1998b), where the possible role of *EDI*, *FLF*, *FLG*, and *FLH* is indicated.

We have shown that the L*er*/Cvi allelic variation probably concerns loci involved in different flowering pathways. Comparison of map positions between identified QTLs and mutant loci might suggest putative candidate genes at which this natural variation occurs. Nevertheless, cautions must be taken given the inaccuracy of the QTL mapping and the large number of known mutant loci affecting flowering behavior, which appeared scattered over the five linkage groups (Koornneef *et al.* 1998b). Similar considerations must be taken when comparing QTL positions in different populations, and fine-scale mapping and allelism tests are required to determine the locus (or tightly linked loci) involved in the corresponding allelic variation. Two mutant loci, *LHY* and *FHA* (Simon and Coupland 1996; Koornneef *et al.* 1991), assigned to the photoperiod flowering promotion pathway, have been mapped on chromosome *1* in the *EDI* region, although preliminary fine mapping has left *LHY* out. Furthermore, a flowering QTL has been mapped on this genomic region in the cross between L*er* and Col ecotypes (Jansen *et al.* 1995). The *FLF* locus maps in the same region as *FLC*, and the

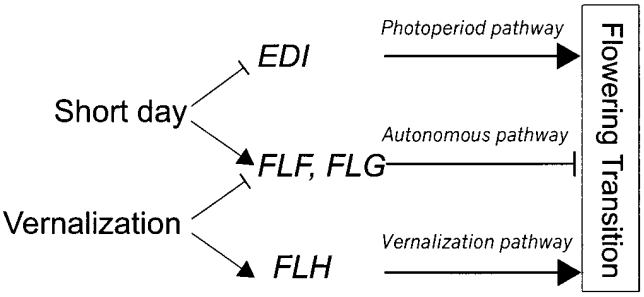

Figure 9.—A model for the control of flowering by *EDI*, *FLF*, *FLG*, and *FLH*. The pathways affected by them and the effect of the environmental conditions on these genes and pathways are indicated. →, promotive effect; ⊣, inhibitory effect.

similar physiological and genetic behavior of late alleles at both loci suggests they are probably the same locus. Several other loci have been identified by mutagenesis close to *FLC*, such as *FY* and *CO* (Koornneef *et al.* 1994). Allelic natural variation has been also assigned to this region in all crosses analyzed previously (Kowalski *et al.* 1994; Clarke and Dean 1994; Jansen *et al.* 1995; Kuittinen *et al.* 1997). It is unknown whether this ecotype variation belongs only to the *FLC* locus, which would indicate the existence of multiple alleles with different flowering effects, or to several closely linked loci. One natural variant, *ART*-Sy0, has been mapped in the region of *FLG* (Grbic and Bleecker 1996; Grbic and Gray 1997). *ART*-Sy0 gives rise to aerial rosettes when combined with late alleles at another locus on chromosome *4*, probably *FRI*. In addition, it seems to produce lateness in the absence of late alleles at the chromosome *4* locus, but taking into account the genetic linkage to *FLC* it is unclear whether this lateness involved *FLC* and whether late *FLC* alleles are also necessary to produce the aerial rosette phenotype. Nevertheless, aerial rosette phenotypes were not observed in late plants of the crosses *FLG*-Cvi and *FLF*-Cvi,*FLG*-Cvi with *FRI*-M73. No known mutant locus maps at the *FLH* position, although QTLs have been identified in this region in most crosses analyzed previously (Kowalski *et al.* 1994; Clarke *et al.* 1995; Mitchell-Olds 1996; Jansen *et al.* 1995).

It is expected that part of the natural variation will correspond to alleles of mutant flowering genes. Nevertheless, it is evident that the spectrum of natural genetic variation will be different from the spectrum obtained by artificial mutational analyses, partly due to the limitations of the small number of ecotypes used to generate mutants. Some alleles might not be functional in some ecotypes, as is likely to be the case for *FRI* alleles in many early ecotypes (Koornneef *et al.* 1994), and the epistatic interactions hamper the detection by mutagenesis of flowering loci, such as was shown previously with *LD* or *FLD*, which interact with *FLC* (Koornneef *et al.* 1994; Lee *et al.* 1994a; Sanda and Amasino 1996b), and therefore their mutations were detected in Col but not in L*er*. Further analysis of the loci identified in this study and in other populations is to come and the final identification of individual natural alleles at the molecular level is still needed. This will provide tools not only for the developmental and physiological dissection of the flowering process, but also for understanding the molecular mechanisms and the ecological and evolutionary significance of this quantitative natural variation.

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LITERATURE CITED

Alonso-Blanco, C., A. J. M. Peeters, M. Koornneef, C. Lister, C. Dean *et al.*, 1998 Development of an AFLP based linkage map of L*er*, Col and Cvi *Arabidopsis thaliana* ecotypes and construction of a L*er*/Cvi recombinant inbred line population. Plant J. **14:** 259–271.

Amasino, R. M., 1996 Control of flowering time in plants. Curr. Opin. Genet. Dev. **6:** 480–487.

*At*DB. Arabidopsis thaliana database. http://www-genome.stanford.edu.

Bagnall, D. J., 1992 The control of flowering of *Arabidopsis thaliana* by light, vernalisation and gibberellins. Aust. J. Plant Physiol. **19:** 401–409.

Bagnall, D. J., R. W. King and R. P. Hangarter, 1996 Blue-light promotion of flowering is absent in *hy4* mutants of *Arabidopsis*. Planta **200:** 278–280.

Bell, C. J., and J. R. Ecker, 1994 Assignement of 30 microsatellite loci to the linkage map of Arabidopsis. Genomics **19:** 137–144.

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

Carré, I. A., N. Ramsay, R. Schaffer and G. Coupland, 1997 Characterisation of a putative circadian clock gene in *Arabidopsis*. European Symposium on Photomorphogenesis, Leicester, UK.

Chandler, J., A. Wilson and C. Dean, 1996 *Arabidopsis* mutants showing an altered response to vernalization. Plant J. **10:** 637–644.

Clarke, J. H., and C. Dean, 1994 Mapping *FRI*, a locus controlling flowering time and vernalization response in *Arabidopsis thaliana*. Mol. Gen. Genet. **242:** 81–89.

Clarke, J. H., R. Mithen, J. K. M. Brown and C. Dean, 1995 QTL analysis of flowering time in *Arabidopsis thaliana*. Mol. Gen. Genet. **248:** 555–564.

Chase, K., F. R. Adler and K. G. Lark, 1997 Epistat: a computer program for identifying and testing interactions between pairs of quantitative trait loci. Theor. Appl. Genet. **94:** 724–730.

Coupland, G., 1995 Genetic and environmental control of flowering time in *Arabidopsis*. Trends Genet. **11:** 393–397.

Goto, N., T. Kumagai and M. Koornneef, 1991 Flowering responses to light-breaks in photomorphogenic mutants of *Arabidopsis thaliana*, a long-day plant. Physiol. Plant. **83:** 209–215.

Grbic, V., and A. B. Bleecker, 1996 An altered body plan is conferred on Arabidopsis plants carrying dominant alleles of two genes. Development **122:** 2395–2403.

Grbic, V., and J. Gray, 1997 Aerial rosette 1, *ART1*, is a new late flowering gene of *Arabidopsis thaliana*. 8th International Conference on Arabidopsis Research, Madison, WI.

Guo, H., H. Yang, T. Mockler and C. Lin, 1998 Regulation of flowering time by Arabidopsis photoreceptors. Science **279:** 1360–1363.

Hicks, K. A., A. J. Millar, I. A. Carré, D. E. Somers, M. Straume *et al.*, 1996 Conditional circadian dysfunction of the Arabidopsis early-flowering 3 mutant. Science **274:** 790–792.

Jansen, R. C., and P. Stam, 1994 High resolution of quantitative traits into multiple loci via interval mapping. Genetics **136:** 1447–1455.

Jansen, R. C., J. W. van Ooijen, P. Stam, C. Lister and C. Dean, 1995 Genotype by environment interaction in genetic mapping of multiple quantitative trait loci. Theor. Appl. Genet. **91:** 33–37.

Karlsson, B. H., G. R. Sills and J. Nienhuis, 1993 Effects of photoperiod and vernalization on the number of leaves at flowering in 32 *Arabidopsis thaliana* (*Brassicaceae*) ecotypes. Am. J. Bot. **80:** 646–648.

Konieczny, A., and F. M. Ausubel, 1993 A procedure for mapping Arabidopsis mutations using co-dominan ecotype specific PCR-based markers. Plant J. **4:** 403–410.

Koornneef, M., C. J. Hanhart and J. H. Van der Veen, 1991 A genetic and physiological analysis of late flowering mutants in *Arabidopsis thaliana*. Mol. Gen. Genet. **229:** 57–66.

Koornneef, M., H. Blankestijn-De Vries, C. J. Hanhart, W. Soppe and A. J. M. Peeters, 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.

Koornneef, M., C. J. Hanhart, P. van Loenen-Martinet and H. Blankestijn-De Vries, 1995 The effect of daylength on the

transition to flowering in phytochrome deficient, late-flowering and double mutants of *Arabidopsis thaliana*. Physiol. Plant. **95:** 260–266.

Koornneef, M., C. Alonso-Blanco, H. Blankestijn-De Vries, C. J. Hanhart and A. J. M. Peeters, 1998a Genetic interactions among late flowering mutants of Arabidopsis. Genetics **148:** 885–892.

Koornneef, M., C. Alonso-Blanco, A. J. M. Peeters and W. Soppe, 1998b Genetic control of flowering time in Arabidopsis. Annu. Rev. Plant Physiol. Plant Mol. Biol. **49:** 345–370.

Kowalski, S. P., T. H. Lan, K. A. Feldmann and A. H. Paterson, 1994 QTL mapping of naturally-occurring variation in flowering time of *Arabidopsis thaliana*. Mol. Gen. Genet. **245:** 548–555.

Kuittinen, H., M. J. Sillanpaa and O. Savolainen, 1997 Genetic basis of adaptation: flowering time in *Arabidopsis thaliana*. Theor. Appl. Genet. **95:** 573–583.

Laibach, F., 1951 Über sommer und winterannuelle rasse von *Arabidopsis thaliana* (L.) Heynh: ein beitrag zur atiologie der blutenbildung. Beitr. Biol. Pflantz. **28:** 173–210.

Lee, I., A. Bleecker and R. M. Amasino, 1993 Analysis of naturally occurring late flowering in *Arabidopsis thaliana*. Mol. Gen. Genet. **237:** 171–176.

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

Lee, I., M. J. Aukerman, K. N. Lohman, S. D. Michaels, L. M. Weaver *et al.*, 1994b Isolation of *LUMINIDEPENDENS*: a gene involved in the control of flowering time in *Arabidopsis*. Plant Cell **6:** 75–83.

Lee, I., and R. M. Amasino, 1995 Effect of vernalization, photoperiod, and light quality on the flowering phenotype of *Arabidopsis* plants containing the *FRIGIDA* gene. Plant Physiol. **108:** 157–162.

Lobin, W., 1983 The occurrence of *Arabidopsis thaliana* in the Cape Verde Islands. Arab. Inf. Serv. **20:** 119–123.

MacKnight, R., I. Bancroft, T. Page, C. Lister, R. Schmidt *et al.*, 1997 *FCA*, a gene controlling flowering time in *Arabidopsis*, encodes a protein containing RNA-binding domains. Cell **89:** 737–745.

Martinez-Zapater, J. M., G. Coupland, C. Dean and M. Koornneef, 1994 The transition to flowering in *Arabidopsis*, pp. 403–434 in *Arabidopsis*, edited by E. M. Meyerowitz and C. R. Somerville. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Mitchell-Olds, T., 1995 The molecular basis of quantitative genetic variations in natural populations. Trends Ecol. Evol. **10:** 324–328.

Mitchell-Olds, T., 1996 Genetic constraints of life-history evolution: quantitative-trait loci influencing growth and flowering in *Arabidopsis thaliana*. Evolution **50:** 140–145.

Murfet, I. C., 1977 Environmental interaction and the genetics of flowering. Annu. Rev. Plant Physiol. **28:** 253–278.

Napp-Zinn, K., 1957 Untersuchungen zur Genetik des Kältebedürfnisse bei *Arabidopsis thaliana* L. Heynh. Zeitschrift Induktion Abstammungs und Vererbungslehre **88:** 253–285.

Napp-Zinn, K., 1969 *Arabidopsis thaliana* (L.) Heynh, pp. 291–304 in *The Induction of Flowering: Some Case Histories*, edited by L. T. Evans. Macmillan, Melbourne.

Napp-Zinn, K., 1987 Vernalization: environmental and genetic regulation, pp. 123–132 in *Manipulation of Flowering*, edited by J. G. Atherton. Butterworths, London.

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

Rédei, G. P., 1970 *Arabidopsis thaliana* (L.) Heynh: a review of the genetics and biology. Bibliogr. Genet. **2:** 1–151.

Rédei, G. P., 1992 A heuristic glance to the past of Arabidopsis genetics, pp. 1–15 in *Methods in Arabidopsis Research*, edited by C. Koncz, N. Chua and J. Schell. World Scientific, Singapore.

Roldán, M., C. Gómez-Mena, L. Ruiz-García, M. Martín-Trillo, J. Salinas *et al.*, 1997 Effect of darkness and sugar availability to the apex on morphogenesis and flowering time of Arabidopsis. Flowering Newsletter **24:** 18–24.

Sanda, S. L., and R. Amasino, 1995 Genetic and physiological analysis of flowering time in the C24 line of *Arabidopsis thaliana*. Weeds World **2:** 2–8.

Sanda, S. L., and R. M. Amasino, 1996a Interaction of *FLC* and late-flowering mutations in *Arabidopsis thaliana*. Mol. Gen. Genet. **251:** 69–74.

Sanda, S. L., and R. M. Amasino, 1996b Ecotype-specific gene expression of a flowering mutant phenotype in *Arabidopsis thaliana*. Plant Physiol. **111:** 641–644.

Sanda, S. L., M. John and R. M. Amasino, 1997 Analysis of flowering time in ecotypes of *Arabidopsis thaliana*. J. Heredity **88:** 69–72.

Simon, R., and G. Coupland, 1996 *Arabidopsis* genes that regulate flowering time in response to day-length. Sem. Cell Dev. Biol. **7:** 419–425.

Simon, R., M. I. Igeno and G. Coupland, 1996 Activation of floral meristem identity genes in Arabidopsis. Nature **384:** 59–62.

Van der Schaar, W., C. Alonso-Blanco, K. Léon-Kloosterziel, R. C. Jansen, J. W. van Ooijen *et al.*, 1997 QTL analysis of seed dormancy in Arabidopsis using recombinant inbred lines and MQM mapping. Heredity **79:** 190–200.

Van der Veen, J. H., 1965 Genes for late flowering in *Arabidopsis thaliana*. pp. 62–71 in *Arabidopsis Research, Proceedings of the Göttingen Symposium*, edited by G. Röbbelen. Wasmund, Gelschenkirchen, Germany.

Van Ooijen, J. W., 1992 Accuracy of mapping quantitative trait loci in autogamous species. Theor. Appl. Genet. **84:** 803–811.

Van Ooijen, J. W., and C. Maliepaard, 1996 MapQTL (tm) version 3.0: Software for the calculation of QTL positions on genetic maps. CPRO-DLO, Wageningen, Netherlands.

Van Ooijen, J. W., and C. Maliepaard, 1996 MapQTL (tm) version 3.0: software for the calculation of QTL positions on genetic maps. Plant Genome IV Abstracts.

Vos, P., R. Hogers, M. Bleeker, M. Reijans, T. Van de Lee *et al.*, 1995 AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res. **23:** 4407–4414.

Westerman, J. M., 1970 Genotype-environment interaction and developmental regulation in *Arabidopsis thaliana*. Heredity **25:** 93–106.

Wilson, R. N., J. W. Heckman and C. R. Somerville, 1992 Gibberellin is required for flowering but not for senescence in *Arabidopsis thaliana* under short days. Plant Physiol. **100:** 403–408.

Whitelam, G. C., and N. P. Harberd, 1997 Action and function of phytochrome family members revealed through the study of mutant and transgenic plants. Plant Cell Environ. **17:** 615–625.

Zeng, Z-B., 1994 Precision mapping of quantitative trait loci. Genetics **136:** 1457–1468.

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