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QTL analysis of flowering time in *Arabidopsis thaliana*

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Abstract Quantitative trait loci (QTL) analyses based on restriction fragment length polymorphism maps have been used to resolve the genetic control of flowering time in a cross between two *Arabidopsis thaliana* ecotypes H51 and Landsberg *erecta*, differing widely in flowering time. Five quantitative trait loci affecting flowering time were identified in this cross (*RLN1-5*), four of which are located in regions containing mutations or loci previously identified as conferring a late-flowering phenotype. One of these loci is coincident with the *FRI* locus identified as the major determinant for late flowering and vernalization responsiveness in the *Arabidopsis* ecotype Stockholm. *RLN5*, which maps to the lower half of chromosome five (between markers mi69 and m233), only affected flowering time significantly under short day conditions following a vernalization period. The late-flowering phenotype of H51 compared to Landsberg *erecta* was due to alleles conferring late flowering at only two of the five loci. At the three other loci, H51 possessed alleles conferring early flowering in comparison to those of Landsberg *erecta*. Combinations of alleles conferring early and late flowering from both parents accounted for the transgressive segregation of flowering time observed within the F_2 population. Three QTL, *RLN1*, *RLN2* and *RLN3* displayed significant genotype-by-environment interactions for flowering time. A significant interaction between alleles at *RLN3* and *RLN4* was detected.

Key words Flowering time · Vernalization · Quantitative trait loci · *Arabidopsis* · RFLP

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Introduction

Extensive natural variation for flowering time and the ease with which mutations may be produced has meant that *Arabidopsis thaliana* (L.) Heynh. (Arabidopsis) has been used as a model for the study of floral induction for over 40 years (Atherton 1987). Recently, Arabidopsis has received renewed attention due to its suitability for molecular genetic analysis and gene cloning (Meyerowitz 1987). Studies on flowering in Arabidopsis have adopted two approaches. First, flowering time has been studied in progeny from crosses between different ecotypes (Napp-Zinn 1969). The number of loci involved in regulating flowering time has been shown to depend upon both the ecotype and the environment in which the plants are grown (Dierks 1958; Härer 1950; Seyffert 1960). Genetic analysis indicated that four or five loci were responsible for the difference in flowering time between the late-flowering, vernalization-responsive ecotype Stockholm (St) and the early flowering Limburg-5 (Li-5) (Napp-Zinn 1957). The late-flowering phenotypes of two inbred lines, H51 and H55 *früh* developed from an Li5 × St cross, were controlled by single loci (Napp-Zinn 1979). H51 was homozygous for the dominant St allele at the *FRIGIDA* (*FRI*) locus, while H55 *früh* was homozygous for the recessive St allele at the *KRYOPHILA* (*KRY*) locus. The St alleles at both loci conferred late flowering. The delayed

flowering time of both lines could be reversed by vernalization (Napp-Zinn 1979). Plants homozygous for the St allele at a third locus, *JUVINALIS* (*JUV*) flowered early.

A locus, termed *FLA*, that determines most of the flowering time differences between the ecotypes San Feliu or Leiden and Columbia (Col) has recently been mapped relative to RFLP markers to chromosome 4 (Lee et al. 1993). Another locus with a similar function, mapping to a similar position, has also been identified in the ecotypes Pitztal and Innsbruck (Burn et al. 1993). Clarke and Dean (1994) also mapped the *FRI* locus to a similar position on chromosome 4. The coincidence of the map positions of all these loci, suggest that they are allelic. If this is the case, then the majority of the flowering time differences found, at least between late- and early-flowering ecotypes, could be attributed to the segregation of alleles at this one locus. The late-flowering phenotype conferred by alleles at the *FRI* locus was modified towards early flowering by Landsberg *erecta* alleles at an unknown number of loci (Clarke and Dean 1994).

The second approach to the genetic analysis of flowering in Arabidopsis has used induced mutations. Over 50 mutations that confer a later-flowering phenotype have been identified. In a study by Koornneef et al. (1991), 42 independent mutants were found to represent mutations at 11 loci which were distributed on all chromosomes, apart from chromosome 3. The mutants were classified according to their differential response to environmental factors and subdivided into epistatic groups. It has not been established whether the loci identified through mutagenesis correspond to those regulating the flowering time in different ecotypes.

The development of restriction fragment length polymorphism (RFLP) mapping enables quantitative trait loci (QTL), which are not amenable to Mendelian analysis, to be resolved in segregating populations (Paterson et al. 1988). Furthermore, QTL analyses have been extended to resolve genotype × environment (G × E) interactions by comparing QTL maps derived from the analysis of the same population grown in a range of environments (Stuber et al. 1992). In this manner, it has been possible to attribute G × E interactions to single QTL. These techniques are ideally suited to the analysis of flowering time in Arabidopsis where extensive RFLP maps are available. Comparison of map positions of QTL with those of induced mutations will also help integrate all the information on loci controlling flowering time in Arabidopsis. The first QTL analysis of flowering time in Arabidopsis has recently been reported (Kowalski et al. 1994). Two independently segregating loci that affected days to first flowering were mapped to chromosome 5. These QTL appear also to influence node number, leaf length at flowering and leaf length at 35 days.

In the present paper, we identified and mapped loci segregating in a cross between the late-flowering, vernalization responsive line, H51 and the early-flowering line Landsberg *erecta* (Ler), and examined G × E interactions for flowering time in response to photoperiod and vernalization.

Materials and methods

Plant material and growth conditions

The experimental material was developed by crossing the Arabidopsis inbred line H51 (Napp-Zinn 1957) with Ler (Redei 1992). These lines exhibited significant differences in flowering time when grown in a range of environments, and displayed differential responses to vernalization in an environment-dependent manner (Table 1). The F_1 plants from this cross were selfed and 50 F_2 plants grown. Each F_2 was selfed to produce an F_3 family. Approximately 15 F_3 plants were bulk-harvested for DNA isolation to infer the genotype of the F_2 parent, and the flowering time and leaf number of 30 plants were scored after growth in controlled environment chambers.

Genotypes at 31 RFLP loci were determined using methods previously described (Clarke and Dean 1994). The 31 RFLP markers used are shown in Fig. 1.

Phenotypes were evaluated for the 50 F_3 families, the parents and the F_1 in each of three controlled environments in Sanyo Gallenkamp walk-in controlled environment rooms at relative humidity (RH) 75% ±2.5%, temperature 20°C ±1°C, with or without vernalization:

1. *Continuous light* (*CD*): 24 h photoperiod from 400 W, Wotan, metal halide power star lamps giving photosynthetically active radiation (PAR) of 134.7 μmol photons m^2per s and a red: far-red light ratio (R: FR) of 2.3.
2. *Extended short day* (*ESD*): 10 h photoperiod from 100 W tungsten lamps giving PAR of 92.9 μmol photons/m^2per s; R: FR of 1.49; 8 h photoperiod of PAR of 14.27 μmol photons/m^2 per s and R: FR 0.66.
3. *Short day* (*SD*): 10 h photoperiod of PAR of 113.7 μmol photons/m^2 and R: FR 2.41.

Vernalization was provided by a Sanyo-Gallenkamp CE with Phillips TLD58W cool white fluorescent lamps, 8 h photoperiod of PAR of 9.49 μmol photons $m^{-2}s^{-1}$, R: FR 3.9, 75% ±2.5% RH, 5°C ±1°C.

Fluence rates and spectral distributions were measured with a LI–1800 spectroradiometer (Li-Cor, Lincoln, Neb., USA).

Seeds were sown into 7.5 cm pots containing a 1.5:1:1 (by volume) soil mix of John Innes No. 1 compost: vermiculite: grit. Pots were covered with clingfilm to maintain a high humidity. Seed dormancy was broken by incubation at 4°C for 3 days in the dark and then the seedlings were transferred to a range of controlled environments. After 7 days in the controlled environments, seedlings were either transferred for 8 weeks to the vernalization cabinet or transplanted into 60-cell compartment seed trays and covered in polythene for 2 days. Vernalized seedlings were transplanted in the same manner. Thirty plants of each F_3 family were sown per treatment. Plants were randomized within seed trays.

Data for the SD environment without a vernalization treatment were incomplete as a result of aphid damage, and could not be repeated due to limited seed. This treatment was, therefore, excluded from the analysis.

Measurement of flowering time

Flowering time responses to daylength and vernalization were measured as days to flowering (FT) and rosette leaf number (LN). FT was scored as the number of days between sowing and the appearance of the first bud (by eye). These measurements were made twice a week.

For FT after vernalization, FT was recorded as the number of days from the first day at 20 C. LN was scored as the number of rosette leaves (excluding cotyledons, secondary leaves produced from below the main rosette leaves and inflorescence cauline leaves) counted once the bolt had extended.

Linkage analysis

The RFLP markers used in this study had previously been mapped (Chang et al. 1988; Lister and Dean 1993), in crosses between the ecotypes Ler and Col. Polymorphisms, therefore, needed to be established between the H51 and Ler parents. The genetic map was then calculated and checked for consistency with previously reported maps.

Thirty-one RFLP markers, chosen to cover the Arabidopsis genome at intervals of approximately 20 cM, were used to construct the linkage map of the F_2 population, which served as the basis for the QTL analysis (Fig. 1). An F_2-derived map was constructed by pairwise and multipoint linkage analysis with MAPMAKER computer software (Lander et al. 1987) for Macintosh version 1.0 (a gift from S. Tingey, DuPont) using the Haldane map function (Haldane 1919). Linkage groups were determined using pairwise analyses with a LOD threshold of 3.0 and a maximum distance of 80 cM. Multipoint analyses comparing candidate orders were used to determine the most likely map.

QTL mapping

Both single-point analysis and interval mapping using MAPMAKER QTL analysis (Lincoln and Lander 1990) were carried out on the data to identify QTL linked to RFLP markers, which affected flowering time. In the single point analysis, a one-way analysis of variance (ANOVA) was used to test whether the F_3 family means of flowering time or leaf number of the two homozygous classes, at every RFLP marker, were significantly different. MAPMAKER QTL version 1.0 was run on a VAX/VMS system. A LOD score of 2.0 was used as the significance threshold for the presence of a QTL at each location. This is the suggested LOD score for a genome of five chromosomes and a density of RFLP markers of one every 20 cM (Lander and Botstein 1989). Analyses were first performed on untransformed phenotypic data. The data was also transformed ($\log_{10}$) to fit a more normal distribution and the analysis repeated. Such analyses did not alter any of the conclusions and are, therefore, not shown.

The dominance of alleles at each RFLP marker was assessed by comparing F_3 family means for homozygous and segregating families. In one-factor ANOVA, a linear model of the number of H51 alleles (0, 1 or 2) was fitted to leaf number, and the effect of the genotype partioned into a linear effect and deviations from the linear model. This analysis was done for each environment separately.

Interaction of two QTLs, *RLN3* and *RLN4* was studied by using a two-factor ANOVA, with genotypes at *RLN3* and *RLN4* as classifying factors. Only homozygous genotypes were included in the analysis.

Analysis of genotype-by-environment interaction

G × E interaction was assessed by a two-factor ANOVA of F_3 family means, using presumed F_2 genotypes and environments as classifying factors. Genotypes were fitted as a linear model, as described above. The analyses were repeated separately for five RFLP loci which had been identified by single-point analysis as being linked to a QTL.

Variance component analysis

The contribution of each of five identified QTL to the variance was estimated by residual maximum likelihood (REML) analysis of variance components. In each environment a model was fitted in which the genotype at each of the five identified QTL were independent random factors. Variance components were constrained to be non-negative.

ANOVA and REML were done using version 5.3.1 of the Genstat statistical package, run on a VAX VMS computer (Genstat 5 Committee, 1993).

Results

RFLP segregation and map construction

The RFLP linkage map derived from analysis of the F_3 families covered 470.6 cM and the average distance between markers was 18.1 cM (Fig. 1). The most likely marker order (show in Fig. 1) agreed with the marker order on the Landsberg *erecta* × Columbia recombinant inbred map (Lister and Dean 1993, C. Lister personal communication). Six (19%) of the 31 marker loci deviated significantly ($P \leq 0.05$) from the expected 1:2:1 frequency. These loci represented four segments spread over four of the five chromosomes (Fig. 1).

Phenotypic evaluation

There was a significant correlation between LN and FT (correlation coefficient of 0.975) for F_3 families grown in CD. LN was thus adopted for subsequent analysis of flowering time.

Ler and H51 differed significantly for LN in three different photoperiods with and without vernalization (Table 1). In all cases, F_3 family means were observed to fall beyond the means of the two parents (Fig. 2) suggesting transgressive segregation (DeVicente and Tanksley 1993). H51 was observed to flower earlier than Ler under SD after vernalization (as measured by both LN and FT) but flowered consistently later in all other environments/vernalization treatments.

QTL determining flowering time

Two methods of QTL mapping were employed in this study, the single marker one-factor analysis and interval mapping. The advantages and disadvantages of the two methods are clearly described by Tanksley (1993). The results of the two QTL analyses are presented in Fig. 3. A total of five QTL (LOD > 2) was detected by interval mapping for LN at flowering time (Fig. 1), with the two methods of analysis giving similar overall pictures. The QTL mapping to the upper arm of chromosome 5 may well be two linked loci. With the population size used, this could not be resolved and so for the

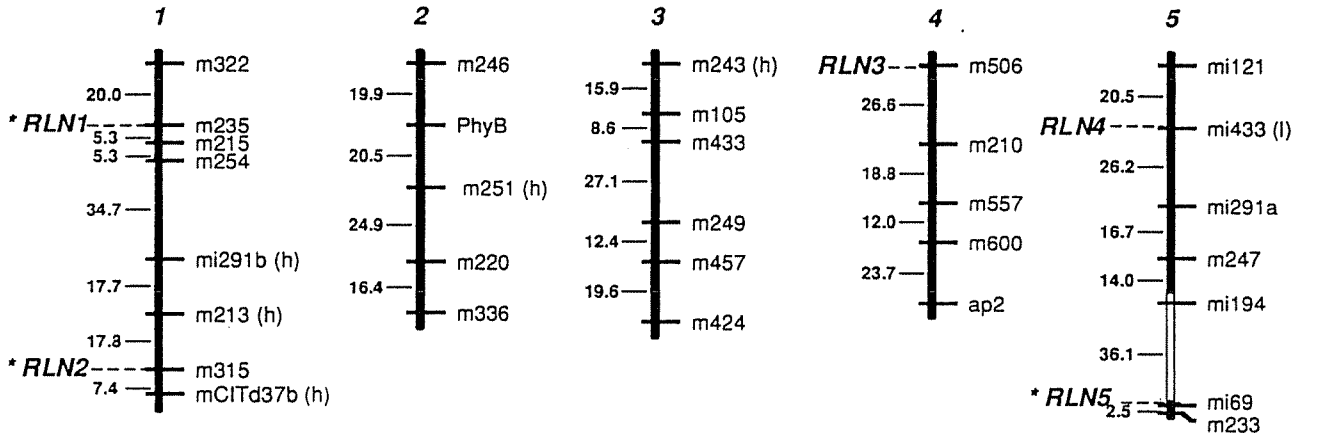

Table 1 Mean rosette leaf numbers ± SE for Landsberg *erecta* (Ler) and H51 grown at different daylengths with or without vernalizations (*V*) (*CD* continuous light, *ESD* extended short day, *SD* short day)

Treatment	Ler	H51	Population size
CD − V	7.8 ± 0.19	32.5 ± 0.72	30
CD + V	7.8 ± 0.27	10.30 ± 0.19	30
ESD − V	6.3 ± 0.10	28.2 ± 1.91	30
ESD + V	8.1 ± 0.20	9.3 ± 0.20	30
SD + V	16.6 ± 0.56	13.3 ± 0.35	15

Fig. 1 RFLP map derived from a Landsberg *erecta* × H51 F_2 population. Locations of 31 RFLP markers are shown as calculated using MAPMARKER (Lander et al. 1987) and a LOD score of >3.0, except where indicated by the *hollow bar*, where a LOD of 2.6 was used. Distances between markers are given in centimorgans calculated using the Haldane mapping function (Haldane 1919). RFLP markers are designated by clone number and either m... (Chang et al. 1988) or mi... (Y-G. Lui, N. Mitsukawa and R.F. Whittier, in preparation). (*h*), segregation significantly skewed in favour of the H51 allele; (*l*), segregation significantly skewed in favour of the Landsberg *erecta* allele. Symbols to the left of the chromosome represent of the Landsberg *erecta* allele. Symbols to the left of the chromosome represent approximate positions of significant QTL (LOD >2) detected for rosette leaf number. * denotes QTL displaying allelic effects opposite to those predicted from the parental values

rest of the analysis presented here it is considered as one QTL. Within each environment, either two or three QTL were detected. The full results are presented in Fig. 3 and summarized in Table 2.

Genotype-by-environment interaction

The interaction of five environments with the five detected QTL was evaluated. *RLN1* was detected in ESD; *RLN2* was detected in ESD and ESDV; *RLN3* was detected in CD, CDV, ESD and ESDV; *RLN4* was detected in CD, CDV, ESDV and SDV; *RLN5* only influenced flowering time in SDV. ANOVA confirmed a significant ($P \leq 0.001$) G × E interaction for m315 (*RLN2*) and m506 (*RLN3*) corresponding to changes in the LN at flowering time in response to photoperiod/vernalization treatments. A G × E interaction for m235 of reduced significance ($P \leq 0.01$) was detected.

Allelic effects of QTL

Analysis of allelic effects of QTL was assessed by ANOVA analysis of mean leaf number of F_3 families that were homozygous or segregating for the RFLP markers most closely associated with a QTL. The mean leaf number of the three genotypes, at each RFLP marker, in five separate environments and also pooled across environments, is shown in Table 3. By comparing the difference between the means of the parental controls with the genotype of each QTL allele, it was possible to determine whether each allele had the expected effect relative to the parental means. For H51, three of the five QTL (*RLN 1, 2, 5*) had allelic effects that reduced LN.

Dominance for significant QTLs in the different environments is also indicated in Table 3. This was determined by comparing the effects of the three genotypes with that expected from a fully linear effect of the number of H51 alleles. Dominance relationships were generally consistent across the different environments, with just minor differences probably caused by the relatively small population sizes.

Variance component analysis

Table 4 shows approximate relative contributions of each QTL to the overall phenotypic variation in the five different environments. It is clear that they vary dramatically. *RLN3* is the only QTL that contributes in

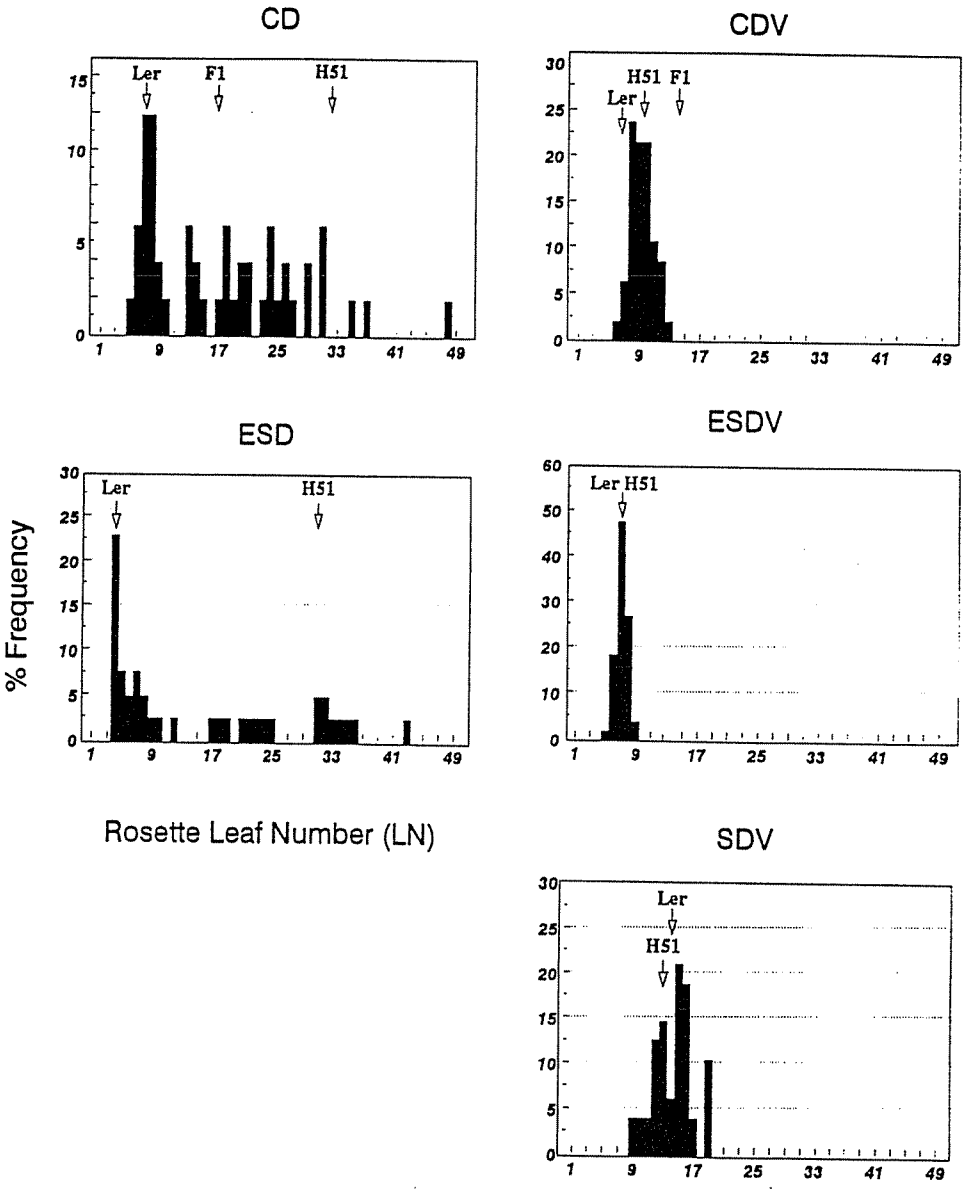

Fig. 2 Percentage frequency distribution of rosette leaf number (*LN*) for F_3 family means ($n \leq 30$). Conditions are: 24 h photoperiod (*CD*); an 8 h extended, 10 h short day (*ESD*); and a 10 h short day (*SD*), with and without an 8 week vernalization at 5 C ± 1 C (*V*). Means for parental [Landsberg *erecta* (Ler) and H51] and F_1 controls are shown by *arrows*

all environments. *RLN5* has a maximal contribution in SDV, with no apparent effect in CD, CDV or ESD (Table 3). *RLN4* has a significant contribution in all the environments except ESD (Table 3).

Interaction of RLN3 and RLN4

Indications that there was an interaction between the *FRI* locus and a locus on chromosome 5, termed *FLC* (Koornneef et al. 1994; Lee et al. 1994b) prompted us to analyze whether there was a significant interaction between *RLN3* and *RLN4*. ANOVA of mean leaf numbers of F_3 families homozygous at m506 and mi433 showed there to be a significant interaction ($P \leq 0.01$) between the loci that was not significantly altered by the environment. Families homozygous for the Ler allele at *RLN3* flowered earlier than those homozygous for the H51 allele (Table 5). Based on means across environments, the genotype at *RLN4* did not significantly affect the phenotype of families which had the homozygous H51 genotype at *RLN3*, but families with the homozygous Ler genotype at *RLN3* flowered earlier if they had the Ler, rather than the H51 allele at *RLN4*. Although the *RLN3*, *RLN4* interaction with the environment was just not significant ($P = 0.101$) it is interesting to examine the values for the CD environment (Table 5). Here, the genotype at *RLN4* did affect the phenotype of families which had the homozygous H51 genotype at *RLN3*. Families with the homozygous H51 genotype at *RLN3* flowered earlier if they had the Ler, rather than the H51 allele at *RLN4*.

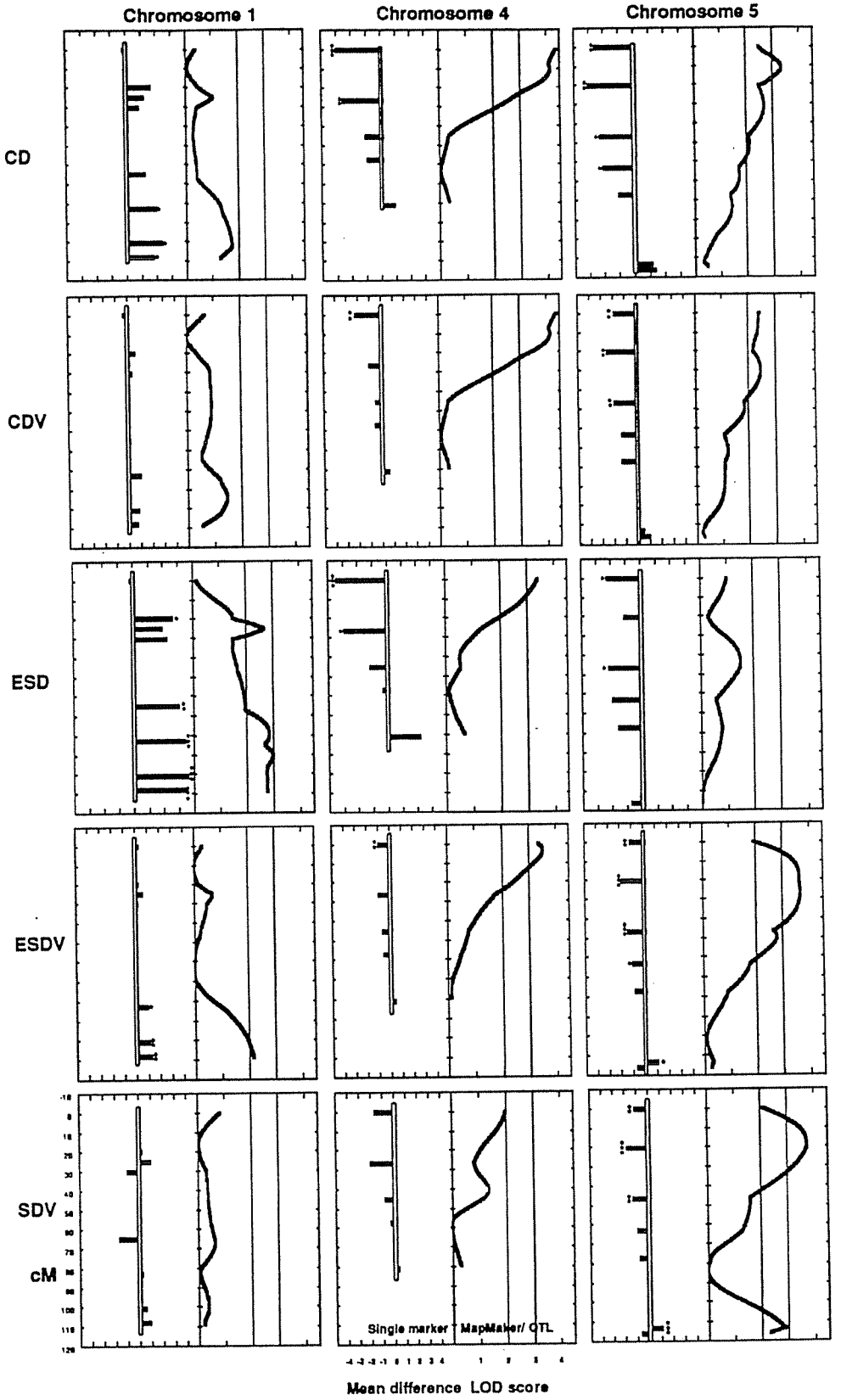

Fig. 3 QTL plots for rosette leaf number (*LN*) monitored in F_3 families from a cross between Landsberg *erecta* and H51. Plots are shown for the three chromosomes showing QTL in the five environments. The right half of each plot shows LOD scores calculated by the MAPMAKER QTL programme. *Vertical lines* indicate the LOD thresholds of 2.0 and 3.0. The left half of the plot shows the mean difference between the two classes of homozygous plants in the F_3 families, calculated by a single marker method. Negative values denote that late-flowering is conferred by H51 alleles, positive values denote the late flowering is conferred by Ler alleles. Significance levels based on ANOVA are denoted by: * ($P \leq 0.05$), ** ($P \leq 0.01$), *** ($P \leq 0.001$)

Table 2 Summary of quantitative triat loci (*QTL*) identified in a 24 h photoperiod (*CD*), an 8 h extended 10 h short day (*ESD*), and a 10 h short day (*SD*) with and without 8 weeks vernalization at 5°C ± 1°C (*V*). Only loci scoring LOD > 2 were classified as a QTL

Environment	*RLN1*	*RLN2*	*RLN3*	*RLN4*	*RLN5*
CD			+	+	
CDV			+	+	
ESD	+	+	+		
ESDV		+	+	+	
SDV				+	−

Discussion

In total, five (or possibly six, if *RLN4* is actually two QTL) QTL affecting flowering time were identified across the photoperiod and vernalization treatments. The number of QTL identified varied from two to three depending on the environment/vernalization treatment. Some of the QTL extended over 40 cM (Fig. 3) so it is likely that additional QTL within these regions having minor effects on the LN at flowering would have been obscured. The number of loci influencing flowering agrees with previous genetic analyses where four or more loci have been suggested to determine the differences between early summer and winter annuals (Barthelmess 1960; Seyffert 1960; Napp-Zinn 1961). The variation in the number of QTL identified between different environments was again consistent with the observations of a number of authors (Dierks 1958; Härer 1950; Seyffert 1960) who reported the action of additional modifying loci in non-inductive environments.

Transgressive segregation observed in the F_2 population could be accounted for by QTL with effects opposite to their predicted parental phenotype. We predict that the individuals with the lowest LN would be homozygous for H51 alleles at *RLN1*, *RLN2* and *RLN5* and for Ler alleles at *RLN3* and *RLN4*. This genotype was, however, not observed in our population as it has a probability of occurrence of 1.1024.

Based upon both the degree of late flowering conferred by the H51 allele at *RLN3* and its location, above m506 on chromosome 4, this QTL is thought to correspond to the *FRI* locus mapped in a cross between H51 and Li-5 (Clarke and Dean 1994). This locus is likely to be allelic with the *FLA* locus mapped by Lee et al. (1993) which maps approximately 5 cM from the late-flowering mutation *ld* (Lee et al. 1994a). Mutations

Table 3 Mean leaf number of plants homozygous or segregating for each marker most closely associated with the observed QTL. Only QTL scoring > LOD2 were analyzed. Numbers of plants for each genotype are shown in *brackets*. The environments were treated separately and also pooled. Allelic effects are described as: *A* additive, where the linear effect of the number of H51 alleles but not the deviation from this linear model was significant at the 5% level or lower; *D* dominant, where the linear effect and deviation were both significant; *H* heterotic, where the deviation but not the linear term was significant. Three QTL showed significant genotype by environment interactions, m235-*RLN1* ($P \leq 0.05$), m315-*RLN2* and m506-*RLN3* ($P \leq 0.001$)

QTL	Environment	Ler Ler	Allele Ler H51	H51 H51	Dominance
m235 (*RLN1*)	CD	21.15 (11)	16.27 (25)	14.10 (10)	
	CDV	9.79	9.22	9.35	
	ESD	23.30	12.42	10.87	A
	ESDV	7.36	7.15	7.28	
	SDV	14.51	14.4	14.54	
	Pooled	15.22 (55)	11.89 (125)	11.23 (50)	A
m315 (*RLN2*)	CD	26.64 (8)	15.18 (23)	15.95 (17)	D
	CDV	10.34	8.96	9.66	
	ESD	30.69	11.77	13.41	D
	ESVD	8.08	7.04	7.19	H
	SDV	15.19	14.1	14.9	
	Pooled	18.19 (40)	11.41 (115)	12.22 (85)	D
m506 (*RLN3*)	CD	8.23 (14)	19.92 (25)	22.93 (9)	A
	CDV	8.13	9.8	10.27	A
	ESD	6.66	19.28	23.44	A
	ESVD	6.55	7.65	7.37	D
	SDV	13.08	15.57	14.61	H
	Pooled	8.53 (70)	14.44 (125)	15.72 (45)	D
mi433 (*RLN4*)	CD	12.11 (14)	19.3 (13)	26.99 (7)	A
	CVD	8.83	9.35	11.15	A
	ESD	14.26	17.99	19.46	
	ESDV	6.71	7.29	8.36	A
	SDV	12.94	14.31	17.74	A
	Pooled	10.97 (70)	13.65 (65)	16.74 (35)	A
mi69 (*RLN5*)	CD	20.08 (8)	14.8 (24)	15.02 (9)	
	CVD	9.8	9.15	9.46	
	ESD	13.28	12.9	13.44	
	ESVD	7.56	7.23	6.68	
	SDV	16.76	14.62	11.8	A
	Pooled	13.5 (40)	11.74 (120)	11.28 (45)	

Table 4 Variance component (*VC*) analysis using residual maximum likelihood analysis (*REML*). The approximate percentage of the total variance in rosette leaf number (*LN*) attributable to the effects of genotypes at each QTL is shown. Data from the five environments were analyzed separately. The remaining VC, not shown in the table, is due to effects other than those of the five identified QTL including random variation within genotypes

Environment	m235 (*RLN1*)	m315 (*RLN2*)	m506 (*RLN3*)	mi433 (*RLN4*)	mi69 (*RLN5*)
CD	0	9	27	24	0
CDV	0	0	29	44	0
ESD	5	29	29	2	0
ESDV	0	18	24	32	0
SDV	0	1	5	13	71

Table 5 Mean leaf number of plants homozygous for Ler or H51 parental alleles at either *RLN3* or *RLN4* in the five different environments and then pooled over all environments – denotes a missing value

RLN3	*RLN4*	CD	CDV	ESD	ESDV	SDV	Pooled
A	A	6.54	7.43	4.24	6.16	11	7.06
	B	10.4	10.1	8.7	7	16.4	10.52
B	A	21.7	10.25	31.8	7.45	14.6	17.16
	B	30.8	11.4	–	8.5	16.1	16.7

affecting flowering time (Koornneef 1990; Koornneef et al. 1991) have been identified in the regions containing *RLN1*, *RLN2*, and *RLN4*. *RLN1* maps in a region containing two loci, *FHA* and *G1*, *RLN2* in the region containing *FT* and *FE*, while *RLN4* maps in the region containing *CO*, *FY* and the *FLC* locus, a modifier of *FLA* and also *LD* (Koornneef et al. 1994), see below. *RLN5* mapped to a region of chromosome 5 where no loci affecting flowering time had previously been identified. However, it did map close to the *DFF-b* QTL identified as influencing days to flowering in a cross between *Arabidopsis* ecotypes Hannover/Münden and WS (Kowalski et al. 1994). *DFF-a* could also be allelic to *RLN4* (Kowalski et al. 1994). In order to test whether the QTL correspond to these various loci, it will be necessary to produce near-isogenic lines in order to do the allelism tests.

Recent results have demonstrated that the late-flowering phenotype confered by two loci *FLA* (now termed *FRI*) (Lee et al. 1993) and *LD* (Redei 1962) is modified (toward early flowering) by Ler alleles at a locus *FLC* or two closely linked loci mapping in the vicinity of *RLN4* (Lee et al. 1994b; Koornneef et al. 1994). In both cases, *FLA* and *LD* had segregated as single loci in crosses with the Columbia ecotype. Based on map position and the interaction of *RLN3* and *RLN4*, it seems likely that *RLN4* may be the same locus as *FLC*. The interaction appears to explain the absence of *fri* mutations as late-flowering mutations in the Ler background, however, the interaction is slightly different in the different crosses. As described here, the greater interaction is the influence of H51 alleles at *RLN4* delaying flowering in individuals homozygous for Ler alleles at *RLN3*. As described by Koornneef et al. (1994) and Lee et al. (1994b), individuals that would be equivalent to individuals homozygous for H51 alleles at *RLN3* are only late flowering when homozygous for H51 alleles at *RLN4*. Whether these differences are explained by *RLN4* or *FLC* representing different loci or whether other loci influence this interaction remains to be elucidated.

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References

Atherton JG (1987) Manipulation of flowering. Butterworths Scientific Publications, London

Barthelmess I (1960) Merkmalskorrelation und Selektion bei *Arabidopsis thaliana* (L.) Heynh. Z Pflanzenzücht 52:273–332

Burn JE, Smyth DR, Peacock WJ, Dennis ES (1993) Genes conferring late flowering in *Arabidopsis thaliana*. Genetica 90:147–155

Chang C, Bowman JL, DeJohn AW, Lander ES, Meyerowitz EM (1988) Restriction fragment length polymorphism linkage map for *Arabidopsis thaliana*. Proc Natl Acad Sci USA 85:6856–6860

Clarke JH, Dean C (1994) Mapping *FRI*, a locus controlling flowering time and vernalization. Mol Gen Genet 242:81–89

De Vicente MC, Tanksley SD (1993) QTL analysis of transgressive segregation in an interspecific tomato cross. Genetics 134:585–596

Dierks W (1958) Untersuchungen zum Heterosisproblem. Z Pflanzenzücht 40:67–102

Haldane JBS (1919) The combination of linkage values, and the calculation of distances between the loci of linked factors. J Genet 8:299–309

Härer L (1950) Die Verebung des Blähalters früher and später sommereinjähriger Rassen von *Arabidopsis thaliana* (L.) Heynh. Beitr Biol Pflanzen 28:1–35

Koornneef M (1990) *Arabidopsis thaliana* In: O'Brian ST (ed) Genetic maps (locus maps of complex genomes) Book 6, Plants. Cold Spring Harbor Laboratory Press, Cold Spring Harbor New York, pp 6.94–6.97

Koornneef M, Hanhart CJ, van der Veen JH (1991) A genetic and physiological analysis of late flowering mutants in *Arabidopsis thaliana*. Mol Gen Genet 299:57–66

Koornneef M, Blankestijn-de Vries H, Hanhart C, Soppe W, 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

Kowalski SP, Lan T-H, Feldmann KA, Paterson AH (1994) QTL mapping of naturally occurring variation in flowering time of *Arabidopsis thaliana*. Mol Gen Genet 245:548–555

Lander ES, Botstein D (1989) Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121:185–199

Lander ES, Green P, Abrahamson J, Barlow J, Daly MJ, Lincoln SE, Newburg L (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181

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

Lee I, Aukerman MJ, Gore SL, Lohman KN, Michaels SD, Weaver LM, John MC, Feldmann KA, Amasino RM (1994a) Isolation of *LUMINIDEPENDENS*: a gene involved in the control of flowering time in *Arabidopsis*. Plant Cell 6:75–83

Lee I, Michaels SD, Masshardt AS, Amasino RM (1994b) The late flowering phenotype of *FRIGIDA* and mutations in *LUMINIDEPENDENS* is suppressed in the Landsberg *erecta* strain of *Arabidopsis*. Plant J 6:903–909

Lincoln SE, Lander ES (1990) Mapping genes controlling quantitative traits using MAPMAKER QTL. A Whitehead Institute for Biomedical Research technical report, 1st edn

Lister C, Dean C (1993) Recombinant inbred lines for the mapping of RFLP and phenotypic markers in *Arabidopsis thaliana*. Plant J 4:745–750

Meyerowitz EM (1987) *Arabidopsis thaliana*. Annu Rev Genet 21:93–111

Napp-Zinn K (1957) Untersuchungen zur Genetik des Kältebedürfnisses bei *Arabidopsis thaliana*. Z Indukt Abst Vererb 88:253–285

Napp-Zinn K (1961) Über die Bedeutung genetischer Untersuchungen an kältebedürftigen Pflanzen für die Aufklärung von Vernalisationserscheinungen. Züchter 31:128–135

Napp-Zinn K (1969) *Arabidopsis thaliana* (L.) Heynh In: Evans LT (ed) Introduction to flowering. MacMillan, Melbourne, pp 291–304

Napp-Zinn K (1979) On the genetical basis of vernalization requirement in *Arabidopsis thaliana* (L.) Heynh In: Physiologie de la floraison. No. 285. Editions du CNRS, pp 217–220

Paterson AH, Lander ES, Hewitt JD, Peterson S, Lincoln SE, Tanksley SD (1988) Resolution of quantitative traits into Mendelian factors by using a complex linkage map of restriction fragment length polymorphisms. Nature 335:721–726

Redei GP (1962) Supervital mutants of *Arabidopsis*. Genetics 47:433–460

Redei GP (1992) A heuristic glance at the past of *Arabidopsis* genetics In: Koncz C, Chua N-H, Schell J (eds) Methods in Arabidopsis research. World Scientific, Singapore, pp 1–15

Seyffert W (1960) Untersuchungen über die Vererbung quantitativer Charaktere an *Arabidopsis thaliana* (L.) Heynh. Z Pflanzenzücht 42:356–401

Stuber CW, Lincoln SE, Wolff DW, Helentjaris T, Lander ES (1992) Identification of genetic factors contributing to heterosis in a hybrid from two elite maize inbred lines using molecular markers. Genetics 132:823–839

Tanksley SD (1993) Mapping polygenes. Annu Rev Genet 27:205–233