Novel Loci Control Variation in Reproductive Timing in *Arabidopsis thaliana* in Natural Environments

Cynthia Weinig,*,†,[1] Mark C. Ungerer,‡ Lisa A. Dorn,* Nolan C. Kane,* Yuko Toyonaga,* Solveig S. Halldorsdottir,‡ Trudy F. C. Mackay,‡ Michael D. Purugganan‡ and Johanna Schmitt*

**Department of Ecology and Evolutionary Biology, Brown University, Providence, Rhode Island 02912, †Department of Plant Biology, University of Minnesota, St. Paul, Minnesota 55108 and ‡Department of Genetics, North Carolina State University, Raleigh, North Carolina 27695*

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ABSTRACT

Molecular biologists are rapidly characterizing the genetic basis of flowering in model species such as *Arabidopsis thaliana*. However, it is not clear how the developmental pathways identified in controlled environments contribute to variation in reproductive timing in natural ecological settings. Here we report the first study of quantitative trait loci (QTL) for date of bolting (the transition from vegetative to reproductive growth) in *A. thaliana* in natural seasonal field environments and compare the results with those obtained under typical growth-chamber conditions. Two QTL specific to long days in the chamber were expressed only in spring-germinating cohorts in the field, and two loci specific to short days in the chamber were expressed only in fall-germinating cohorts, suggesting differential involvement of the photoperiod pathway in different seasonal environments. However, several other photoperiod-specific QTL with large effects in controlled conditions were undetectable in natural environments, indicating that expression of allelic variation at these loci was overridden by environmental factors specific to the field. Moreover, a substantial number of QTL with major effects on bolting date in one or more field environments were undetectable under controlled environment conditions. These novel loci suggest the involvement of additional genes in the transition to flowering under ecologically relevant conditions.

THE transition to flowering is a central event in plant life history, and the timing of reproduction is a primary determinant of fitness in many species (Cohen 1976; Geber 1990; Stratton 1998). Information on the genetic mechanisms underlying this transition is therefore critical for understanding the evolution of life histories in natural plant populations. The developmental pathways leading to flowering are rapidly being elucidated by molecular genetic studies in model systems such as *Arabidopsis thaliana* (Coupland 1997; Koornneef *et al.* 1998; Levy and Dean 1998; Weigel 1998; Mockler *et al.* 1999; Simpson and Dean 2002). However, genes identified by studies of mutant or transgenic plants in controlled experimental settings may not be the loci primarily controlling variation in flowering time in natural populations. A valuable complementary approach, therefore, is the identification of quantitative trait loci (QTL) underlying natural variation (Koornneef *et al.* 1998). For molecular biologists, QTL mapping can help to identify additional genes important in pathways to flowering (Koornneef *et al.* 1998; El-Assal *et al.* 2001). For evolutionary biologists, QTL mapping can provide important information about the genetic basis of life history evolution in natural populations (Mitchell-Olds 1996). To answer questions at both levels, it is important to know whether QTL effects differ in different natural environments.

The natural history and genetic basis of developmental pathways to flowering in *A. thaliana* suggest that different loci may contribute to variation in reproductive timing in different ecological settings. *A. thaliana* is a predominantly self-fertilizing, colonizing annual species, native to Eurasia but now widely naturalized in the United States and elsewhere. Plants initially grow as a rosette and then bolt to produce an indeterminate inflorescence from the apical meristem. *A. thaliana* populations occur over a wide latitudinal gradient, and ecotypes differ substantially in life history and reproductive phenology (Nordborg and Bergelson 1999). Northern populations often possess multiple germination cohorts with seeds emerging in either fall (short-day photoperiod) or spring (long-day photoperiod). This germination polymorphism is thought to be a "bet-hedging strategy" (Venable 1985; Silvertown 1988) against environmental heterogeneity; fall germinants risk overwinter mortality, but those that survive the winter have a longer growth period and are expected to produce more fruit relative to spring germinants. Southern populations tend to exhibit a fixed fall-germinating annual

[1]*Corresponding author:* Department of Plant Biology, 220 Biosciences Ctr., University of Minnesota, 1445 Gortner Ave., St. Paul, MN 55108. E-mail: cweinig@tc.umn.edu

life history, which maximizes fitness when winters are mild and spring growing conditions are unfavorably hot or dry.

Flowering in Arabidopsis is accelerated by long days (mediated by the photoperiod developmental pathway) and by exposure to cold (mediated by the vernalization pathway) (Koornneef *et al.* 1998; Levy and Dean 1998; Johanson *et al.* 2000). Flowering is also promoted by the so-called autonomous pathway, which interacts with the vernalization pathway through the central regulatory gene *FLOWERING LOCUS C* (*FLC*; Sheldon *et al.* 2000). Different populations and seasonal cohorts experience different photoperiods and temperatures during the transition to flowering, depending on latitude and timing of germination. Such environmental variation in inductive cues may cause differential activation or repression of certain pathways in different sites or seasons (Simpson and Dean 2002). If so, genetic variation at loci associated with these pathways may contribute to variation in flowering time only in certain natural environments.

Here we report the first study of QTL for date of bolting (the transition from vegetative to reproductive growth) in *A. thaliana* in natural field environments and compare the results with those obtained under typical controlled, growth-chamber conditions. A few QTL were common to controlled experimental settings and the field, and a few had large effects under controlled conditions that were undetectable in natural environments. However, a substantial number of QTL had major effects on bolting date in one or more field environments that were undetectable under controlled environment conditions. These novel loci suggest the involvement of additional genes in the transition to flowering under ecologically relevant conditions, beyond those detected under controlled conditions. The genetic basis of the bolting date differed between geographic locations and between spring and fall cohorts. Thus, the expression of genetic variation and the potential for life history evolution in natural Arabidopsis populations depend upon the ecological setting.

MATERIALS AND METHODS

Study species and mapping population: Quantitative genetic analyses utilized recombinant inbred lines (RILs) of *A. thaliana* (L.) Heynh. (Brassicaceae) developed from a cross between the Landsberg *erecta* and Columbia accessions advanced through single-seed descent to the F_8 (Lister and Dean 1993). Residual heterozygosity has been estimated as 0.42% in these lines (Lister and Dean 1993). Because siblings within a line are homozygous and genetically uniform, we could replicate these RILs across environments and experiments to examine environmental variation in QTL expression.

Controlled-environment experiments: RILs were grown in growth chambers at 20° under long, 14-hr days (LD; Ungerer *et al.* 2002) and short, 10-hr days (SD) at the North Carolina State University (NCSU) Phytotron Facility. The photoperiod treatments were chosen to approximate the range of natural photoperiods experienced by natural populations of *A. thaliana* in our study areas. For both the short- and the long-day experiments, seeds of 97 RILs were sown at random with respect to genotype into pairs of 72-cell flats. A single replicate of each RIL was represented in each flat, thereby randomizing flat and positional effects across the lines with 15 replicates of each line. Seeds were imbibed and cold stratified in darkness for 4 days at 4° to induce germination. Additional details of the experimental design and culture conditions are given by Ungerer *et al.* (2002).

Field experiments: RILs were planted into field sites in North Carolina and Rhode Island in fall 1999 and spring 2000. In spring and fall in Rhode Island, and in fall in North Carolina, we planted the RILs when naturally emerging seedlings in local populations were at a similar developmental stage. Since natural North Carolina populations exhibit a fixed winter annual life history with no spring germination, we planted the North Carolina spring cohort when average seasonal temperatures were similar to those in Rhode Island at the time of spring germination in natural populations.

Seeds of 98 RILs were stored under dry conditions at room temperature until planting and initially sown into damp Metromix 350 soil medium in each of 30, 98-cell plug trays. Seeds for the fall plantings were cold stratified in the plug trays at 4° for 4 days. Seeds for the spring plantings were cold stratified for 14 days to simulate the cold experienced by overwintering seeds. Following cold stratification, seeds were germinated in the watered plug trays under natural day lengths in the greenhouses of Brown University and NCSU. Seedlings were then transplanted into prepared field plots at Brown's Haffenreffer Grant, Bristol, Rhode Island (latitude 41°N 41′W) and NCSU's Clayton Agricultural Test Field, Clayton, North Carolina (latitude 35°N 39′W). In both Rhode Island and North Carolina, field plots were prepared via plowing and raking or disking, respectively; no effort was made to modify local soil conditions. For the Rhode Island fall germination cohort, 3–5 seeds of all 98 RILs were sown into each of 10 blocks. After 14 days in the greenhouse, seedlings were thinned to one per cell and transplanted on November 4–6, 1999, with the intact soil plug into the native soil in 30 field blocks with each flat corresponding to a block. Seedlings were spaced at 10-cm intervals in 70 × 140-cm blocks to prevent competitive interactions. Following the stratification treatment, the spring germination cohort remained in the greenhouse for 2 weeks until April 5–7, 2000, at which point it was transplanted to the field. Blocks for the fall and spring plantings were arranged at 1-m spacing in a 6 × 10 checkerboard array such that neighboring blocks alternated seasonal cohorts.

The North Carolina plantings were prepared in the same way. The fall germination cohort was transplanted to the field between November 16–18, 1999. The spring germination cohort was transplanted March 7–9, 2000. Plants in all cohorts were watered only on the day of transplanting, after which natural rainfall was the only source of water.

We used the number of days between germination and the onset of bolting as our estimate of time to flowering and the transition from vegetative to reproductive function. Bolting refers to the differentiation of an inflorescence, rather than additional rosette leaves, from the apical meristem. Inflorescences were visibly distinguishable from leaves at a size of ~1 mm. Plants were monitored weekly for the onset of bolting and twice weekly on average once the first plants bolted. In all quantitative-genetic and QTL mapping analyses described below, we refer to the focal trait as "bolting date." Although we planted 30 replicate germination plugs per RIL, the actual number of replicates for which we recorded bolting date was somewhat lower, due to low germination in some plugs and seedling mortality in the field. The number of replicate plants

within a given RIL ranged from 10 to 30 in the field sites and from 11 to 15 in the greenhouse.

Analysis of variance of bolting date: Within each environment, random-effects ANOVA was used to partition variance for bolting into sources originating from line (L) and error according to the model, $y = \mu + L +$ error, where μ is the overall mean. This model was expanded to account for variation attributable to environment by the mixed-model ANOVA: $y = \mu + L + E + E \times L +$ error, where E is the growth environment and is treated as a fixed effect. SAS VARCOMP was used to estimate variance components, and significance tests for F ratios were obtained from SAS GLM (SAS 1999). The among-line variance, V_L, is an estimate of the genetic variance between parental lines, given that gene frequencies are 0.5 at all segregating loci affecting the trait. Thus, QTL mapping should be carried out only in traits with significant among-line variance components; bolting date exhibited significant V_L in all field and controlled environments (see RESULTS below).

From the components of variance, we calculated across-environment correlations (r_{GE}) as $cov_{12}/\sqrt{(V_{L1} \times V_{L2})}$, where cov_{12} represents the covariance of a bolting date across two environments and V_{L1} and V_{L2} are the among-line variance components within each of the two environments (ROBERTSON 1959). Significant correlations across the fall and spring seasonal environments within each geographic region are of particular relevance because genetic similarity of bolting date across seasonal cohorts could constrain adaptive evolution within each environment. Results from the two-way ANOVA including line and season as main effects are used to determine whether r_{GE} differ significantly from zero or unity: across-environment genetic correlations are significantly different from 1 if the $E \times L$ interaction terms are significant and are significantly different from 0 if the among-line variance is significant in the same analysis.

Linkage-map construction: Lines present only in the phytotron or the field experiment were excluded from the analyses, resulting in a total of 95 lines. A large number of polymorphic marker loci have been identified in the L*er* × Col recombinant inbred lines, but not all lines have been genotyped at all loci. A subset of marker loci was therefore used to construct the linkage map. Markers genotyped in 80% or more of the lines were selected to provide even coverage of the genome. Maps were constructed using Mapmaker/Exp 3.0 (LANDER *et al.* 1997); exact methods are described in greater detail elsewhere (UNGERER *et al.* 2002).

QTL analyses: For each experimental environment, we used the phenotypic mean of all measured replicates within an RIL as our estimate of average bolting date. Individual values and RIL means for bolting date were non-normally distributed in the North Carolina fall cohort. Log transformation improved normality. However, we present QTL mapped with the means of the original rather than of the transformed data, because transformation did not significantly affect the mapping results and comparisons of QTL mapped in different environments are facilitated by the similar scale. QTL for bolting date were mapped using the composite interval mapping (ZENG 1994) procedure of QTL Cartographer (BASTEN *et al.* 1994, 1999). QTL cofactors were initially selected using forward-backward stepwise regression. Within each experimental environment, the significance threshold of the likelihood-ratio (LR) test statistic for a QTL was determined through permutation analyses (DOERGE and CHURCHILL 1996). QTL whose peaks were separated by LR values below the significance threshold are shown as separate QTL (Figure 1). All QTL are shown with the 2-LOD support limits, where LOD = 0.217 × LR. When comparing across environments, overlap of the 2-LOD support limits provides evidence that similar QTL determine the expression of bolting date. Additive effects were calculated as the difference of the two homozygous classes divided by two and are standardized to the line variance within each environment to facilitate comparisons of effect size among QTL.

To estimate the total variance contributed by QTL detected within an environment, we used ANOVA to calculate the sums of squares for each marker locus closest to a QTL peak; total variance was calculated by adding the sums of squares for the markers and dividing the total by the model sums of squares. QTL × environment interactions were tested using analysis of variance (FRY *et al.* 1998) in which season and all significant QTL detected in the genome-wide screen were included in the model. When all possible two-way interactions are tested, ANOVA effectively corrects for potential type I error resulting from multiple tests. Maximum-likelihood techniques are also available to test for QTL × environment interactions, but these analyses were inappropriate for the structure of our data. ANOVA was similarly used to test for epistasis; the model included all two-way interactions between significant QTL detected in the genome-wide screen. There was, however, no evidence of epistasis (data not shown). To identify potential candidate genes, markers defining the limits of QTL regions were determined on the L*er* × Col genetic map, as were their positions on the Arabidopsis physical map. Exact methods for scanning the annotated sequence within these regions are described elsewhere (UNGERER *et al.* 2002).

RESULTS AND DISCUSSION

RILs differ in responsiveness of bolting date to photoperiod, seasonal environment, and geographic site: As expected in this long-day species, plants grown under long days in the phytotron bolted earlier than those grown under short days, 20 and 29 days on average after planting, respectively. In the field, the experimental plants bolted at different ages and times in different seasonal cohorts and geographic regions (Table 1). As a result, they experienced different photoperiods in the four planting treatments. Plants in the fall cohorts began bolting in Rhode Island on March 1 and in North Carolina by December 19, at which point day lengths were <12 hr (Table 1). Thus, Arabidopsis plants clearly initiate reproduction under short days in natural seasonal environments. Plants in the Rhode Island spring cohort began bolting on April 28, while plants in the North Carolina spring cohort bolted on April 15. Photoperiods were >12 hr at the time of bolting in both of these cohorts (Table 1).

One-way ANOVA demonstrated that RILs differed significantly in bolting date within each field and controlled environment (Table 2). Heritabilities ranged from 0.10 to 0.22 in the field and from 0.44 to 0.49 in the phytotron. Higher-order ANOVA revealed significant differences among lines in the response of bolting date to photoperiod in the phytotron ($F_{1,94} = 5.94$, $P < 0.0001$ for line × photoperiod interaction) and to the combination of season and site in the field ($F_{1,95} = 92.94$, $P < 0.0001$ for line × season × site interaction). The three-way interaction of line × season × site was explained at least in part by line × season interactions within each geographic region (Table 3, A and B). Across-season

TABLE 1

Differences in photoperiod at time of germination and bolting among the seasonal environments and geographic regions

Environment	Approximate date of germination	Date of first bolting	Days to first bolting	Average days to bolting	Shortest photoperiod between germination and bolting	Photoperiod at bolting
Fall						
Rhode Island	Oct. 17–19	March 1	134	150	9 hr 09 min	11 hr 14 min
North Carolina	Oct. 31–Nov. 3	December 19	49	73	10 hr 26 min	9 hr 45 min
Spring						
Rhode Island	March 21–23	April 28	38	44	12 hr 10 min	13 hr 53 min
North Carolina	Feb. 22–24	April 15	52	60	11 hr 09 min	13 hr 05 min

Dates of germination are the dates on which the seed stratification treatments ended and seed trays were placed under ambient photoperiods. Date of first bolting indicates the date the first plants in a cohort began bolting, that is, differentiated a flowering inflorescence, while days to bolting is the absolute number of days elapsed between the time of germination to the time of bolting. The following column provides the average number of days to bolting for a cohort. Because changes in day length may influence bolting date, the shortest photoperiod experienced by a cohort after planting, in addition to the photoperiod on the date of bolting, are presented in the last two columns. Note that the North Carolina fall and Rhode Island spring cohorts experienced day lengths consistently shorter and longer than 12 hr, respectively, from the time of germination to bolting, while the other two cohorts experienced increasing day lengths prior to bolting.

genetic correlations within each geographic region were positive, $r_{GE} = 0.29$ and 0.50 for Rhode Island and North Carolina, respectively.

Several QTL of large effect account for much of the variation in bolting date within each environment: Six significant QTL explained ~69% of the variation in bolting date in both the long-day and the short-day chamber environments (Table 4) with individual contributions ranging from 7 to 19%. In each of the four field environments, eight to nine significant QTL accounted for 68–82% of the observed variation, with individual loci accounting for 4–18%. Within each environment, the Col and L*er* parents each contributed alleles with positive as well as negative additive effects (Table 4). The absolute magnitude of these additive effects ranged from 1.1 to 2.0 days under short days and from 0.5 to 1.0 day under long days in the chambers (Table 4). In Rhode Island, effect sizes were 0.4–0.9 day in the fall cohort and 0.7–1.1 days in the spring (Table 4). In North Carolina, effect sizes were 3.0–6.5 days in the fall cohort and 0.9–1.2 days in the spring (Table 4). Thus, natural allelic variation at specific loci had important phenotypic effects in natural environments.

TABLE 2

Restricted maximum-likelihood estimates of the among-line variance component, V_L, and associated *P* value within each field and controlled environment

Environment	V_L	V_{error}	*P*
Fall			
Rhode Island	2.21	13.62	<0.0001
North Carolina	77.40	435.04	<0.0001
Spring			
Rhode Island	5.31	18.51	<0.0001
North Carolina	5.69	50.21	<0.0001
Long days	2.66	2.77	<0.0001
Short days	11.68	14.70	<0.0001

Only one QTL for bolting date is common to all environments: This locus, at a map position of ~125 cM on chromosome 5 (Figure 1, QTL peaks shown in pink), accounted for 16–19% of the observed variation in bolting date in the chamber environments and 4–12% of variation in the field environments. The importance of this QTL in both short days and long days in the phytotron and in prior studies manipulating irradiance (Stratton 1998), as well as in different seasonal environments, suggests that it may act as a central regulator of all flowering-time pathways. Its expression in both spring and fall environments may serve as a genetic constraint on the evolution of reproductive timing in populations with two seasonal germination cohorts, because selection on variation at this locus in one seasonal cohort will produce a correlated response in bolting date in the other cohort.

Several photoperiod-specific QTL expressed in the controlled environments influence bolting in seasonal field environments: Four QTL for bolting date were detected under long days but not short days, suggesting that these are involved in the photoperiod pathway. These included significant QTL at map positions of 1 cM on chromosome 1, at 17 and 25 cM on chromosome 2, and at 20 cM on chromosome 5. Two of these four putative photoperiod-pathway QTL also controlled variation in bolting date in the field, but only in spring cohorts, which experienced long days at bolting (Figure 1, QTL peaks shown in green). The long-day QTL at the top of chromosome 1 was detected in both the Rhode Island and the North Carolina spring cohorts. The long-day QTL at 19 cM on chromosome 5 was expressed only in the Rhode Island spring cohort, the

TABLE 3

Two-way ANOVA for bolting date responses to seasonal environments in Rhode Island and North Carolina

Source	d.f.	Mean square	*P*	Variance component
A. Rhode Island				
Line	94	118.30	0.01	1.05
Season	1	1.20×10^6	<0.0001	—
Line × season	94	75.29	<0.0001	2.80
Error	4047	18.06		18.04
B. North Carolina				
Line	94	1527.74	0.05	10.57
Season	1	5.00×10^6	<0.0001	—
Line × season	94	1080.54	<0.0001	38.55
Error	3880	250.26		250.59

only cohort to experience long days throughout its life history from the time of germination to bolting (Table 1). Thus, some loci involved with the photoperiod pathway are important under natural spring conditions, when plants are experiencing long days at the time of bolting.

Four other QTL were detected under short days but not long days. These include loci at 49 cM on chromosome 1, at 80 cM on chromosome 2, and at 59 and 69 cM on chromosome 4. Allelic variation at these loci appears to be expressed only when not overridden by the photoperiod pathway. Like the long-day photoperiod QTL, two of the four loci for bolting date under short days also controlled variation in the field, but only in fall cohorts, which started bolting when day lengths were <12 hr (Figure 1, QTL peaks shown in blue; Table 1). Significant QTL at 80 cM on chromosome 2 for the North Carolina fall cohort and at 57 cM on chromosome 4 for both Rhode Island and North Carolina fall cohorts overlapped with QTL for bolting date under short days in the phytotron. Thus, allelic variation expressed only under short days (in the absence of a long-day signal acting through the photoperiod pathway) can be important under fall conditions in natural environments.

Several QTL detected in controlled environments have little effect on bolting date in natural seasonal environments: Of 12 QTL detected in the phytotron, 4 were not apparent in any field environment. Two QTL for bolting date specific to LD in the phytotron (at 17 and 25 cM on chromosome 2) were undetectable in the field, despite the fact that power analyses (Soller and Beckman 1990; Lynch and Walsh 1997) showed comparable statistical power between LD in the phytotron and the spring field environments. Thus, expression of allelic variation at these photoperiod-related loci was apparently overridden by some other environmental factor in the field experiment. Similarly, two QTL for bolting date detected under SD (at 49 cM on chromosome 1 and at 69 cM on chromosome 4) were not apparent under field conditions. Again, expression of allelic variation at these loci was probably overridden by environmental factors other than photoperiod in the field experiment. One possibility is that the cold experienced by the overwintering fall cohorts, and the cold stratification of seeds to simulate winter conditions for the spring cohorts, acted through the vernalization pathway to suppress expression of this variation. Interestingly, two of the QTL detected here (~110 and 123 cM on chromosome 1 in both the North Carolina cohorts and the Rhode Island spring cohorts) appear to overlap with a QTL for vernalization effects on rosette leaf number at flowering found in a prior study using the L*er* × Col lines (Jansen *et al.* 1995). However, other unmeasured environmental factors may also be at play. Whatever the mechanism, our results indicate that photoperiod-specific QTL detected under controlled conditions may not always be important in the field, where other ecological factors may override the expression of variation at these loci.

It is also noteworthy that the *ERECTA* marker, an induced phenotypic mutation in L*er*, was associated with large phenotypic effects on bolting date under both long days and short days in the phytotron, but had no detectable effect in the field except for the Rhode Island fall cohort. These differences cannot be explained by greater power in the Rhode Island fall cohort relative to the other field cohorts because statistical power was similar among the field cohorts. If *ERECTA* rather than a linked gene is the QTL at 42 cM on chromosome 2, our results suggest that a major developmental mutation may have no phenotypic effect in certain natural environments and thus may be sheltered from natural selection in those environments.

A number of novel QTL determine bolting date in the field: Fourteen QTL were detectable in one or more of the field environments, but not in the phytotron environments (Figure 1, QTL peaks shown in red). With the exception of one QTL on chromosome 5 (position ~84 cM in the Rhode Island fall cohort), all of the QTL unique to the field showed significant QTL × environment interactions as determined by ANOVA analyses ($P < 0.05$ for QTL marker × environment interaction terms). Five QTL were unique to different geographical/seasonal field cohorts, while the rest were found in two or more cohorts. Of the QTL common to two or more cohorts, the QTL in the middle of chromosome 3 was expressed only in the Rhode Island fall and spring cohorts, suggesting that it may interact with some environmental factor (such as soil characteristics or herbivore load) unique to our Rhode Island field site. Two adjacent QTL on chromosome 1 (84–93 cM) controlled bolting in both the Rhode Island fall and the North Carolina spring cohorts. These two cohorts germinated when day lengths were <12 hr and experienced a transi-

TABLE 4

QTL for bolting date under short and long days in the phytotron and in fall and spring seasonal cohorts in Rhode Island and North Carolina

Trait	Chromosome	QTL map position in cM (nearest marker)	2-LOD support limit (cM range)	LR	Additive effect/σ_G	[r^2]
Short days in phytotron	1	49.45 (*CATTS039*)	41.64–54.66	31.6	−0.12	0.14
	2	41.55 (*er*)	39.24–45.05	31.8	−0.15	0.14
	2	79.76 (*RRS2*)	69.05–79.76	21.9	0.10	0.08
	4	58.74 (*JGB9*)	47.70–69.39	20.6	0.10	0.09
	4	69.31 (*mi232*)	47.70–69.39	19.2	0.09	0.08
	5	126.05 (*SNP153*)	121.54–129.65	34.1	0.17	0.16
Fall cohort in Rhode Island	1	88.81 (*agp1e*)	82.71–97.71	15.1	−0.14	0.04
	1	93.15 (*CH.215L*)	91.01–96.87	19.7	−0.17	0.06
	2	40.89 (*er*)	39.24–44.05	44.2	−0.28	0.15
	3	53.40 (*ve021*)	47.39–56.90	39.2	0.20	0.12
	4	0.01 (*mi51*)	0.01–3.32	25.1	0.19	0.07
	4	56.40 (*AG*)	53.02–58.74	24.2	0.16	0.08
	5	84.01 (*mi83*)	69.67–90.96	17.4	−0.14	0.04
	5	128.15 (*CATHHANK*)	122.54–132.15	37.9	0.21	0.12
Fall cohort in North Carolina	1	5.40 (*apx1A*)	0.01–11.17	14.9	−0.04	0.06
	1	15.27 (*ATTS0477*)	11.17–15.57	14.6	−0.04	0.06
	1	111.73 (*mi103*)	111.73–114.24	24.9	0.07	0.16
	1	123.82 (*ve011*)	121.62–127.09	34.1	0.09	0.18
	2	60.27 (*ve018*)	42.05–79.76	14.8	0.04	0.06
	2	76.26 (*RRS2*)	42.05–79.76	14.1	0.04	0.06
	4	50.23 (*mi260*)	43.45–62.10	16.2	0.05	0.07
	4	56.90 (*AG*)	43.45–62.10	14.5	0.05	0.07
	5	58.40 (*mi291b*)	54.91–63.17	23.8	−0.06	0.10
	5	127.05 (*ve032*)	106.45–132.15	10.1	0.03	0.04$^+$
Long days in phytotron	1	0.01 (*ve001*)	0.01–11.17	14.3	−0.18	0.07
	2	16.93 (*g4133*)	10.20–29.15	18.3	0.20	0.08
	2	25.15 (*mi398*)	10.20–29.15	16.3	0.20	0.07
	2	42.55 (*er*)	36.74–50.05	23.2	−0.25	0.13
	5	19.60 (*ve033*)	16.60–24.42	26.7	0.35	0.15
	5	125.05 (*SNP153*)	120.09–128.15	35.2	0.28	0.19
Spring cohort in Rhode Island	1	0.01 (*ve001*)	0.01–3.01	32.9	−0.20	0.15
	1	111.73 (*mi103*)	111.73–136.37	15.3	0.14	0.07
	1	123.32 (*ve011*)	119.04–131.37	25.5	0.18	0.10
	2	0.05 (*ve012*)	0.01–12.00	15.2	0.13	0.06
	3	61.07 (*g4564b*)	49.89–71.94	13.9	0.12	0.06
	5	14.34 (*g3837*)	10.01–31.42	18.5	0.15	0.08
	5	19.60 (*ve033*)	9.73–31.27	18.5	0.15	0.08
	5	110.99 (*emb514*)	109.45–132.15	13.8	0.13	0.07
	5	125.05 (*SNP153*)	116.33–128.15	21.3	0.15	0.09
Spring cohort in North Carolina	1	0.01 (*ve001*)	0.01–11.17	13.6	−0.16	0.07
	1	5.40 (*apx1A*)	0.01–11.17	14.3	−0.16	0.08
	1	84.35 (*mi209*)	79.82–96.37	17.5	−0.19	0.10
	1	89.81 (*agp1e*)	79.82–97.71	14.1	−0.18	0.09
	1	126.59 (*agp64*)	120.04–134.87	20.4	0.21	0.13
	4	9.77 (*mi301*)	6.08–13.34	16.7	−0.22	0.10
	5	122.04 (*g2368*)	114.99–132.15	14.1	0.17	0.08
	5	127.55 (*CATHHANK*)	114.99–132.15	14.1	0.16	0.08

QTL for each trait were mapped using composite interval mapping within each geographic region and season. The first three columns indicate the chromosomal location of the QTL, the nearest marker locus, and the centimorgan range defining the 2-LOD support limits around the QTL. The LR is the test statistic for composite interval mapping, the significance of which is determined through permutation analyses (Doerge and Churchill 1996); for all traits, the significance threshold for an experiment-wide error rate of $\alpha = 0.05$ was <14.2. The "+" symbol denotes a QTL significant at $\alpha < 0.15$. The second to last column denotes the additive effects. Effects are positive if the Col allele confers bolting later than the L*er* allele and negative if the Col allele confers bolting earlier. The final column shows the proportion of variance explained by a QTL, which was calculated as the proportion of the model sums of squares explained by a given QTL in an ANOVA including all significant QTL as main effects.

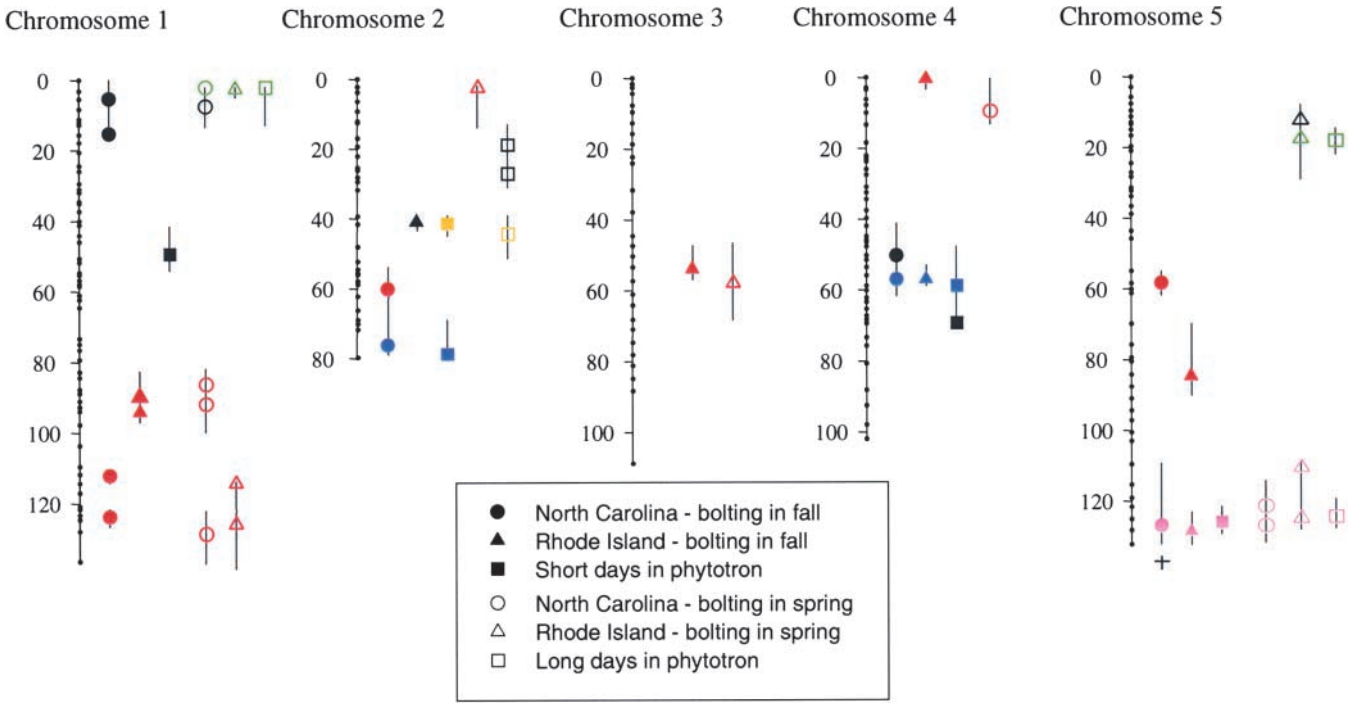

FIGURE 1.—QTL detected under short and long days in the phytotron at NCSU and in experimental field plantings in Rhode Island and North Carolina. Solid symbols represent QTL detected under SD conditions in the phytotron and SD field conditions experienced by the fall seasonal cohorts. Open symbols represent QTL detected under LD conditions in the phytotron and LD conditions experienced in the field by the spring seasonal cohorts. Lines bracketing the QTL symbols denote the 2-LOD support limits (see Table 4 for exact values). Significance of individual QTL at an experiment-wide error rate of $\alpha = 0.05$ was determined through permutation tests, with the exception that the QTL with a "+" is significant only at $\alpha < 0.15$. Multiple symbols with overlapping support limits within an experimental treatment indicate that the LR test statistic dropped below the significance threshold between the two QTL peaks. Colors highlight specific results: pink denotes QTL for bolting date common to all environments; green denotes QTL common to long days in the phytotron and long-day field settings (>12 hrs); blue denotes QTL common to short days in the phytotron and short-day field settings (<12 hrs); gold denotes QTL common to long and short days in the phytotron; and red denotes QTL that significantly affected bolting date only in one or more field environments.

tion to long days prior to bolting (Table 1). One possibility is that the QTL common to these cohorts are somehow involved in sensing changing day length as an environmental cue for bolting.

On the basis of the marker loci nearest the QTL peaks, only 3 of the 14 QTL detected in one or more field cohorts (at ~110 and 123 cM on chromosome 1 and at 1 cM on chromosome 2) may correspond to QTL detected in prior studies mapping QTL for reproductive timing in the same RILs grown in controlled settings (JANSEN *et al.* 1995; MITCHELL-OLDS 1996; STRATTON 1998). None of these studies is strictly comparable. Each was conducted under slightly different light conditions: continuous light (MITCHELL-OLDS 1996), 16-hr long days (JANSEN *et al.* 1995), and variable irradiance under 15-hr days (STRATTON 1998). Moreover, the linkage maps were made with slightly different sets of markers, as well as different RILs ($n = 95$ or $n = 100$, depending on the study). These differences, although slight, can affect map positions of the QTL. Nevertheless, 11 QTL detected in the field in our study appear to have no counterpart in controlled environments. The detection of many QTL unique to the field provides strong evidence that experiments performed in controlled environments will provide an incomplete picture of the genetic mechanisms underlying the transition to bolting in natural populations.

The genetic basis of variation in bolting date differed dramatically between seasons and sites: Of the eight QTL for bolting date detected in the Rhode Island fall cohort, only two loci (in the middle of chromosome 3 and the bottom of chromosome 5) overlapped with the nine QTL observed in the Rhode Island spring cohort. These common QTL together explain 25% of variation in bolting date in the fall cohort (out of 60% explained by all QTL) and 14% (out of 67% total) in the spring cohort. Similarly, we observed only two loci in common between seasonal cohorts in North Carolina (bottom of chromosomes 1 and 5) out of nine loci in the fall cohort and eight in the spring cohort. These two QTL together explained 10% out of 64% of the total variance in the fall and 11% out of 47% in the spring. Thus, consistent with the small across-season genetic correlations, most of the loci controlling variation in timing of bolting

differ between spring and fall generations in the same geographical location.

Expression of allelic variation also differed between North Carolina and Rhode Island. Out of 18 total QTL detected in the fall and 17 QTL detected in the spring seasonal cohorts, only 2 QTL were common to both sites within each seasonal cohort. The observation that different QTL determine bolting date across seasonal environments is consistent with the small, positive genetic correlations. Thus, the genetic basis of life history variation in this colonizing species can vary with geographic location, even in populations composed of identical genotypes.

Environmentally dependent expression of allelic variation may have important implications for life history evolution in natural plant populations. Seasonal differences in the genetic basis of bolting date are particularly relevant in northern populations of *A. thaliana*, which often have both fall and spring seasonal cohorts, as in Rhode Island. If the genetic potential for response to natural selection on reproductive life histories differs among seasonal cohorts, phenotypes expressed in fall and spring may evolve independently in response to divergent selection across seasons (Y. Toyonaga, C. Weinig and J. Schmitt, unpublished results). However, QTL expressing allelic variation in both seasonal environments will constrain such independent evolutionary response; that is, evolutionary responses to selection in the fall seasonal cohort will result in a correlated response in the spring cohort.

Geographic differences in the genetic basis of bolting date suggest that colonizing populations with similar initial genetic composition may respond to selection on allelic variation at entirely different loci in different sites. If these geographic differences in expression of allelic variation are related to climate, then climate change within a site could conceivably alter the genetic potential for life history evolution in response to changing selective pressures. Conditional expression of QTL effects has also been observed in *Drosophila melanogaster* and several crop species, where effects of individual QTL vary with the physical environment (Brummer *et al.* 1997; Sari-Gorla *et al.* 1997; Gurganus *et al.* 1998; Leips and MacKay 2000; Vieira *et al.* 2000) and genetic background (Nuzhdin *et al.* 1997; Leips and MacKay 2000). If QTL × environment interactions are common in diverse organisms, they may have important consequences for evolutionary dynamics.

Positional candidates that influence bolting: The availability of mapping data, coupled with the whole-genome sequence map available for *A. thaliana* (Arabidopsis Genome Initiative 2000), provides an opportunity to identify candidate genes for ecological QTL using both functional and positional information. Several QTL detected in this study span regions containing candidate genes with known function in photoperiod signaling or pathways to bolting. For those QTL observed only under long-day conditions, genes active in the photoperiod pathway are likely candidates. Based on the Arabidopsis genome map, 37 genomic Arabidopsis bacterial artificial chromosome (BAC) clones are localized to the top of chromosome 5 within the 95%-support limits for the photoperiod-dependent QTL detected in the Rhode Island spring seasonal cohort. The marker locus nearest to the QTL peak (*ve033*) is positioned on the BAC clone adjacent to the one containing *FLC*, which is polymorphic in this cross. This gene, which encodes a MADS-box transcriptional activator, mediates flowering time via the autonomous and vernalization pathways (Michaels and Amasino 1999, 2001), but is also regulated by genes in the photoperiod pathway (Rouse *et al.* 2002). Another candidate in the region spanning this QTL is *CONSTANS* (*CO*), which encodes a zinc-finger protein and regulates flowering time in response to photoperiod (Putterill *et al.* 1995; Reeves and Coupland 2001). *FLOWERING TIME* (*FT*) is a possible candidate for the QTL at 93 cM on chromosome 1, detected in cohorts experiencing a switch from short to long days in the field. *FT*, a *TFL1* paralog encoding a protein similar to Raf kinase inhibitor, is necessary for early flowering responses to long days mediated by *CONSTANS* and is also regulated by the autonomous pathway (Onouchi *et al.* 2000; Samach *et al.* 2000). *CRYPTOCHROME 2* (*CRY2*) is an attractive candidate for the QTL at the top of chromosome 1. This blue-light photoreceptor plays a central role in perception of long days (Guo *et al.* 1998; Mockler *et al.* 1999). *CRY2* has been identified as the QTL originally referred to as *EDI* in RILs from the L*er* × Cvi cross (El-Assal *et al.* 2001) and differs between the parents in the L*er* × Col cross used in this study by three nonsynonymous substitutions in the coding region (El-Assal *et al.* 2001).

Interesting candidate genes for the QTL on chromosome 4 expressed only in SD and fall-germinating cohorts include *EARLY UNDER SHORT DAYS 4* (*ESD4*; Reeves *et al.* 1997; Koornneef *et al.* 1998) and *EARLY BOLTING UNDER SHORT DAYS* (*EBS*; Reeves *et al.* 1997; Gómez-Mena *et al.* 2001); mutants at both loci flower early under short days. Mutants at *EBS* also increase expression at the floral homeotic genes *APETAL3*, *PISTILLATA*, and *AGAMOUS* (Gómez-Mena *et al.* 2001). The observed mutant phenotypes suggest that naturally occurring alleles at these loci could similarly affect reproductive timing. Candidate-gene analyses must be viewed cautiously, due to the large genomic regions defined by QTL mapping, but these results suggest a starting point for further research identifying the loci that underlie reproductive timing in natural environments.

The finding that many QTL control variation in reproductive timing only in certain natural environments has important implications for gene discovery. QTL mapping is a first step toward identifying developmental loci (Alonso-Blanco *et al.* 1998; El-Assal *et al.* 2001;

Borevitz *et al.* 2002) that may not be found through mutant screens. If allelic variation at some of these loci is expressed only in natural environments, then those loci may never be identified by studies in controlled settings. Field studies of natural allelic variation in realistic ecological settings can therefore be a powerful complement to molecular tools for elucidating the genetic architecture of developmental pathways.

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