# The Association of Flowering Time Quantitative Trait Loci with Duplicated Regions and Candidate Loci in *Brassica oleracea*

**E. J. R. Bohuon,† L. D. Ramsay,* J. A. Craft,* A. E. Arthur,† D. F. Marshall,* D. J. Lydiate† and M. J. Kearsey***

**Plant Genetics Group, School of Biological Sciences, The University of Birmingham, Birmingham B15 2TT, United Kingdom and †Cambridge Laboratory, The John Innes Centre for Plant Science Research, Norwich NR4 7UH, United Kingdom*

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ABSTRACT

A population of 150 doubled haploid lines of rapid cycling *Brassica oleracea*, derived from an $F_1$ from a var. *alboglabra* × var. *italica* cross, was scored for flowering time in two trials. Using information on 82 mapped molecular markers, spread evenly across the nine linkage groups, QTL were identified at six locations; one each on linkage groups O2 and O3 and two each on linkage groups O5 and O9. In total, these QTL explained 58 and 93% of the genetical variation in the two trials. Three of these QTL, on linkage groups O2, O3, and O9, were situated in regions showing considerable homology both with each other and with chromosome regions of *B. nigra* that have been shown to affect flowering time. These same regions are all homologous to a single tract of Arabidopsis chromosome 5, which contains a number of the flowering-related genes, one or more of which may be candidates for the QTL found in Brassica.

THE integration of molecular marker linkage maps with quantitative trait data in segregating populations is now yielding valuable information on the range of quantitative trait loci (QTL) effects and locations in humans and in many plant and animal species (Tanksley 1993; Haley 1995; Stuber 1995; Weekes and Lathrop 1995; Bezant *et al.* 1996, 1997). However, most currently employed methods are limited in the number of QTL that can be detected given experimental populations of realistic size; the more QTL there are underlying a trait, the lower the genetical contribution of each to the total additive genetic variance and hence the lower the probability of detection. Few individual studies have detected more than ten QTL per trait in a single population sample at a given time, while most report around four (Kearsey and Farquhar 1998). Furthermore, which QTL are detected in a given population can vary across environments. The reliability of the results from such experiments on $F_2$, recombinant inbred, or doubled haploid populations are further compromised by the wide confidence intervals associated with QTL position and effect (Darvasi *et al.* 1993; Hyne *et al.* 1995). In order to use the results of such experiments for the introgression of useful QTL into superior breeding lines or, in particular, for map-based gene cloning, greater confidence is needed in the position and action of the putative QTL. The emphasis of such work has thus been to integrate the results with other QTL experiments and with mutation and physiological studies (Touzet *et al.* 1995).

The comparison of the QTL found in experiments involving different crosses and/or different environments can provide additional confidence in their locations (Beavis *et al.* 1991) but such comparisons can be complicated by G × E effects, a lack of common markers, and the likelihood that different crosses will be segregating for different QTL. Comparisons have been possible in cereals and tomato where a large number of QTL experiments have been carried out (Paterson *et al.* 1991; Lin *et al.* 1995; Thomas *et al.* 1995). These comparisons have repeatedly implicated equivalent regions of the genome in the control of particular quantitative traits. Comparisons have also been made between species (Lin *et al.* 1995) using the syntenic relationship between the cereal genomes and the close relationship found in the relative position and effect of some major loci (Laurie *et al.* 1994). Such studies also highlight the control of traits by homologous chromosomal regions within the cereals.

The association of putative QTL with "candidate" genes identified from either physiological or mutation studies has also been explored as a means of determining more accurately the position and effect of the QTL (Mackay 1995). In plants a rapidly growing number of genes are being identified through insertional mutagenesis, map-based gene cloning, or expressed sequence tag (EST) programs, and these are likely to provide a growing resource of potential candidate genes for QTL associated with important agronomic traits such as flowering time and disease resistance. However, the correspondence of such candidate genes with QTL is com-

*Corresponding author:* M. J. Kearsey, Plant Genetics Group, School of Biological Sciences, The University of Birmingham, Birmingham, B15 2TT, UK. E-mail: m.j.kearsey@bham.ac.uk

plicated by the problems of the detection and accurate positioning of the QTL involved. It is likely that there may be several potential candidate genes within the confidence limits of QTL location and care must be taken not to blindly accept the first suitable candidate with a plausible function.

Brassicas are of major international agricultural significance, for example, as vegetables (cauliflower, Brussels sprouts, cabbages, broccoli), animal fodder (kales and swedes), oils (rapeseed), etc. They are also known to have important anti-cancer activity (Beecher 1994). These crop species are also diverse in their morphology and developmental regulation. A greater knowledge of their genetics is therefore long overdue. The present report describes part of a major project to study QTL in Brassicas, using not only conventional mapping populations but also engineered single segment substitution lines, derived by backcrossing, to allow the accurate positioning of QTL (Ramsay *et al.* 1996). The program was developed to explore the extensive genetical variation in this important crop genus, capitalizing on the synteny between and within Brassica genomes and *Arabidopsis thaliana* to facilitate the mapping and identification of potential candidate loci.

In this article, the QTL analyses are presented from two trials of a population of doubled haploid lines of *B. oleracea* used to construct a detailed linkage map (Bohuon *et al.* 1996). Of the six QTL affecting flowering time discovered, three were found in duplicated, homologous regions of the *B. oleracea* genome, which are syntenic with a region of Arabidopsis chromosome *5* known to contain several flowering-related candidate genes.

## MATERIALS AND METHODS

**Plant material:** A population of 169 doubled haploid (DH) lines was developed from four identical $F_1$ plants produced from the cross between two doubled haploid parents of diverse origin: a rapid cycling line, *B. oleracea* var. *alboglabra* (A12DHd) and a calabrese, *B. oleracea* var. *italica* (GDDH33). For simplicity, we will refer to these parental lines as A12 and GD, respectively. Details of the construction of the population and the derived linkage map as well as the nomenclature and orientation of the linkage groups used in this article haves been given elsewhere (Bohuon *et al.* 1996). A sample of 149 of these lines was available for the trials.

**Trial design:** Two trials were planted adjacent to one another in 1994 in the trial field at the John Innes Centre, Norwich, UK. Both trials consisted of individually randomized plants in a single block, a design chosen to maximize the power of QTL detection (Kearsey and Pooni 1996). The first trial, sown on April 11, contained five replicates from each of 148 lines while the second trial, sown on the May 31, was based on 109 lines (108 of which were a subset of the 148 lines used in trial one) but with double the replication. Parental lines were also incorporated into each trial. The plants for both trials were raised from seed germinated on moist filter paper in petri dishes placed in an incubator at 18° for 2 days. The seeds were then directly sown into pots, in their random positions, in an unheated glasshouse and transplanted in the field at the four- to five-leaf stage. The plants were arranged in the field 40 cm apart in rows spaced 1 m apart. The trial was surrounded by a row of guard plants to minimize edge effects and was protected from bird damage by a netted cage. Flowering time was measured on each individual plant as the number of days from sowing to the appearance of the first open flower.

**TABLE 1**

**Summary of numbers and positions marker loci used for QTL mapping**

| Linkage group | Length (Haldane cM) | No. of markers | Average distance between markers (Haldane cM) |
|---|---|---|---|
| 01 | 118.2 | 8 | 16.9 |
| 02 | 118.9 | 9 | 14.9 |
| 03 | 157.5 | 15 | 11.3 |
| 04 | 110.1 | 11 | 11.0 |
| 05 | 104.2 | 8 | 14.9 |
| 06 | 73.2 | 5 | 18.3 |
| 07 | 88.9 | 7 | 14.8 |
| 08 | 85.3 | 9 | 10.7 |
| 09 | 112.8 | 10 | 12.5 |
| Total | 969.1 | 82 | 13.3 |

**QTL analyses:** The results were analyzed using the genetic linkage map derived from the original population of 169 DH lines (Bohuon *et al.* 1996), which contained 310 loci arranged into nine linkage groups covering a total length of 875 cM (Kosambi) (969 cM Haldane). These consensus map distances were used, rather than those estimated from the particular subset of lines and markers in the trials, because the consensus map is more useful for comparisons across studies. Moreover, simulation studies (Hyne *et al.* 1995) have shown that, given the number of lines used, both maps would lead to very similar QTL locations.

For QTL mapping we used a subset of 82 loci chosen to provide an even coverage of the genome. These were spaced approximately 10–18 cM apart (Table 1). On average, for any given locus marker information was available for 91% of the DH lines, as only those loci with reliable genotype data in a high proportion of the lines were used.

The main analysis was based upon the marker regression approach of Kearsey and Hyne (1994) using the software on web site http://web.bham.ac.uk/g.g.seaton/. ANOVAs test for the presence of one or more QTL and the probabilities associated with *F* values of items in the ANOVA, as well as the confidence intervals of the estimated positions and gene effects, are obtained by carrying out 1000 simulations. Adequate models are accepted when the residuals are no longer significant. It is important to note that, because the *F* tests are assessed by simulation, normal significance levels, *i.e.*, 5%, etc., can be applied. This is because the multiple tests at different putative QTL positions are subsumed within the simulations and cease to be a factor. For comparison, the data were also analyzed using the SIM function in the MQTL program (Tinker and Mather 1995), which uses the interval mapping approach of Haley and Knott (1992), and using MAPMAKER/QTL (Lander *et al.* 1987; Lander and Botstein 1989; see Bohuon 1996). The results of these analyses gave essentially similar results but are not reported here.

**Comparative mapping:** Comparisons within the *B. oleracea* genome were performed using the multiple loci found by the restriction fragment length polymorphism (RFLP) probes used to construct the linkage map derived from the DH lines

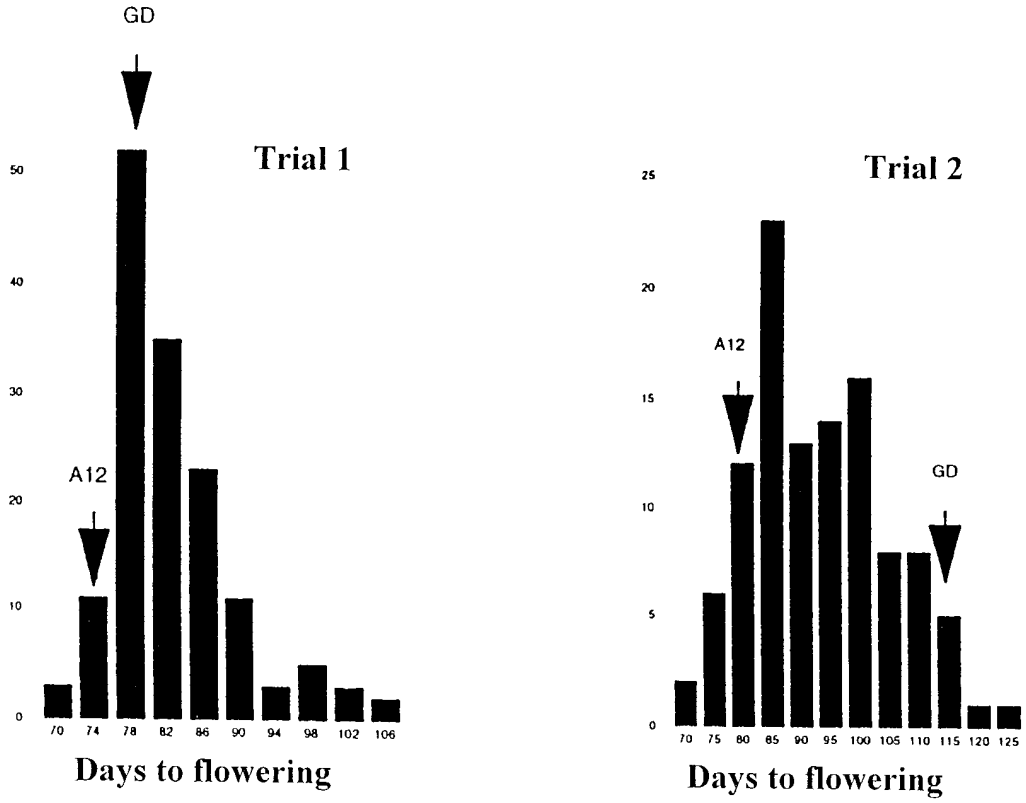


Figure 1.—Distribution of flowering times among the DH lines in the two trials. The parental (A12 and GD) means are indicated with arrows.

(Bohuon *et al.* 1996). *B. oleracea* linkage groups are described using the notation O1 through O9, the O standing for *oleracea*. The genetic map was also compared with that of *B. nigra* reported by Lagercrantz and Lydiate (1995) because a subset of RFLP probes detected polymorphism in both populations. This comparison allowed the position of the Arabidopsis homologues in *B. oleracea* to be inferred from the fine mapping comparison made between *A. thaliana* and *B. nigra* (Lagercrantz *et al.* 1996). In addition, some of the Arabidopsis clones used for the fine comparative mapping were also used on the *B. oleracea* DH population to confirm the position of the homologous regions. The clones used included the cDNA c339 and the YAC end fragments Labi8E7, LEW6G7, and LEW21F12 (see Lagercrantz *et al.* 1996). In addition a cDNA of the late allele, *co* of the *CONSTANS* gene, *CO* (Coupland 1995; Putterill *et al.* 1995), kindly supplied by G. Coupland, (John Innes Centre, Norwich, UK), has recently been hybridized to a subset of our *B. oleracea* DH mapping population.

## RESULTS

**QTL analyses:** The distribution of flowering times among the DH lines in each trial are shown in Figure 1 and their ANOVAs in Table 2. There were highly significant flowering time differences between lines on both occasions although the estimates of additive genetic variance, $V_A$ ($= \frac{1}{2}\Sigma a^2$) and environmental variance, $V_E$, were much larger in the later trial (see Kearsey and Pooni 1996 for definition of terms).

Table 3 presents a summary of the marker regression ANOVAs. There was no evidence for QTL on linkage groups O1, O4, O6, O7, and O8 in either trial, because neither the regression nor the residual items were significant. Moreover, single marker ANOVAs (data not shown) also failed to detect any significant effects on these chromosomes. Linkage groups, O2, O3, and O5 gave evidence for no more than one QTL in either trial, as indicated by significant regression but nonsignificant residual items, based on a single QTL model. Different, nonoverlapping QTL were located on linkage group O5 in the two trials. Linkage group O9 showed evidence for more than one QTL in one or both trials (significant residual and regression), and one additional QTL was fitted, resulting in the residual becoming nonsignificant. Figure 2 illustrates graphically the fit of a one- and two-QTL model to the marker means for linkage groups O2 (trial one) and O9 (trials one and two), respectively.

Table 4 summarizes the locations and additive effects of the QTL identified in each trial and this information is illustrated as chromosome ideograms in Figure 3. We have identified five QTL in each trial, with three QTL being significant in both. The sign of the additive effect is negative when the A12 allele causes earlier flowering and is positive otherwise. Because the A12 parent was selected for early flowering, we would expect most additive effects to be negative but one late flowering allele is still present towards the end of linkage group O9. It is important to note that, when there is more than one QTL on a chromosome (as in O9), the true additive effects, as shown in Table 4, do not match the size of the peaks in Figure 2. This is because the effects on the marker means of individual QTL are inflated or deflated by the other QTL to which they are linked—inflated if

**TABLE 2**

**ANOVA of the DH lines for flowering time from the two trials together with estimates of genetical and environmental components**

| | Trial 1 | | | | Trial 2 | | | |
|---|---|---|---|---|---|---|---|---|
| Source | d.f. | MS | *P* | ems | d.f. | MS | *P* | ems |
| DH lines | 147 | 219.0 | <0.001 | $\sigma^2 + 4.83\ \sigma^2 b$ | 108 | 1068.2 | <0.001 | $\sigma^2 + 8.06\ \sigma^2 b$ |
| Error | 567 | 10.0 | | $\sigma^2$ | 770 | 62.2 | | $\sigma^2$ |
| | | | | Estimates | | | | |
| $V_A (= \frac{1}{2}\sigma^2 b)$ | | 21.6 | | | | 62.4 | | |
| $V_E (= \sigma^2)$ | | 10.0 | | | | 62.2 | | |

the increasing alleles are in coupling, but deflated if in repulsion as they are on O9. The QTL are "named" according to their chromosomes and positions in these two trials simply to facilitate comparisons between Table 4 and Figure 2. These names should not be assigned any other, more permanent, significance.

Table 4 also shows the percentage of the additive genetical variation in each trial, $V_A (= \frac{1}{2}\Sigma a^2)$, accounted for by each individual QTL, as well as the overall percentage of the $V_A$ explained. Over all QTL, we have explained 58 and 93% of the additive genetical variation in trials one and two, respectively. It should be noted that, when there are two (or more) QTL on a chromosome, the expected contribution of that chromosome to the additive variance is not simply the sum of the individual QTL, because of the bias introduced by linkage disequilibrium (see Kearsey and Pooni 1996). The corrected contributions of each chromosome are shown in parentheses in Table 4.

**Comparative mapping:** Examination of multiple loci detected with the same RFLP probe allowed the comparison of the arrangement of homologous loci on different linkage groups. Figure 4 shows the *B. oleracea* linkage groups O2, O3, and O9 and the *B. nigra* linkage groups LG2 and LG8 to illustrate the collinearity that exists over large sections of the linkage groups. The degree of collinearity for linkage groups O2, O3, and O9 is considerable with 73, 41, and 53 cM (Haldane) bound by loci detected by the probes W116 or W200 at one end and by R34 and/or N120 and W133 at the other. Our ability to define the precise ends of the homologous tracts is limited by the availability of probes which will detect *Eco*RI polymorphisms in all tracts. Linkage groups LG2 and LG8 from *B. nigra* have been shown to have close fine scale collinearity with a region of chromosome 5 of *A. thaliana* (Langercrantz *et al.* 1996). A subset of the Arabidopsis probes (c339, Labi8E2, LEW6G7), used to demonstrate that collinearity, has also been located on our *B. oleracea* map and this is shown in Figure 4. These probes map to the expected regions given the larger scale homology between *B. oleracea* and *B. nigra* shown by the Brassica RFLP markers. More recently, we have mapped an *Eco*RI polymorphism detected by an Arabidopsis *co* cDNA to the expected region of group O2 adjacent to the C339 locus. This confirms that groups O2, O3, and O9 have homologous terminal tracts that contain a central region corresponding to the location of *CO* on chromosome 5 of Arabidopsis (see box illustrating Arabidopsis CO contig in Figure 4). This has been confirmed by the location, adjacent to C339, of a locus homologous to a cDNA of the Arabidopsis *CONSTANS* gene (see Figure 4). *Brassica oleracea* linkage group O4 also has interstitial markers in common with this same Arabidopsis region, but its limits are less clear and no QTL were identified on this chromosome. The regions syntenic to Arabidopsis are also indicated on the chromosome ideograms in Figure 3. Comparison of the QTL locations in Figure 3 with the Arabidopsis syntenic regions in Figure 4 shows them to be located within the Arabidopsis regions and to overlap the region containing the *CO* contig for FT02.1, FT03.1, and FT09.2.

**TABLE 3**

**Items from marker regression ANOVAs for all linkage groups for which significant effects were detected in either trial**

| Linkage group | Trial | Regression[a] | Residual for one QTL[a] |
|---|---|---|---|
| 02 | 1 | <0.01 | 0.81 |
| | 2 | 0.25 | 0.64 |
| 03 | 1 | 0.03 | 0.10 |
| | 2 | <0.001 | 0.27 |
| 05 | 1 | <0.001 | 0.76 |
| | 2 | <0.001 | 0.83 |
| 09 | 1 | <0.001 | 0.02 |
| | 2 | 0.04 | 0.007 |

[a] Data are probabilities associated with regression and residual means squares, based on one QTL.

## DISCUSSION

The data from the two trials suggest that there are at least six QTL that affect flowering time segregating in

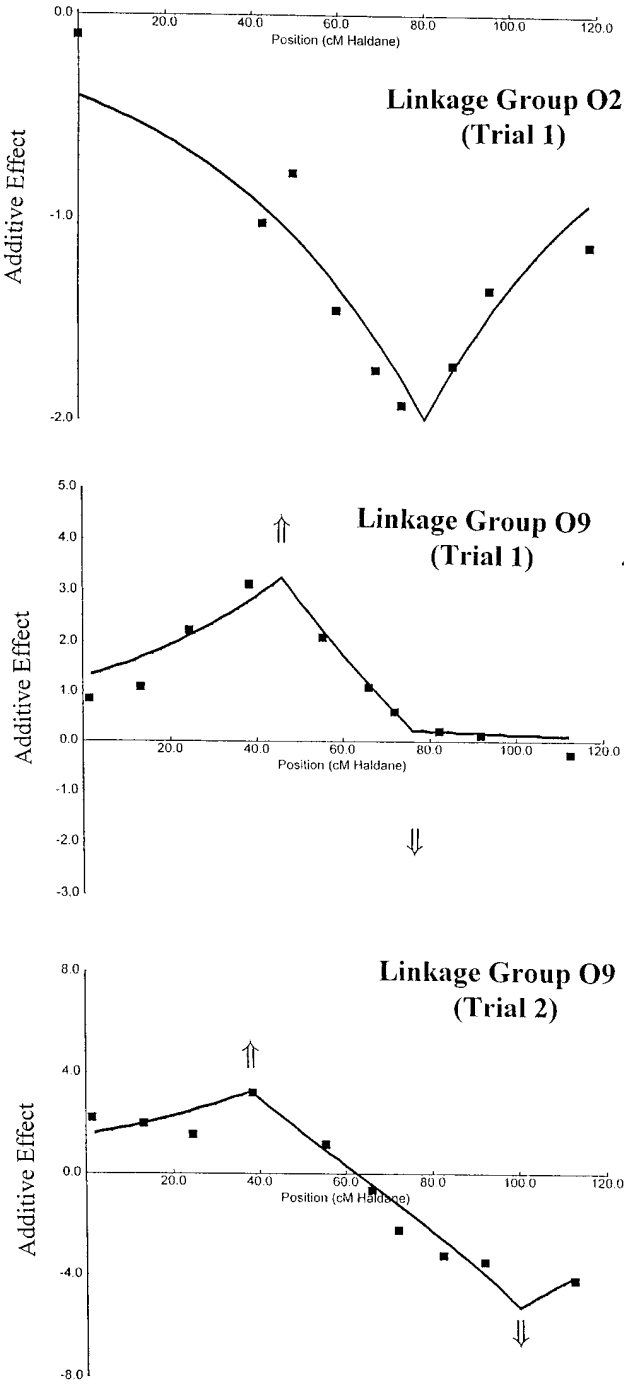


Figure 2.—The fit of the additive effects of individual markers to a one- and two-QTL model. The abscissa represents the position on the chromosome in centimorgans (Haldane), the ordinate represents the additive effect (half the difference between the marker means). The observed and expected values associated with each marker are shown by the solid squares and lines, respectively. Top, a single QTL on linkage group O2 in trial one (QTL FT02.1 at 80 cM); center and bottom, two QTL in repulsion at opposite ends of linkage group O9 (QTL FTO9.1 and FTO9.2) at 46 and 76 cM in trial one and at 38 and 100 cM in trial two. In the latter two, the arrows indicate the position and effect of each QTL. See Table 4 for QTL names.

this cross: one each on linkage groups O2 and O3 and two each on linkage groups O5 and O9 (Table 4; Figure 3). It has been argued, on statistical grounds (Hyne and Kearsey 1995), that it would be difficult to detect more than 12 QTL in any single trial and our results are consistent with this.

The large 95% confidence intervals (Figure 3) for QTL locations are much as expected (Darvasi *et al.* 1993; Hyne *et al.* 1995) and do not conflict with the close similarity of gene locations in the two trials. This latter similarity arises because the same set of DH lines is used in both trials, not a different sample from the same cross: the confidence intervals, on the other hand, are obtained by the simulated resampling of different DH lines from the same cross and hence give the relevant estimates of precision. These wide confidence intervals emphasize the value of alternative experimental designs, such as the use of backcross substitution lines (Ramsay *et al.* 1996), to improve precision.

The range of flowering times shown by the extreme DH lines ($2\Sigma|a|$) is 33 and 55 days in trials one and two, respectively. The corresponding additive effects of the detected QTL, $2\Sigma|a|$ (Table 4) from each trial are 24.8 and 49.4 days; *i.e.*, they explain a large part but by no means all the total spread of flowering observed in the DH lines. However, the earlier flowering A12 parent contains at least one late-flowering allele for which GD contains the alternative, early allele. Allowing for the sign of the $a$'s gives $\Sigma a$ of 3.4 and 14.3 days which explains the much smaller difference in flowering times between the two parental lines in trial one (Figure 1) and the transgressive segregation in the DH lines. The preponderance of earlier-flowering alleles in the A12 parent presumably reflects the history of past selection for early flowering in this rapid cycling line compared with that in a commercial calabrese variety.

The QTL identified explain 58 and 93% of the genetic variation in trials one and two, respectively. There is, thus, still considerable genetical variation to be explained in trial one, and so there are probably more than five QTL for flowering time segregating in this cross. Those QTL detected all have very similar sized effects within each trial. Thus they vary between 1.8 to 4.5 days in trial one and between 2.0 and 6.8 days in trial two (Table 4).

Two previous studies using mapped populations of *B. oleracea* have found significant QTL for flowering time. Kennard *et al.* (1994), using single factor ANOVA on an $F_2$ from a cabbage $\times$ broccoli cross, found regions on two linkage groups that had a strong effect on flowering time and evidence for smaller effects on another three linkage groups. Camargo and Osborn (1996) found three linkage groups with significant QTL for flowering time using $F_3$ families from a different cross between cabbage and broccoli. Unfortunately, because of the lack of a standardized nomenclature for linkage groups in *Brassica* spp. and a lack of common probes,

**TABLE 4**

**Properties of QTL located; position, additive effects and % genetic variance explained**

| Linkage group | QTL name | Position cM | | Additive effect ($a$) | | $\%V_A$ explained | |
|---|---|---|---|---|---|---|---|
| | | Trial 1 | Trial 2 | Trial 1 | Trial 2 | Trial 1 | Trial 2 |
| O2 | FTO2.1 | 80 | 82[a] | −2.0 | −2.0[a] | 9 | 3[a] |
| O3 | FTO3.1 | 12 | 12 | −1.8 | −6.3 | 8 | 32 |
| O5 | FTO5.1 | 16 | 46 | −1.9 | −4.4 | 8 | 16 |
| O9 | FTO9.1 | 46 | 38 | +4.5 | +5.2 | 47 | 22 |
| | FTO9.2 | 76 | 100 | −2.2 | −6.8 | 11 | 37 |
| | | | | | | (33) | (42) |
| No. of QTL: | | 5 | 5 | | | | |
| $\Sigma a^2$ | | | | 35.94 | 136.33 | | |
| ($\Sigma a^2$ allowing for linkage on O9) | | | | (25.07) | (115.86) | | |
| $2V_A$ = Exp $\Sigma a^2$ | | | | 43.2 | 124.8 | | |
| Total $V_A$ explained (%): | | | | (58) | (93) | 58 | 93 |
| $\Sigma\|a\|$ | | | | 12.4 | 24.7 | | |
| $\Sigma a$ | | | | −3.4 | −14.3 | | |

The sign before the additive effect is negative when A12 carries the "early" allele and positive if A12 carries the "late" allele; for individual QTL, the % variance explained is $a^2 \times 100/2V_A$, except for that for linkage group O9 with two QTL, which allows for the effect of linkage and is shown in parentheses. The estimates of $V_A$ are taken from Table 2.

[a] Item not significant but most likely QTL position and effect added for comparison.

it is not yet possible to compare the QTL found in these two studies with each other or with those found in the present experiment.

The use of common probes has revealed the essential identity of the C genomes of *B. oleracea* and *B. napus* (Bohuon *et al.* 1996) and the extensive collinearity of the large segments of the A and C genomes in *B. napus* (Parkin *et al.* 1995). Field experiments involving *B. napus* populations genotyped with these common probes have indicated that regions on linkage groups in the A genome that show homology to the regions in O2, O3, and O9 also carry QTL for flowering time (Keith 1996; Osborn *et al.* 1997; Salinas-Garcia 1996). This is also true for *B. nigra* where two homologous regions on LG2 and LG8, collinear with the same *B. oleracea* linkage groups, explained 53 and 12% of the total variation for flowering time (Lagercrantz *et al.* 1996). Teutonico and Osborn (1995), in a comparison of the results of an experiment with an unvernalized $F_2$ population of *B. rapa* with those from a similar experiment using DH lines in *B. napus* (Ferreira *et al.* 1995), found some evidence that two linkage groups that showed homology between the genomes contained flowering time QTL. From common probes it is likely that these QTL map to either the *B. napus* homologues of the *B. oleracea* linkage groups O2, O3, and O9 found to be associated with flowering time in this study or to the equivalent A genome regions. It seems, therefore, that the same homologous regions within and between the genomes of three different Brassica species control a significant proportion of the genetic variance of flowering time.

This correspondence of QTL, across and within genomes, has also been observed in cereals. For example, a review of recent work showed that homologous regions within the maize genome carry similar QTL and there is also evidence for equivalent regions being of importance for the same traits in sorghum (Lin *et al.* 1995).

The large scale collinearity observed between the *B. oleracea* linkage groups O2, O3, and O9 and the *B. nigra* linkage groups LG2 and LG8 (Figure 4) is of particular interest given the fine scale collinearity between the *B. nigra* linkage groups and chromosome *5* of *A. thaliana* (Lagercrantz *et al.* 1996). The present study shows that the fine scale collinearity also extends to the homologous regions of *B. oleracea* (Figure 4). Moreover, these homologous regions within the *B. oleracea* genome, linkage groups O2, O3, and O9, also affect the same quantitative trait. Thus QTL FTO3.1, FTO9.2, and possibly FTO2.1 fall within the homologous Arabidopsis sequence (Table 4; Figures 3 and 4), while all have decreasing and similar effects in A12. They also overlap the positions syntenic with the Arabidopsis *CO* contig. Although the Arabidopsis tracts found on linkage groups O2, O3, and O9 are probably the same physical length, it is interesting to note that they have different genetic map lengths, 73, 41, and 53 cM, in the three *B. oleracea* regions. These differences between relative physical and genetic lengths have implications

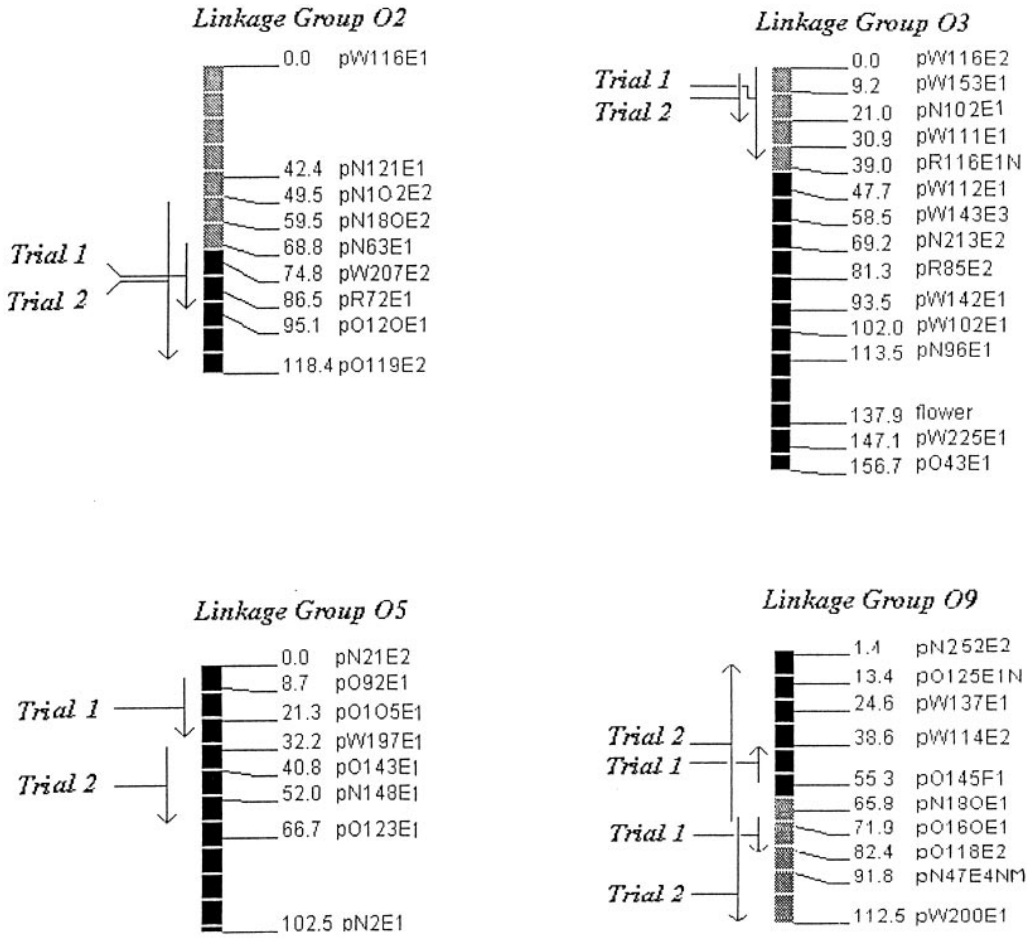


Figure 3.—Ideograms of those linkage groups showing the positions of the flowering time QTL in each trial. The most likely position of each QTL is indicated by the horizontal line attached to the arrows. The direction of the arrow (down or up) indicates whether A12 has the early- or late-flowering allele, respectively; the length of the arrows indicates the 95% confidence interval. The regions of homology with Arabidopsis chromosome 5 are shown by the gray regions of linkage groups 02, 03, and 09. The markers and their map positions are shown to the right of each linkage group.

for candidate locus searches. The region on chromosome O4 is shorter and less well defined, although it does contain the *CONSTANS* gene, *CO*.

The *CO* gene is in the center of this region of chromosome *5* in Arabidopsis and we know from the current study that it is present in at least the homologous O2 and O4 regions in *B. oleracea*. The sequence of *CO* has recently been obtained by map-based gene cloning and it appears by homology to be a zinc-finger-based transcription factor that is involved in the photoperiod-sensitive regulation of flowering time (Putterill *et al.* 1995). Plants carrying the *co* mutation are late flowering under long days but flower at the same time as the wild type under short days (Koornneef *et al.* 1991).

In Arabidopsis the region of chromosome *5* containing *CO* has been shown to play a significant role in the control of flowering time and the highly correlated character, leaf number at flowering, in natural ecotypes. QTL for these characters have been found in this region in the analyses of segregating populations derived from several crosses between different ecotypes, and G × E effects for these QTL have also been observed (Clarke *et al.* 1995; Jansen *et al.* 1995). The region around *CO* on chromosome *5* also contains several other known flowering time mutants (Koornneef *et al.* 1994). The interaction with vernalization and another QTL (possibly the *FRI* locus) positioned on chromosome *4* led Clarke *et al.* (1995) to postulate that the candidate locus for the QTL found on chromosome *5* in their study was *FLC* rather than *CO*.

Because of the increased precision that will be available, we expect to be able to account for the variation in flowering time in this cross more fully as trial data become available from the set of backcross substitution lines we have created based on the introgression of short tracts from the GD parent into A12 (Ramsay *et al.* 1996). Further rounds of recombination using suitable lines as parents will enable us to locate the QTL more precisely relative to *CO* and other potential candidate genes. We are also currently performing crosses between the substitution lines to construct a derived set of lines that contain all possible allelic combinations of the three *CO* homeologous tracts. The analysis of such interactions will be of particular interest in light of the observations of Putterill *et al.* (1995), which showed that increasing the copy number of *co* in transformation experiments led to earlier flowering. This suggests that the *co* gene product is limiting. Interactions between flowering time at the QTL level have already been observed in segregating populations of Arabidopsis (Clarke *et al.* 1995)

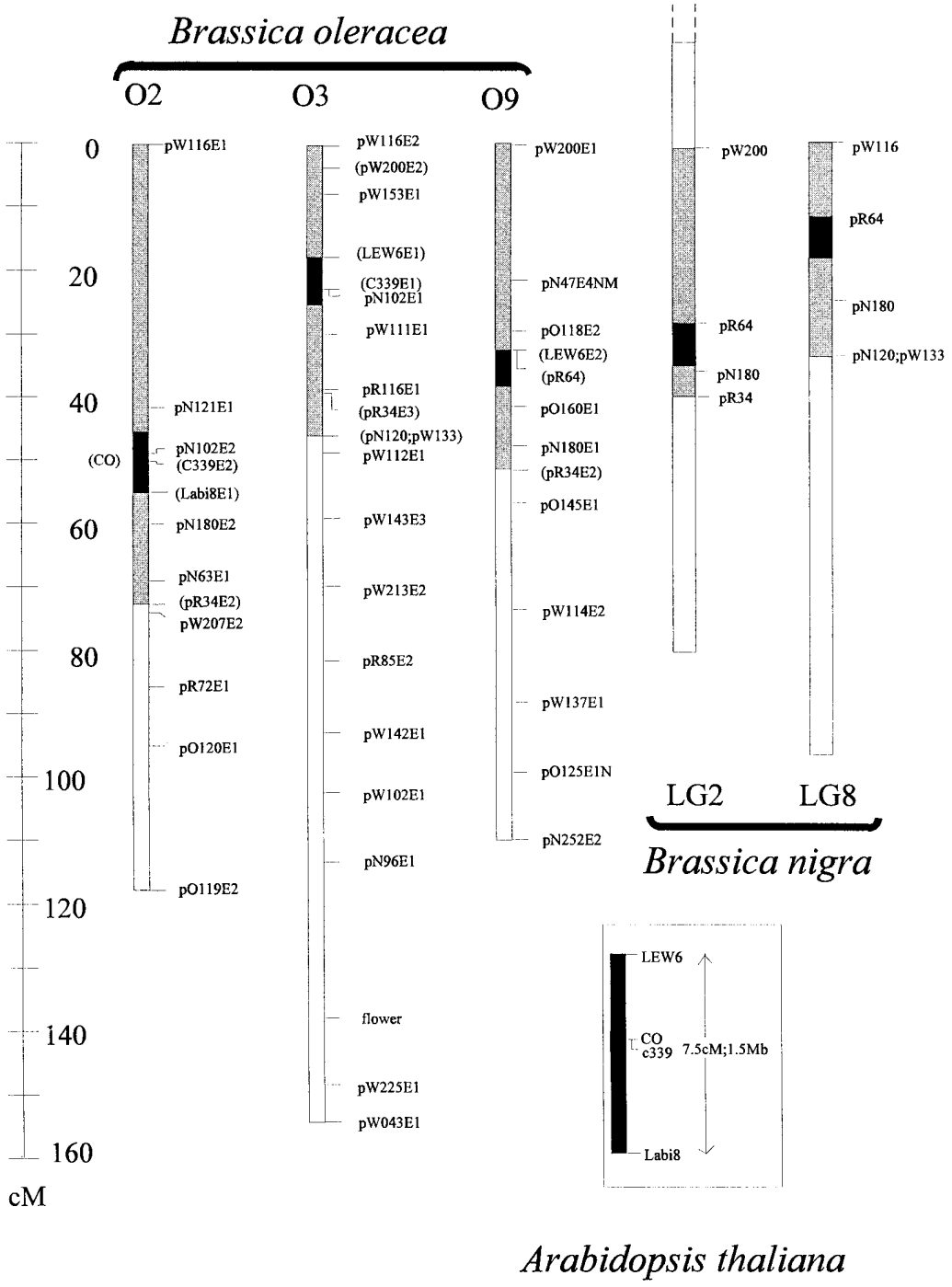


Figure 4.—A comparison of the *B. oleracea* linkage groups O2, O3, and O9 with *B. nigra* linkage groups LG2 and LG8 and part of Arabidopsis chromosome *5*. Syntenic regions with Arabidopsis chromosome *5* are shown in gray. The black regions are those corresponding to the Arabidopsis *CO* contig shown in the box at the bottom of the figure. N.B.: Linkage groups O9, LG2, and LG8 are inverted for ease of comparison. Key loci for defining syntenic regions, including the *CONSTANS* gene (*CO*), but not used as markers in the QTL analyses are shown in brackets. For LG2 only part of the chromosome is shown.

and *Brassica* spp. (Camargo and Osborn 1996; Lagercrantz *et al.* 1996) but such experiments are intrinsically insensitive because of the low probability of occurrence of certain multi-locus genotypes.

The present study has highlighted the potential of integrating QTL analyses with comparative genome studies and suggested candidate loci. This approach, however, is dependent on the further elucidation of the Brassica/Arabidopsis synteny and of the genome evolution within Brassica genus (Lagercrantz *et al.* 1996; Teutonico and Osborn 1995). The comparison of experiments is also dependent on the adoption of a standard nomenclature for the linkage groups with a framework of shared marker loci.

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