Comparison of Flowering Time Genes in *Brassica rapa*, *B. napus* and *Arabidopsis thaliana*

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ABSTRACT

The major difference between annual and biennial cultivars of oilseed *Brassica napus* and *B. rapa* is conferred by genes controlling vernalization-responsive flowering time. These genes were compared between the species by aligning the map positions of flowering time quantitative trait loci (QTLs) detected in a segregating population of each species. The results suggest that two major QTLs identified in *B. rapa* correspond to two major QTLs identified in *B. napus*. Since *B. rapa* is one of the hypothesized diploid parents of the amphidiploid *B. napus*, the vernalization requirement of *B. napus* probably originated from *B. rapa*. Brassica genes also were compared to flowering time genes in *Arabidopsis thaliana* by mapping RFLP loci with the same probes in both *B. napus* and Arabidopsis. The region containing one pair of Brassica QTLs was collinear with the top of chromosome *5* in *A. thaliana* where flowering time genes *FLC*, *FY* and *CO* are located. The region containing the second pair of QTLs showed fractured collinearity with several regions of the Arabidopsis genome, including the top of chromosome *4* where *FRI* is located. Thus, these Brassica genes may correspond to two genes (*FLC* and *FRI*) that regulate flowering time in the latest flowering ecotypes of Arabidopsis.

THE genus Brassica includes species with many morphological forms that are cultivated for vegetables, oils, fodder and condiments. Major differences among crop types are regulated, in part, by genes that control flowering time. Forms such as broccoli and spring oilseed rape are annuals and flower in the seeding year; other forms, such as cabbage and winter oilseed rape, are biennials and require several weeks of exposure to low temperatures (vernalization) to induce flowering.

We previously identified genes controlling vernalization-responsive flowering time in *B. napus* (FERREIRA *et al.* 1995) and *B. rapa* (TEUTONICO and OSBORN 1995) by analyzing segregating populations derived from crosses of annual and biennial oilseed cultivars. Two major quantitative trait loci (QTL) were positioned on linkage groups (LG) 2 and 8 of a *B. rapa* RFLP map. *For B. napus*, we identified a single major QTL on LG 9 and minor QTL effects associated with LG 12 and LG 16. We also found preliminary evidence that the QTLs in the two species might represent equivalent genes (TEUTONICO and OSBORN 1995).

Many loci with late-flowering alleles have been identified in the related crucifer *Arabidopsis thaliana*, some of which confer phenotypes that are responsive to vernalization (KOORNNEEF *et al.* 1991). The latest flowering ecotypes of Arabidopsis have late-flowering alleles at the *FRI* locus on the top of chromosome *4* (BURN *et al.* 1993; LEE *et al.* 1993; CLARKE and DEAN 1994) and at *FLC* on the top of chromosome *5* (KOORNNEEF *et al.* 1994; LEE *et al.* 1994a). These two loci account for the late-flowering phenotype in progeny of crosses to Landsberg *erecta*, and plants with the late-flowering alleles are responsive to vernalization. The tops of chromosomes *4* and *5* also contain other loci (*LD* on chromosome *4* and *FY* and *CO* on chromosome *5*) for which recessive (*LD* and *FY*) or semidominant (*CO*) mutations confer late-flowering phenotypes (KOORNNEEF *et al.* 1991; LEE *et al.* 1994b). Mutants with late-flowering alleles at *FY* and *LD* are responsive to vernalization, but the *CO* late-flowering mutant is only weakly responsive.

Arabidopsis is an attractive species for studying the genetics of flowering time because of the relative ease of cloning specific genes. Two genes controlling flowering time have been cloned: *LD* (LEE *et al.* 1994b) and *CO* (PUTTERILL *et al.* 1995), and there are ongoing efforts to clone other genes. Some of the genes that have been or will be cloned in Arabidopsis may have homologues that regulate this trait in Brassica species. LAGERCRANTZ *et al.* (1996) presented evidence for homology of *CO* with flowering time genes in *B. nigra*. Information on the homology of Arabidopsis and Brassica genes provides insight on the evolution of traits and new avenues for manipulating traits in Brassica crops.

In this article, we report on a further analysis of the *B. napus* flowering time data using additional RFLP and AFLP marker loci detected in this population and on a new analysis of a recombinant inbred population derived from the same *B. rapa* cross studied previously.

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The map positions of flowering time QTL detected in these populations are compared between the Brassica species and to the map positions of flowering time genes in *A. thaliana*. Our results provide strong evidence that homologous genes control vernalization-responsive flowering time in both *B. rapa* and *B. napus* and that these genes may be homologous to flowering time genes identified in Arabidopsis.

MATERIALS AND METHODS

QTL controlling flowering time were identified in a population of 89 oilseed *B. napus* double haploid (DH) lines and a population of 72 oilseed *B. rapa* recombinant inbred (RI) lines. The *B. napus* population was developed from a cross of the winter rapeseed cultivar Major by the spring canola cultivar Stellar. An RFLP map based on segregation in this population (FERREIRA *et al.* 1994) was expanded from 132 to 480 loci by adding 71 RFLP loci, five isozyme loci, two disease resistance loci, two erucic acid loci (THORMANN *et al.* 1996), and 268 AFLP loci using previously described methods (VOS *et al.* 1995). The expanded map provided marker genotype information in previously unexplored regions of the genome. The *B. rapa* RI population was developed by single-plant descent to the F_6 generation using F_2 plants derived from a cross of the winter turnip rape cultivar Per by the spring sarson cultivar R500. Eighty-seven RI lines were used to construct a genetic linkage map (KOLE *et al.* 1997) consisting of three phenotypic marker loci and 143 RFLP loci detected with 102 DNA probes, most of which were used previously to construct a linkage map of an F_2 population derived from this cross (TEUTONICO and OSBORN 1994).

Flowering time data for the *B. napus* population was from a previously described experiment using three treatments: 0, 4 and 8 wk of vernalization (FERREIRA *et al.* 1995). The *B. rapa* population was tested for flowering time in a 1995 Madison, Wisconsin field trial using the experimental approach described by FERREIRA *et al.* (1995), except that four replications with one plant per replication and two treatments (0 and 6 wk of vernalization) were used. Data were recorded as days to flowering (DTF) from transplanting and lines that showed no sign of flowering at the end of the experiment were assigned a value of 100 DTF.

QTLs for flowering time were identified by interval mapping (LANDER and BOTSTEIN 1989) using MAPMAKER/QTL 1.1 (LINCOLN *et al.* 1992). The genomes were scanned initially for potential QTLs using a LOD (log likelihood of the odds ratio that a QTL is present *vs.* absent) threshold of 2.0. The effects of the QTL with the largest LOD score were fixed, and the genomes were rescanned using an increased LOD score of 2.0 to find additional QTLs. This process was repeated until no other significant QTL effects were detected. The LOD score, percentage variation explained and additive effects of each QTL were determined by removing individual QTL effects from the complete multilocus model (ZENG 1994). Map position confidence intervals for each QTL were defined by a 1.0 LOD reduction from the peak LOD score for the complete multilocus model. Additive by additive epistasis was tested by a two-factor analysis of variance (EDWARDS *et al.* 1987) using the genotypes of marker loci closest to the peak LOD scores of QTLs.

Arabidopsis DNA clones detecting RFLP loci at the tops of chromosomes *4* (near *FRI*) and *5* (near *FLC*, *FY*, and *CO*) (LISTER and DEAN 1993; CHERRY *et al.* 1996) were used as probes to identify segregating RFLP loci in a *B. napus* DH population derived from a cross of a resynthesized by a natural *B. napus* that was highly polymorphic for restriction fragments (PARKIN *et al.* 1995). DNAs from 10 DH lines selected for mapping informativeness were digested with six restriction enzymes (*Bgl*II, *Cfo*I, *Cla*I, *Eco*RI, *Eco*RV and *Hin*dIII), and Southern blots were probed with the Arabidopsis DNA clones. Segregation data for the 10 lines were used to approximately position the marker loci with respect to RFLP loci that had been mapped previously in this population (PARKIN *et al.* 1995).

Brassica DNA clones detecting RFLP loci near Brassica flowering time QTLs were used as probes to identify segregating RFLP loci in an Arabidopsis RI population (LISTER and DEAN 1993). Thirty RI lines were selected for mapping informativeness, and DNAs from the lines were digested individually with seven restriction enzymes (*Bgl*II, *Cfo*I, *Cla*I, *Eco*RI, *Eco*RV, *Hin*dIII and *Hpa*II), Southern blotted, and probed with Brassica DNA clones. Segregation data for the 30 lines were used to position the marker loci in an RFLP map of the population (LISTER and DEAN 1993; CHERRY *et al.* 1996).

The *B. napus* map of Major × Stellar was aligned with a *B. napus* map derived from another winter by spring cross (N-o-9 × N-o-1; SHARPE *et al.* 1995). This alignment was based on 39 RFLP loci detected with the same probes in each population, most of which detected at least one allele in common, and 111 AFLP loci showing the same allele for a particular enzyme-primer combination. The N-o-9 × N-o-1 map had been previously aligned with the resynthesized × natural *B. napus* map (SHARPE *et al.* 1995; PARKIN *et al.* 1995; I. A. P. PARKIN and D. J. LYDIATE, unpublished results), and this allowed alignment of the Major × Stellar map to the resynthesized × natural map. *B. napus* linkage groups homologous to the *B. rapa* genome were known from the resynthesized × natural map (PARKIN *et al.* 1995), and these groups were aligned to *B. rapa* linkage groups by searching for the most conserved linkages of RFLP loci detected with the same probes in each population.

RESULTS AND DISCUSSION

Flowering time loci in *B. napus*: The *B. napus* DH population given no vernalization treatment segregated for flowering time, as reported previously (FERREIRA *et al.* 1995). One group and the annual parent (Stellar) flowered earlier than the F_1, a second group flowered later than the F_1, and a third group and the biennial parent (Major) did not flower in this experiment (Figure 1a). After 8 wk of vernalization, all DH lines flowered early (Figure 1b). The biennial parent also flowered under these conditions, but later than the annual parent.

The largest QTL effect for flowering time was detected on LG 9 (Table 1), as reported previously by FERREIRA *et al.* (1995). The additional RFLP and AFLP marker loci extended LG 12 as reported previously (FERREIRA *et al.* 1994); and, in this re-analysis, a second QTL with a large effect was detected in the expanded LG 12 (Table 1). These two QTLs explained much of the phenotypic variation for flowering time in the population with no vernalization. All the early flowering lines had genotypes with alleles from the annual parent at one or both marker loci linked to the two QTLs, and all the late and nonflowering lines had alleles from the biennial parent at one or both of the linked marker

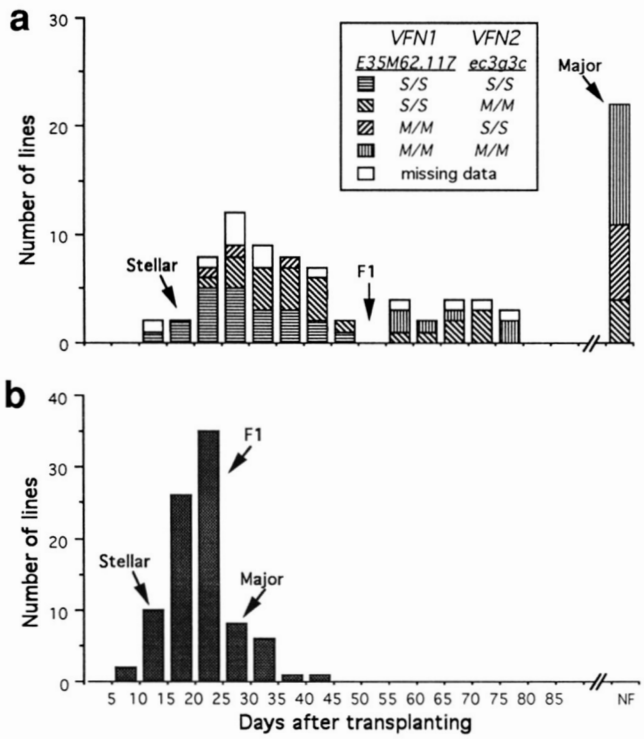

FIGURE 1.—Flowering time distributions of the *Brassica napus* (Major × Stellar) DH population given two treatments: no vernalization (a) and 8 wk of vernalization (b). The genotypes of DH lines at two marker loci closest to the LOD peaks of QTL for *VFN1* and *VFN2* are shown for the nonvernalized population. *S*, allele from the annual parent, Stellar; *M*, allele from the biennial parent, Major; NF, nonflowering.

loci (Figure 1a). After fixing these QTL effects, a third QTL with a smaller effect was detected on LG 16 (Table 1). A multilocus model including all three QTL effects explained nearly two thirds of the variation in flowering time. Marker loci on LG 12 and LG 16 were reported to be associated with flowering time in the previous analysis of this experiment (FERREIRA *et al.* 1995); however, the additional marker loci used in this reanalysis provided better estimates of their positions and effects.

The QTL effects detected on LG 9, LG 12 and LG 16 in the nonvernalized population were either not significant (LG 12 and LG 16) or greatly reduced (LG 9) in the population given 8 wk of vernalization (Table 1). This suggests that the effects of late-flowering alleles at all three loci are responsive to vernalization. We designate these vernalization-responsive flowering time loci in *B. napus* as *VFN1* (LG 9), *VFN2* (LG 12) and *VFN3* (LG 16). A significant QTL effect was detected on LG 9 in the vernalized population (Table 1), but it was in a different position than *VFN1* and may correspond to a separate locus controlling flowering time that is not responsive to vernalization. Thus, we designate this locus as *FN1*. The flowering time data from this experiment was used as an example in describing a Bayesian approach to QTL detection, and evidence for effects of both *VFN1* and *FN1* in the vernalized population are presented (SATAGOPAN *et al.* 1996).

TABLE 1

Linkage group location, LOD scores, percentage variation explained and additive effects of putative QTL for days to flowering in nonvernalized and vernalized populations of *Brassica napus* and *B. rapa*

Treatment	QTL	LG	LOD[a]	Percentage variation[a]	Additive effect[b]
Brassica napus					
Nonvernalized	*VFN1*	9	14.03	46.9	21.91
	VFN2	12	3.96	8.1	9.12
	VFN3[c]	16	2.55	6.4	7.17
			17.41	63.5	
Vernalized	*FN1*	9	6.07	29.1	3.39
Brassica rapa					
Nonvernalized	*VFR2*	8	7.92	34.1	16.72
	VFR1	2	4.45	16.4	11.69
	VFR3[c]	A	2.77	9.5	9.02
			11.53	55.1	
Vernalized	*VFR2*	8	6.97	29.7	2.15
	FR2	3	6.78	29.4	2.09
	FR1	2	2.90	15.9	1.62
	FR3	5	2.89	10.2	1.28
			11.57	58.8	

[a] Individual QTL LOD scores and percentage variation explained determined by removing effects from multilocus models. Values at bottom of each treatment are for complete multilocus model.

[b] Additive effect of biennial parent allele in days.

[c] QTL identified after fixing effects of other QTL.

Flowering time loci in *B. rapa*: In the *B. rapa* RI population given no vernalization treatment, about a quarter of the lines and the annual parent (R500) flowered early, six lines flowered late, and the remaining lines and the biennial parent (Per) did not flower by the end of the experiment (Figure 2a). After 6 wk of vernalization, all lines and the parents flowered early, although Per flowered later than R500 (Figure 2b).

The largest QTL effect was detected on LG 8 in the same position reported previously for an F_2 population from this same cross that was evaluated in a greenhouse for flowering time as unreplicated F_3 lines (TEUTONICO and OSBORN 1995). A second QTL was detected on LG 2, as was reported by TEUTONICO and OSBORN (1995); however, in the current analysis based on RI lines the QTL was in a different position to that reported by TEUTONICO and OSBORN (1995) and probably corresponds to a subthreshold QTL detected in the previous analysis based on F_3 families. The two QTLs on LG 8 and LG 2 explained much of the phenotypic variation for flowering time in the population given no vernalization treatment. Most early flowering lines had alleles from the annual parent at both marker loci linked to the two QTLs, and most nonflowering lines had alleles from the biennial parent at one or both of the linked marker loci (Figure 1a). The exceptions may be due to recombination between the marker loci and QTL alleles or due to other gene effects. After fixing the effects

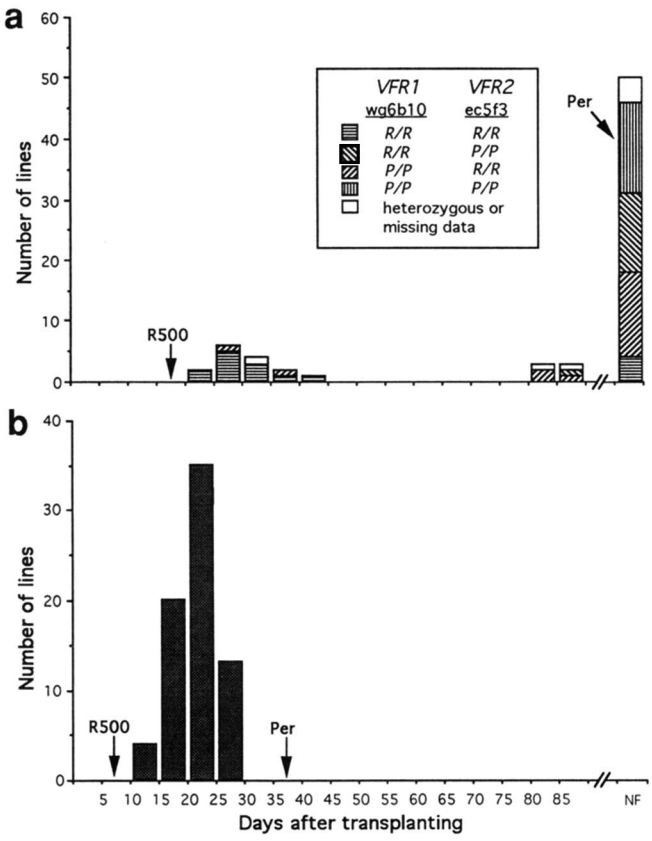

FIGURE 2.—Flowering time distributions of the *Brassica rapa* (Per × R500) RI population given two treatments: no vernalization (a) and 6 wk of vernalization (b). The genotypes of RI lines at two marker loci closest to the LOD peaks of QTL *VFR1* and *VFR2* are shown for the nonvernalized population. *P*, allele from the biennial parent, Per; *R*, allele from the annual parent, R500; NF, nonflowering.

at both QTLs, a third QTL with a smaller effect was detected on LG A, a small linkage group including only three marker loci (Table 1). A multilocus model including the effects of all three QTLs explained over one half of the variation in flowering time.

The QTL effects detected on LG 8, LG 2 and LG A in the nonvernalized population were either not significant (LG 2 and A) or were greatly reduced (LG 8) in the population given 6 wk vernalization treatment (Table 1). This suggests that the effects of late-flowering alleles at all three loci are responsive to vernalization. We designate these vernalization-responsive flowering time loci from *B. rapa* as *VFR1* (LG 2), *VFR2* (LG 8) and *VFR3* (LG A). In addition to a small effect in the *VFR2* region of LG 8, we detected significant QTL effects on LG 3, LG 2 and LG 5 for the vernalized population (Table 1). The effect on LG 2 was not in the same region as *VFR1*, and thus it probably represents a different locus. These three loci control flowering time that is not responsive to vernalization and we designate them as *FR1* (LG 2), *FR2* (LG 3) and *FR3* (LG 5).

Comparative mapping of flowering time loci: Many genes controlling flowering time have been identified and characterized in Arabidopsis. Some of these may be homologous to flowering time genes in Brassica and could be tentatively identified by comparative mapping. Since the tops of chromosomes *4* and *5* in Arabidopsis contain good candidates for genes homologous to the late-flowering genes we identified in Brassica, we used DNA clones that detect RFLP loci in these regions as probes for mapping RFLP loci in *B. napus*. The map positions of these loci in *B. napus* were approximated using 10 DH lines from a population of 50 DH lines that had been used previously to develop a genetic linkage map of 399 RFLP loci (PARKIN *et al.* 1995). The 10 DH lines had a unique combination of marker genotypes for 127 segments of the mapped genome. The same combination of marker genotypes occurred in four pairs of unlinked segments, but none of the segregating RFLP loci detected by Arabidopsis probes mapped to these segments.

Probes from these regions of Arabidopsis detected RFLP loci on several *B. napus* linkage groups (Table 2). This was expected because *B. napus* is an amphidiploid derived from interspecific hybridization of two diploid species, *B. rapa* and *B. oleracea*, which themselves have extensive genome replication (SLOCUM *et al.* 1990; SONG *et al.* 1991; PARKIN *et al.* 1995; LAGERCRANTZ and LYDIATE 1996). The linkage relationships of many loci were conserved in different regions of the *B. napus* genome. Three or more probes from Arabidopsis chromosome *4* detected loci on linkage groups N2, N3, N9, N13 and N19 of *B. napus* and two or more probes from chromosome *5* detected loci on N2, N3, N10, N12, N13 and N19 (Table 2).

The linkage map of the resynthesized × natural *B. napus* population could be aligned to the linkage maps used for mapping flowering time QTLs because many of the same RFLP and/or AFLP loci were identified in the three maps (or in the N-o-9 × N-o-1 *B. napus* map used for alignment), and homologies of *B. napus* linkage groups to those of the progenitor diploid Brassica species were known (PARKIN *et al.* 1995). Based on these alignments, *B. napus* N2 corresponds to *B. napus* LG 9, which contains *VFN1*, and these are homologous to *B. rapa* LG 2, which contains *VFR1* (Figure 3a). The regions containing these QTLs are close to the region on N2 that shows homoeology to the top of Arabidopsis chromosome *4* where *FRI* and *LD* are located.

The Brassica linkage groups containing *VFN2* and *VFR2* were aligned in a similar manner. *B. napus* LG 12, which contains *VFN2*, corresponds to *B. napus* N10, and these are homologous to *B. rapa* LG 8, which contains *VFR2*. This region of N10 shows strong homoeology to the top of Arabidopsis chromosome *5*, where *FLC*, *FY*, *CO* and *EMF1* are located (Figure 3b).

Additional data for aligning these genomic regions were obtained by using Brassica probes that detect RFLP linked to *VFN1* and *VFN2* on N2 and N10 to map RFLP loci in Arabidopsis. Two probes from the region

TABLE 2

Brassica napus* linkage groups (N1–N19) containing RFLP loci detected with *Arabidopsis thaliana* probes that map to the tops of chromosomes *4* and *5

Arabidopsis probe	*Brassica napus* linkage group[a]										
Chromosome *4*											
mi51		N2	N3	N7				N12		N17	
g8802		N2	N3		N9				N13		N19
mi204			N3		N9				N13		N19
g6844		N2	N3		N9				N13		N19
mi122					N9						N19
g3843			N3		N9				N13		N19
Chromosome *5*											
mi97		N2				N10			N13		N19
CHS		N2			N9	N10			N13		N19
mi174		N2	N3			N10		N12	N13		N19
CO	N1	N2	N3			N10	N11	N12		N18	N19
mi438						N10			N13		
mi322						N10					N19

FRI and *LD* are located on chromosome *4* and *CO, FY, FLC* and *EMF1* are located on chromosome *5*.
[a] Linkage group designations based on PARKIN *et al.* (1995).

on N2 showing homoeology to chromosome *4* of Arabidopsis also mapped to the top of chromosome *4*. However, probes from above this region mapped to other Arabidopsis chromosomes (Figure 3a), indicating that linkage arrangements of only short segments are conserved between these species in this region. All probes from the middle of N10 identified RFLP loci at the top of chromosome *5* in Arabidopsis (Figure 3b), indicating a large segment of conserved linkage arrangement between these species in this region of their genomes.

The results from this study suggest that *B. rapa* and *B. napus* have homologous genes with large effects on flowering time. In addition to the QTLs described above, *FN1* and *FR1* were detected in homologous regions of Bn LG 9 and Br LG 2, respectively (Figure 3a), and *VFN3* and *FR2* were detected in homologous regions of Bn LG 16 and Br LG 3, respectively (data not shown). Since *B. rapa* is one of the hypothesized progenitors of the amphidiploid *B. napus*, the vernalization requirement of the biennial *B. napus* cultivar used in this study probably originated from a biennial form of *B. rapa*. Alternatively, it is also possible that the biennial growth habit evolved independently in *B. napus* through mutations at the same, or closely linked, loci as those regulating this trait in *B. rapa*.

We also found evidence that these major Brassica QTLs for flowering time might correspond to Arabidopsis flowering time genes. Brassica genomic regions containing *VFN2* and *VFR2* showed strong homoeology with the top of chromosome *5*, and thus these Brassica loci probably correspond to *FLC, FY* and/or *CO*. Since plants with late-flowering alleles at *FLC* and *FY* are responsive to vernalization, whereas those with *CO* late alleles are only weakly responsive (KOORNNEEF *et al.* 1991, 1994; LEE *et al.* 1994a), the former loci are the best candidates. *FLC* is the more likely of the two to correspond to the Brassica loci we identified, because allelic variation for this locus exists among ecotypes of Arabidopsis (KOORNNEEF *et al.* 1994; LEE *et al.* 1994a). The top of chromosome *5* also contains *EMF1* (YANG *et al.* 1995). This locus may encode a floral inhibitor (SUNG *et al.* 1992), and although alleles conferring later flowering than wild-type alleles have not been identified in Arabidopsis, they may exist in Brassica.

A region near the peak LOD scores for *VFN1* and *VFR1* showed homoeology to the top of chromosome *4* in Arabidopsis where *FRI* and *LD* are located. Homology of *VFN1* and *VFR1* with *FRI* is an attractive hypothesis, because plants with late-flowering alleles at *FRI* are vernalization responsive, and these alleles appear to occur in all of the latest flowering ecotypes tested (BURN *et al.* 1993; LEE *et al.* 1993; CLARKE and DEAN 1994; CLARKE *et al.* 1995; SANDA *et al.* 1997). However, the regions containing the peak LOD scores for *VFN1* and *VFR1* showed homoeology to other Arabidopsis chromosomal regions, including chromosomes *1, 5* and *2*. In each of these regions, other genes controlling flowering time have been identified (KOORNNEEF *et al.* 1991; YANG *et al.* 1995; Figure 3a), but their exact positions on the linkage map of the population we used for mapping are not known.

Alleles at *FRI* and *FLC* interact epistatically to control flowering time, such that late-flowering alleles at both loci are needed to confer late flowering (KOORNNEEF *et al.* 1994; LEE *et al.* 1994a). Significant epistasis also was found for QTLs detected at the tops of chromosomes *4* and *5* in a segregating population from a cross of early and late Arabidopsis ecotypes (CLARKE *et al.* 1995). In our analysis of epistasis, we found significant additive by additive interaction of effects associated with marker

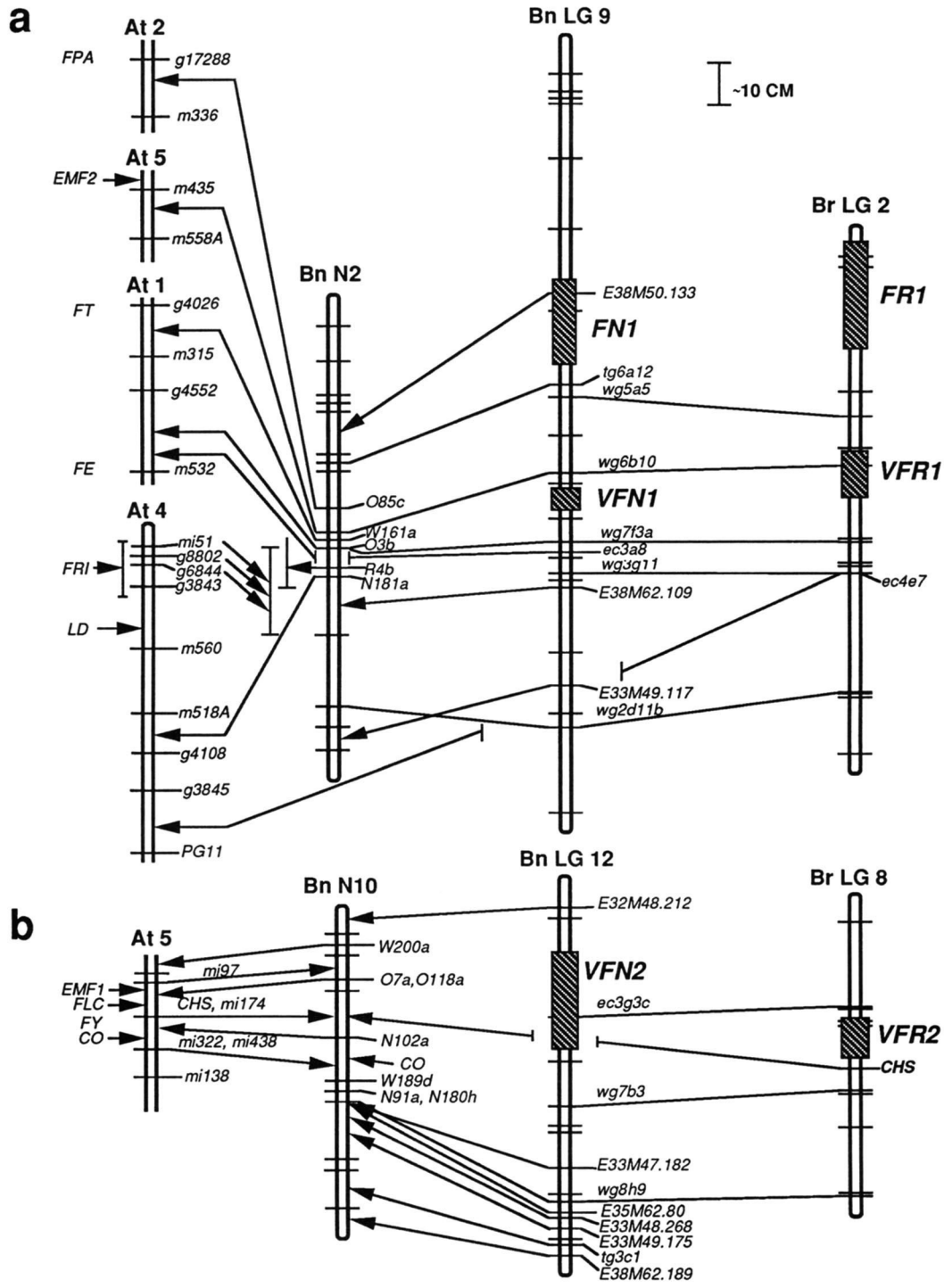

FIGURE 3.—Comparative genetic linkage maps for *Brassica rapa* based on Per × R500 RI lines (Br LG 2 and 8), *B. napus* based on Major × Stellar DH lines (Bn LG 9 and 12) or resynthesized × natural DH lines (Bn N2 and N10), and *Arabidopsis thaliana* based on Landsberg *erecta* × Columbia RI lines (At 1, 2, 4 and 5). Slash marks on vertical lines indicate the relative positions of marker loci. Only those markers used to compare the maps are labeled (marker loci beginning with E are AFLP; all others are RFLP). Lines connect homologous marker loci mapped in each population. Lines ending in arrows show marker interval positions for loci mapped in only a subset of one population (At-Bn comparison) or for loci aligned based on the N-o-9 × N-o-1 *B. napus* map (Bn-Bn comparison; see MATERIALS AND METHODS for a more detailed explanation). The intervals are delineated by the closest marker loci showing recombination with the compared locus; the intervening marker loci (not shown for Arabidopsis chromosomes) showed no recombination with the compared locus. Positions of Brassica flowering time QTL, *FN1*, *VFN1*, *FR1* and *VFR1* (a), and *VFN2* and *VFR2* (b) with 1.0 LOD support intervals are shown. Positions of Arabidopsis flowering time loci (*FPA*, *EMF2*, *FT*, *FE*, *FRI*, *LD*, *EMF1*, *FLC*, *FY* and *CO*) are shown by indicating the marker intervals (arrows) or approximate region of the chromosome (no arrow) where they map.

loci linked to *VFR1* and *VFR2* in *B. rapa*. However, unlike Arabidopsis, this was because genotypes having late alleles at either one or both loci were equally late (Figure 2a). We found no significant additive by additive epistasis for *VFN1* and *VFN2* in *B. napus*. Although interesting, these results must be interpreted cautiously because many of the lines did not flower and were assigned the same late-flowering time for these analyses. If the experiment could be extended so that all lines flowered, the analysis of epistasis might give very different results.

Since the flowering time loci we identified in Brassica were mapped based on quantitative data, we do not know their exact locations or effects or whether each QTL represents only one locus. Therefore, we are backcrossing the late-flowering alleles for each QTL individually into the annual parents in order to obtain Mendelian segregation of the phenotype. Once Mendelized, analysis of large segregating populations with marker loci in these regions will allow us to precisely locate these genes on Brassica linkage maps and determine their specific effects on flowering time. Use of Arabidopsis probes from homoeologous regions, including cloned flowering time genes, will provide stronger evidence about the correspondence of Brassica and Arabidopsis flowering time genes.

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LITERATURE CITED

BURN, J. E., D. R. SMYTH, W. J. PEACOCK and E. S. DENNIS, 1993 Genes conferring late-flowering in *Arabidopsis thaliana*. Genetica **90:** 147–155.

CHERRY, J. M., D. J. FLANDERS, F. X. PETEL and S. WENG, 1996 AtDB: an *Arabidopsis thaliana* database. http://genome-www.stanford.edu/Arabidopsis/.

CLARKE, J. H., and C. DEAN, 1994 Mapping *FRI*, a locus controlling flowering time and vernalization response. Mol. Gen. Genet. **242:** 81–89.

CLARKE, J. H., R. MITHEN, J. K. M. BROWN and C. DEAN, 1995 QTL analysis of flowering time in *Arabidopsis thaliana*. Mol. Gen. Genet. **243:** 555–564.

EDWARDS, M. D., C. W. STUBER and J. F. WENDEL, 1987 Molecular-marker-facilitated investigations of quantitative-trait loci in maize. I. Numbers, genomic distribution and types of gene action. Genetics **116:** 113–125.

FERREIRA, M. E., P. H. WILLIAMS and T. C. OSBORN, 1994 RFLP mapping of *Brassica napus* using doubled haploid lines. Theor. Appl. Genet. **89:** 615–621.

FERREIRA, M. E., J. SATAGOPAN, B. S. YANDELL, P. H. WILLIAMS and T. C. OSBORN, 1995 Mapping loci controlling vernalization requirement and flowering time in *Brassica napus*. Theor. Appl. Genet. **90:** 727–732.

KOLE, C., P. KOLE, R. VOGELZANG and T.C. OSBORN, 1997 Genetic linkage map of a *Brassica rapa* recombinant inbred population. J. Hered. (in press).

KOORNNEEF, M., C. J. HANHART and J. H. VAN DER VEEN, 1991 A genetic and physiological analysis of late-flowering mutants in *Arabidopsis*. Mol. Gen. Genet. **229:** 57–66.

KOORNNEEF, M., H. BLANKESTIJN-DE VRIES, C. HANHART, W. SOPPE and T. PEETERS, 1994 The phenotype of some late-flowering mutants is enhanced by a locus on chromosome 5 that is not effective in the Landsberg *erecta* wild-type. Plant J. **6:** 911–919.

LAGERCRANTZ, U., and D. LYDIATE, 1996 Comparative genome analysis in Brassica. Genetics **144:** 1901–1909.

LAGERCRANTZ, U., J. PUTTERILL, G. COUPLAND and D. LYDIATE, 1996 Comparative mapping in *Arabidopsis* and *Brassica*, fine scale genome collinearity and congruence of genes controlling flowering time. Plant J. **9:** 13–20.

LANDER, E. S., and D. BOTSTEIN, 1989 Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics **121:** 185–199.

LEE, I., A. BLEECKER and R. AMASINO, 1993 Analysis of naturally occurring late-flowering in *Arabidopsis thaliana*. Mol. Gen. Genet. **237:** 171–176.

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

LEE, I., M. J. AUKERMAN, S. L. GORE, K. N. LOHMAN, S. D. MICHAELS *et al.*, 1994b Isolation of *LUMINIDEPENDENS*: a gene involved in control of flowering time in *Arabidopsis*. Plant Cell **6:** 75–83.

LINCOLN, S., M. DALY and E. LANDER, 1992 Mapping genes controlling quantitative traits with MAPMAKER/QTL 1.1. Whitehead Institute Technical Report. 2nd edition, Cambridge, MA.

LISTER, C., and C. DEAN, 1993 Recombinant inbred lines for mapping RFLP and phenotypic markers in *Arabidopsis thaliana*. Plant J. **4:** 745–750.

PARKIN, I. A. P., A. G. SHARPE, D. J. KEITH and D. J. LYDIATE, 1995 Identification of the A and C genomes of the amphidiploid *Brassica napus* (oilseed rape). Genome **38:** 1122–1131.

PUTTERILL, J., F. ROBSON, K. LEE, R. SIMON and G. COUPLAND, 1995 The *CONSTANS* gene of *Arabidopsis* promotes flowering and encodes a protein showing similarities to zinc finger transcription factors. Cell **80:** 847–857.

SANDA, S., M. JOHN and R. AMASINO, 1997 Analysis of flowering time in ecotypes of *Arabidopsis thaliana*. J. Hered. **88:** 69–72.

SATAGOPAN, J. M., B. S. YANDELL, M. A. NEWTON and T. C. OSBORN, 1996 A Bayesian approach to detect quantitative trait loci for complex traits using Markov chain Monte Carlo. Genetics **144:** 805–816.

SHARPE, A. G., I. A. P. PARKIN, D. J. KEITH and D. J. LYDIATE, 1995 Frequent nonreciprocal translocations in the amphidiploid genome of oilseed rape (*Brassica napus*). Genome **38:** 1112–1121.

SLOCUM, M. K., S. S. FIGDORE, W. C. KENNARD, J. Y. SUZUKI and T. C. OSBORN, 1990 Linkage arrangement of restriction fragment length polymorphism loci in *Brassica oleracea*. Theor. Appl. Genet. **80:** 57–64.

SONG, K. M., J. Y. SUZUKI, M. K. SLOCUM, P. H. WILLIAMS and T. C. OSBORN, 1991 A linkage map of *Brassica rapa* (syn. *campestris*) based on restriction fragment length polymorphism loci. Theor. Appl. Genet. **82:** 296–304.

SUNG, Z. R., A. BELACHEW, B. SHUNONG and R. BERTRAND-GARCIA, 1992 EMF, an *Arabidopsis* gene required for vegetative shoot development. Science **258:** 1645–1647.

TEUTONICO, R. A., and T. C. OSBORN, 1994 Mapping of RFLP and qualitative trait loci in *Brassica rapa* and comparison to linkage maps of *B. napus*, *B. oleracea* and *Arabidopsis thaliana*. Theor. Appl. Genet. **89:** 885–894.

TEUTONICO, R. A., and T. C. OSBORN, 1995 Mapping loci controlling vernalization requirement in *Brassica rapa*. Theor. Appl. Genet. **91:** 1279–1283.

THORMANN, C. E., J. ROMERO, J. MANTET and T. C. OSBORN, 1996 Mapping loci controlling the concentrations of erucic and linolenic acids in seed oil of *Brassica napus* L. Theor. Appl. Genet. **93:** 282–286.

VOS, P., R. HOGERS, M. BLEEKER, M. REIJANS, T. VAN DE LEE *et al.*, 1995 AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res. **23:** 4407–4414.

YANG, C. H., L. J. CHEN and Z. R. SUNG, 1995 Genetic regulation of shoot development in *Arabidopsis*: role of the *EMF* genes. Dev. Biol. **169:** 421–435.

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

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