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New Arabidopsis Recombinant Inbred Line Populations Genotyped Using SNPWave and Their Use for Mapping Flowering-Time Quantitative Trait Loci

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ABSTRACT

The SNPWave marker system, based on SNPs between the reference accessions Colombia-0 and Landsberg *erecta* (L*er*), was used to distinguish a set of 92 Arabidopsis accessions from various parts of the world. In addition, we used these markers to genotype three new recombinant inbred line populations for Arabidopsis, having L*er* as a common parent that was crossed with the accessions Antwerp-1, Kashmir-2, and Kondara. The benefit of using multiple populations that contain many similar markers and the fact that all markers are linked to the physical map of Arabidopsis facilitates the quantitative comparison of maps. Flowering-time variation was analyzed in the three recombinant inbred line populations. Per population, four to eight quantitative trait loci (QTL) were detected. The comparison of the QTL positions related to the physical map allowed the estimate of 12 different QTL segregating for flowering time for which L*er* has an allele different from one, two, or three of the other accessions.

FOR the genetic analysis of natural variation, so-called immortal mapping populations are very useful because they allow the localization of many traits that show allelic variation in the same mapping population (Koornneef *et al.* 2004). Furthermore, they allow replication of experiments and testing under various environmental conditions. Since genotypes differ in their genetic composition, the analysis of similar traits in different populations is required to get insight into the genetic variation of a specific trait within a species.

To allow a proper comparison of the locations of genes, it is important to use the same marker framework and markers should preferentially be anchored to the physical map of the species. Various marker systems have been used to genotype Arabidopsis recombinant inbred line (RIL) populations. In Arabidopsis, markers such as single sequence length polymorphisms (SSLPs) and cleaved amplified polymorphic sequences are anchored to the physical map. AFLP markers, as used to genotype the frequently analyzed Landsberg *erecta* (L*er*) × Cape Verde Islands (Cvi) RILs (Alonso-Blanco *et al.* 1998b), can be anchored using bioinformatic tools only when it concerns AFLP bands that are characteristic for the sequenced genome of Columbia (Col-0) (Peters *et al.* 2001). On the basis of available sequence data of Arabidopsis accessions, many SNP markers have been detected [Schmid *et al.* 2003; The Arabidopsis Information Resource (TAIR; http://www.arabidopsis.org); Cereon database (http://www.arabidopsis.org/Cereon/index.jsp); and (http://walnut.usc.edu/2010.html)]. One of the various SNP detection systems (Cho *et al.* 1999) is the recently described SNPWave method (van Eijk *et al.* 2004).

In this article the usefulness of the SNPWave marker system was demonstrated on the basis of SNPs between the reference accessions Col-0 and L*er*, to distinguish Arabidopsis accessions from various parts of the world. In addition, we used these markers to genotype three new sets of RILs derived from crosses between L*er* and Antwerp (An-1), Kashmir (Kas-2), and Kondara (Kond), respectively. The populations studied were developed because their parents showed specific phenotypic differences and represent different geographical origins. To demonstrate their applicability for quantitative trait locus (QTL) mapping as well, we analyzed flowering time (FT) for all lines in the three populations as an example of a quantitative trait. Such analysis using three RIL populations having one common parent allows direct comparison of the loci segregating in these populations and facilitates identification of the different FT loci for which allelic variation is present among Arabidopsis accessions.

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TABLE 1

New markers used to genotype the three populations

Marker name	BAC	Primer 1 (5′ to 3′)	Primer 2 (5′ to 3′)
F12A24b	F12A24	GGTGTGATGTCGACCGGTAAAG	TGCACAACGTGCTCTCCATG
F17A22	F17A22	ACACACGAATATTGATTGTCTAAGG	TCACTTGTCGGTTTGTGTGG
cF7M19	F7M19	AGCTTGTGTCGTTTCCGATAG	AGTTGCAGAAATAAGCAGTGGC
F8D20	F8D21	CTTAAATGCCGATCCAGTCGAGG	TTCATTCGCGCATTTATTGTTGC
K15I22	K15I22	TCGGTGGTTTACTTTCACTTT	GAATTGTAGCTTCTTCTGAACC

MATERIALS AND METHODS

Plant material and growth conditions: *Arabidopsis thaliana* accessions were obtained from the Arabidopsis stock centers Arabidopsis Biological Resource Center (ABRC), Nottingham Arabidopsis Stock Center (NASC), and Sendai (http://www.arabidopsis.org), supplemented with accessions recently collected by members of the Laboratory of Genetics at Wageningen University and deposited at ABRC and NASC. Arabidopsis seeds were sown in petri dishes on water-saturated filter paper, followed by a 4-day cold treatment at 4°, and transferred to a climate room at 25° and 16 hr light for 2 days before planting in 7-cm pots with standard soil. In all descriptions of experiments, time is referred to as days after planting. The plants (12 plants/accession) were grown in an air-conditioned greenhouse with 70% relative humidity, supplemented with additional light (model SON-T plus 400W, Philips, Eindhoven, The Netherlands) providing a day length of at least 16 hr light (long day), with light intensity 125 μmol m^{-2} s^{-1}, and maintained at a temperature between 22°–25° (day) and 18° (night).

New RIL populations were obtained from a cross between the accessions Landsberg *erecta* as a female (L*er*, N20) and both Kashmir (Kas-2, N1264) and Kondara (Kond, CS6175) as a pollen parent, while Antwerp (An-1, N944) was reciprocally crossed with L*er*. The F_1 seeds of the different crosses were grown and allowed for self-fertilization to get F_2 seeds. From the F_2 seeds, sets of 120, 164, and 120 genotyped RILs for, respectively, An-1, Kas-2, and Kond, have been generated by a single-seed procedure until the F_9 generation. To minimize any bias in the selection of plants taken to the next generation, six individuals per RIL were planted and plant number 3 was selected to go on for the next generation with the fifth one as a backup. Two plots containing six plants per RIL of the F_9 generation were planted by the same procedures and under the same conditions mentioned before for growing the accessions. The flower heads of three individuals per RIL were harvested separately for DNA isolation and left for seed harvesting as well as for future use. The recombinant inbred lines with their marker data will be made available through the Arabidopsis stock centers.

To genetically test the allele type for the flowering loci *FRI* and *FLC* in the accessions under study, F_1 plants were made by crossing the five accessions An-1, Kas-2, Kond, L*er*, and Sha with lines that carry either an active *FLC* allele (derived from Col) or an *FRI* allele (*FRI–M73*; derived from line M73) both in a predominantly L*er* genetic background as described in Koornneef *et al.* (1994). The F_1 progeny of the crosses as well as the control plants were grown in a randomized two-plot design with six plants per genotype and under the same conditions mentioned above.

DNA isolation and genotyping: Genomic DNA of 92 accessions was isolated from leaf material of individual plants using a modified CTAB procedure (Stewart and Via 1993). Details of the protocol for genotyping these accessions, using SNP markers, were described previously (van Eijk *et al.* 2004). For the RIL populations, the flower buds of three F_9 plants per genotype were harvested separately for DNA isolation. DNA extraction was performed as described above for the SNP markers. For the SSLP markers, DNA was extracted using the Wizard magnetic 96 (Promega, Madison, WI) DNA isolation kit. SSLP markers were described in Clerkx *et al.* (2004), the TAIR database, or the MSAT database (http://www.inra.fr/qtlat/msat/index.php). Primers used for novel markers that were developed are described in Table 1. In addition, T27K12-SP6 and F5I14-49495 are written as T27K12 and F5I14, respectively. For both markers, the physical position can be found in the TAIR database. All markers used have first been checked to determine if the parental accessions An-1, Kas-2, and Kond were polymorphic with L*er*; thereafter, the polymorphic markers were used to genotype all individual RILs. For SSLP markers, a standard protocol of 30 sec at 94°, 30 sec at 50°, and 30 sec at 72° (35 cycles) was used except for *FRI* (54° annealing, 1-min extension) and *FLC* (52° annealing, 2-min extension). Marker data are presented in supplemental Table 2 at http://www.genetics.org/supplemental/.

Measurement of flowering time: F_{10} generation plants (12 plants/RIL) were grown in the greenhouse in a randomized two-block design to reduce environmental effects. FT for each plant was scored as the number of days from planting until opening of the first flower.

Map construction and QTL analysis: Initially, the three linkage maps have been constructed using only the SNP markers; gaps between markers that were >13 cM were filled using SSLP markers to obtain uniformly distributed markers. The JoinMap program (version 3.0; http://www.kyazma.nl) was used to construct the genetic maps.

The software package MapQTL 5 was used to identify and locate QTL on the linkage map by using interval mapping and multiple-QTL model (MQM) mapping methods as described in its reference manual (http://www.kyazma.nl). In a first step, putative QTL were identified using interval mapping. Thereafter, the closest marker at each putative QTL was selected as a cofactor (van Ooijen and Maliepaard 1996; van Ooijen 2000) and the selected markers were used as genetic background controls in the approximate multiple QTL model of MapQTL. LOD threshold values applied to declare the presence of QTL were estimated by performing permutation tests implemented in MapQTL version 5.0 using at least 1000 permutations of the original data set, resulting in a 95% LOD threshold of 2.4. Two-LOD support intervals were established as 95% QTL confidence interval (van Ooijen 1999) using restricted MQM mapping implemented within MapQTL. The estimated additive genetic effect and the percentage of variance explained by each QTL and the total variance explained by all the QTL affecting a trait were obtained using MQM mapping.

Statistical analysis: Using NTSYSpc version 2.10t. (Rohlf 2001), the tree plot of the 92 Arabidopsis accessions, based on UPGMA cluster analysis using the pattern of polymorphism between 79 SNP markers, was performed (data presented in supplemental Table 1 http://www.genetics.org/supplemental/).

Heritability (broad sense) was estimated as the proportion of variance explained by between-line differences using the general linear model module of the statistical package of SPSS version 11.0.1 (SPSS, Chicago) based on measurements of 6–12 plants per genotype.

Differences in recombination were tested using a chi-square test comparing the number of recombinant and parental lines for two identical markers in two populations where differences were observed.

Two-way interactions among the QTL identified for FT were tested by ANOVA employing the corresponding two markers as fixed factors and the trait as dependent variable and using the general linear model of the statistical package SPSS version 11.5.1. In the L*er* × Kas-2 population, SNP32 was included in this analysis because a suggestive QTL with a LOD of 2.3 was found at that locus. A Bonferroni correction to adjust the 0.05 threshold of significance was applied if multiple tests were performed on the same data set. Only those interactions that were significant after the Bonferroni correction are presented.

RESULTS

Polymorphism between a set of Arabidopsis accessions using SNPWave markers: A 100-plex SNPWave marker set of known SNPs between the two reference Arabidopsis accessions Col-0 and L*er* (van Eijk *et al.* 2004) was used to genotype 92 Arabidopsis accessions. Among markers that could be amplified in most accessions, 37.6–62.4% of the markers were different from the Col-0 allele and 0–37.6% differed from the L*er* allele. The polymorphism data indicated that, for many accessions, crosses made with one of the two reference accessions would yield reasonable numbers of polymorphic SNPWave markers.

A few identical genotypes were detected, of which some have been described for other marker systems as well (*e.g.*, Co-1, C24; L*er*, Di-1; Buckhorn Pass) (Figure 1) (Torjek *et al.* 2003). For some other accessions (*e.g.*, Co-1 and Es-0, Ct-1 and En-2, Be-0 and Tsu-1) this was not expected in view of their different geographical origins. As reported for many marker systems, no obvious structure related to the geographical origin was detected using UPGMA cluster analysis. However, a number of accessions from Central Asia and Russia (Figure 1) seem more related to each other than to accessions from other regions as was reported before (Schmuths *et al.* 2004; Nordborg *et al.* 2005). The data confirm that Kas-1 (N903) and Kas-2 (N1264) are genetically different (Levey and Wingler 2005), although both cluster in the Central Asian group.

L*er* × An-1, L*er* × Kas-2, and L*er* × Kond linkage maps: The accessions used to construct the RIL populations differed for 57.1, 55.8, and 54.5% of the markers from L*er* for An-1, Kas-2, and Kond, respectively. These markers did not completely cover the genome. To construct genetic maps with equally spaced markers for the three different crosses (L*er* × An-1, L*er* × Kas-2, and L*er* × Kond), either additional SNPWave markers had to be developed or publicly available markers (TAIR database) were used as mentioned in materials and methods.

Linkage maps were obtained using 44, 45, and 51 SNP markers supplemented with 20, 31, and 23 SSLP markers for L*er* × An-1, L*er* × Kas-2, and L*er* × Kond, respectively. In addition, the *erecta* mutation segregating in all three populations and the *ga5*-gibberellin-deficient mutation (Xu *et al.* 1995), segregating in the L*er* × Kas-2 population (shown to be present in Kas-2 by the absence of complementation in the cross of the *ga5* mutant and Kas-2), could be scored as morphological markers. This resulted in three genetic maps with 65, 78, and 75 markers for L*er* × An-1, L*er* × Kas-2, and L*er* × Kond, respectively (Figure 2). The markers were assigned to five linkage groups for each population with a total genetic length of 371, 441, and 351 cM for L*er* × An-1, L*er* × Kas-2, and L*er* × Kond, respectively. Most markers were located on the expected linkage groups predicted on the basis of the physical order of the markers in the sequenced Col-0 accession. Exceptions are SNP395, which was expected on chromosome 4 but mapped to chromosome 3 in L*er* × Kond, and two pairs of markers that are inverted in the L*er* × Kas-2 linkage map, *viz.*, C6L9-78 and SNP395 on chromosome 4 and SNP77 and *FLC* on chromosome 5. This deviating order derived from normal recombination patterns (see supplemental Table 2 at http://www.genetics.org/supplemental/), which suggests that genotyping errors are not the reason for this changed order.

Since in each generation heterozygosity per locus is reduced by half after selfing, the probability that a specific locus is heterozygous is 0.39% for the F_9 generation. The average frequency of heterozygosity for all loci is 0.28, 0.25, and 0.25% for L*er* × An-1, L*er* × Kas-2, and L*er* × Kond, respectively, with no locus having a significantly higher value than predicted.

For each marker, the expected segregation ratio would be 1:1 for each parental allele in the case of no bias in the selection of individual plants during the maintenance of the populations. Figure 2 indicates regions with significantly distorted segregation (at $0.0005 < P < 0.05$) for the three populations. Markers showing significant segregation distortion clustered in a certain region of the genome in the three maps, with ratios ranging from 1.4:1 to 2.1:1. These regions either partially overlap in the different populations as on chromosome 1 or are population specific in other chromosome regions. The distortion in most regions favored L*er* alleles, although in four different regions of the three populations, the non-L*er* alleles were in excess (Figure 2).

Comparison of the L*er* × An-1, L*er* × Kas-2, and L*er* × Kond genetic maps: Figure 2 shows the L*er* × An-1, L*er* × Kas-2, and L*er* × Kond genetic maps linked to each other by 41 anchoring markers scored in the three populations. Comparison between the physical map of Col-0 and the three newly generated linkage maps indicates that the overall recombination rates are similar over chromosomes with suppression of recombination observed around the centromeres of chromosomes

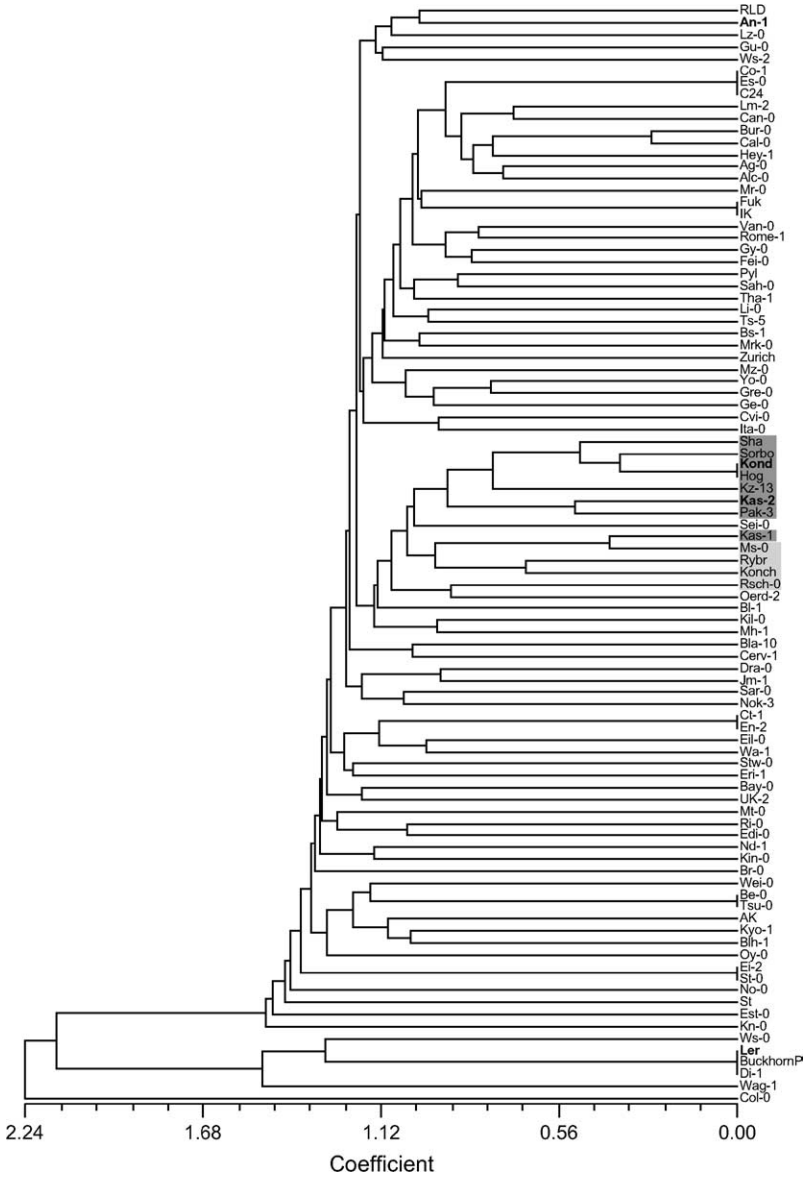

FIGURE 1.—Tree plot of the 92 Arabidopsis accessions based on UPGMA cluster analysis using the pattern of polymorphism among 79 SNP markers. The dark-shaded block indicates accessions from Central Asia, while the light-shaded block refers to accessions from Russia.

2, 3, 4, and 5 (Figure 3). The maps are colinear with the exception of the two inverted pairs of markers in the L*er* × Kas-2 population described above. When comparing recombination frequencies in regions where maps appeared different (Figure 2) among the three populations, recombination was found to be significantly higher ($P = 0.001$) in the Kas-2 cross between SNP71 and SNP203 on chromosome 2 compared to the Kond cross and recombination was not significantly different when compared to the An-1 cross ($P = 0.2$). In other regions differences were not statistically significant.

The pattern of similarity in recombination described above results in similar genetic lengths of the five chromosomes in the three crosses. The largest differences were observed for chromosome 2 where the genetic map of L*er* × Kas-2 is longer than the other two crosses by >20 cM and for chromosome 3 where the L*er* × Kond map is shorter than the other two maps.

QTL mapping of flowering time: Flowering-time QTL have been mapped in several Arabidopsis RIL populations (KOWALSKI *et al.* 1994; CLARKE *et al.* 1995; JANSEN *et al.* 1995; KUITTINEN *et al.* 1997; ALONSO-BLANCO *et al.* 1998a; LOUDET *et al.* 2002; EL-LITHY *et al.* 2004; KOORNNEEF *et al.* 2004 for review). Since different populations may segregate for different loci depending on the genetic composition of their parental lines, a comparison between multiple crosses is needed to obtain information about the variation present among Arabidopsis accessions. This allows the description of the so-called global genetic architecture (SYMONDS *et al.* 2005) of a trait within a species. Comparison between different crosses can be done accurately only when the same markers are used and/or when these markers are anchored to the Arabidopsis physical map, which acts as a reference map.

To illustrate this approach, we analyzed FT in the three RIL populations, which were grown in the same

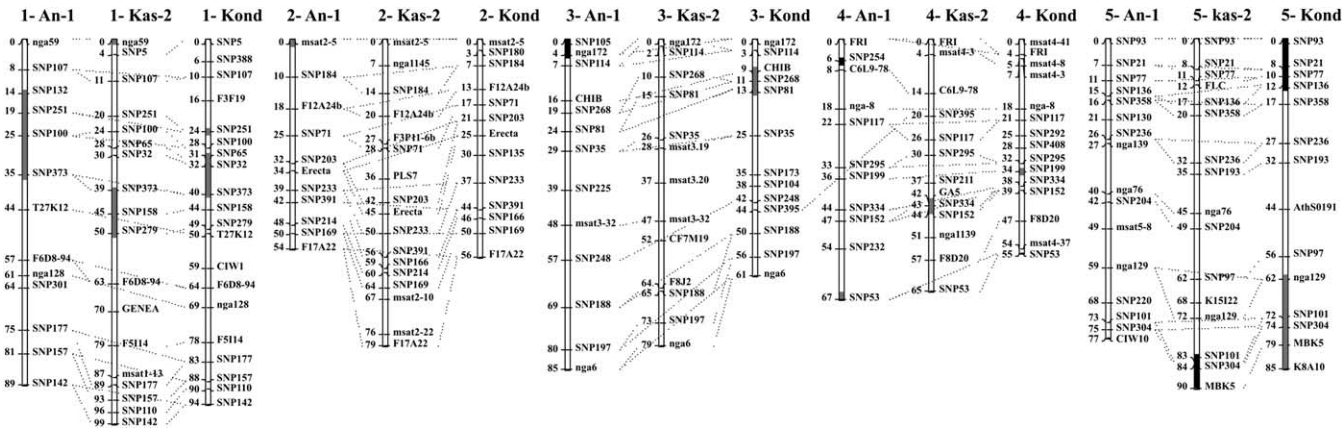

FIGURE 2.—Integrated genetic maps linked through anchoring markers scored in the three populations L*er* × An-1, L*er* × Kas-2, and L*er* × Kond. Distorted regions [regions deviating from the 1:1 ratio ($P < 0.05$)] are indicated by shaded boxes (regions with a significantly higher number of RILs with the L*er* allele) or solid boxes (regions with significantly higher numbers of lines in favor of An-1, Kas-2, or Kond alleles).

greenhouse under long-day conditions but in independent experiments. For all populations, heritabilities were high and transgression beyond the parental values was observed toward both earliness and lateness (Table 2). In total four, six, and eight QTL were identified per population (Figure 4). However, because the populations were not grown in the same experiment, some of the differences among the populations may be due to genotype × environment interactions in cases where specific QTL are expressed only under specific environmental conditions.

Although the FT differences between L*er* and An-1 were very small (Table 2), variation among the RILs is considerable and is explained by four QTL, of which for three the An-1 allele causes early flowering (Figure 4; Table 3). In the Kas-2 and Kond populations, the parents differed much more and the genetic differences could be explained by six and eight QTL, for which in three and five cases the L*er* alleles accelerate flowering, respectively (Figure 4; Table 3). The detected QTL explained 68.3, 78.8, and 84.8% of the phenotypic variance for the L*er* × An-1, L*er* × Kas-2, and L*er* × Kond populations, respectively. Within the three populations significant interactions among several QTL were detected (Table 3).

Relating the map positions and 2-LOD intervals to the physical map on the basis of the Col-0 sequence allows a comparison among the QTL in the three populations. In most cases the comparison was relatively straightforward. However, for QTL with relatively large 2-LOD intervals (top chromosome 1 and 2 for L*er* × An-1 and L*er* × Kond crosses, respectively) and for regions where two linked QTL were detected in the same population, interpretation is more complex.

Two FT QTL are in common among the three populations: the QTL on top of chromosome 3 (around nga172) for which the L*er* allele delays flowering and the QTL located around SNP130 (chromosome 5) for which the L*er* allele accelerates flowering. In addition, there are several QTL common between two of the three populations. These are the QTL located at the *FRI* locus for L*er* × Kas-2 and L*er* × Kond and around SNP136 (chromosome 5) for L*er* × An-1 and L*er* × Kond and around K8A10 (chromosome 5) for L*er* × Kas-2 and L*er* × Kond. For the L*er* × An-1 population the QTL around SNP136 could not be separated from another QTL around SNP236. However, the presence of two distinct QTL in this region was clear for the L*er* × Kond population.

It is known that L*er* carries a weak *FLC* allele in contrast to many other accessions that contain active *FLC* alleles that, together with active *FRI* alleles, confer late flowering, which can be overcome by vernalization (KOORNNEEF *et al.* 1994; MICHAELS *et al.* 2003; SHINDO

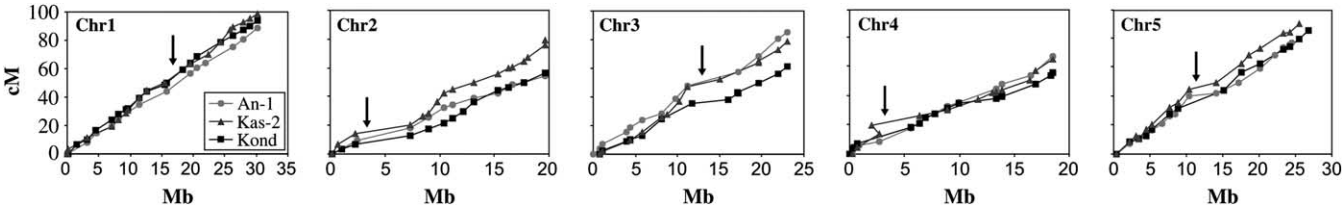

FIGURE 3.—The relationship between the genetic maps of L*er* × An-1, L*er* × Kas-2, and L*er* × Kond and the physical map along the five chromosomes. The arrows indicate the position of the centromeres.

TABLE 2

Parental values, averages, and ranges of flowering time and heritabilities (h^2) in the three populations

RIL population	FT–L*er*	FT non-L*er*	Average FT RILs	Range RILs	h^2
L*er* × An-1	23.4	22.3	23.2	19.1–28.3	0.88
L*er* × Kas-2	33.2	47.0	34.7	24.9–54.0	0.86
L*er* × Kond	29.0	49.3	38.7	26.7–66.2	0.95

et al. 2005). Various authors (Koornneef *et al.* 1994; Gazzani *et al.* 2003; Michaels *et al.* 2003) used hybrids of accessions with lines containing only either an active *FRI* allele or an active *FLC* allele, expecting that when both active copies are present in the hybrids the plants will be late flowering. To test whether active alleles of *FRI* and *FLC* are present, we also applied this procedure in the accessions that we have used to construct our populations. In addition, we included the accession Shakdara (Sha) that is assumed to contain a weak *FLC* allele (Gazzani *et al.* 2003; Michaels *et al.* 2003). This is in agreement with the absence of a late-flowering Sha allele at the *FLC* locus in populations made from crosses with Sha (Loudet *et al.* 2002; El-Lithy *et al.* 2004). Flowering-time data of the hybrids and the parents are shown in Figure 5 and confirm that all accessions except An-1 contain active *FRI* alleles resulting in late flowering, as compared to the parents, when combined with a line containing an active *FLC* allele. Hybrids from crosses made with *FRI–M73* are less late than the *FLC* hybrids but slightly later than the hybrids with L*er* or the accession parents. This observation suggests that all accessions have weak *FLC* alleles (compared to the Col alleles) but stronger than L*er*, except An-1, where the *FRI* hybrid is later flowering than the hybrid with *FLC*.

In addition, population-specific QTL (Table 3) were detected around markers SNP107 and SNP254 for L*er* × An-1, where L*er* alleles delayed flowering. For L*er* × Kas-2, two QTL with different allele effects were identified around the markers SNP110 and SNP295. In the L*er* × Kond population-specific QTL could be identified around markers CIW1, F5I14, and msat2-5 with different allele effects. The number of colocating QTL might be higher since in a few cases suggestive QTL (LOD between 1.5 and 2.4) were detected in one population at a position where significant QTL were detected in another population (data not shown). This was true for two suggestive QTL at SNP301 and GENEA for L*er* × An-1 and L*er* × Kas-2, respectively, which colocate with a

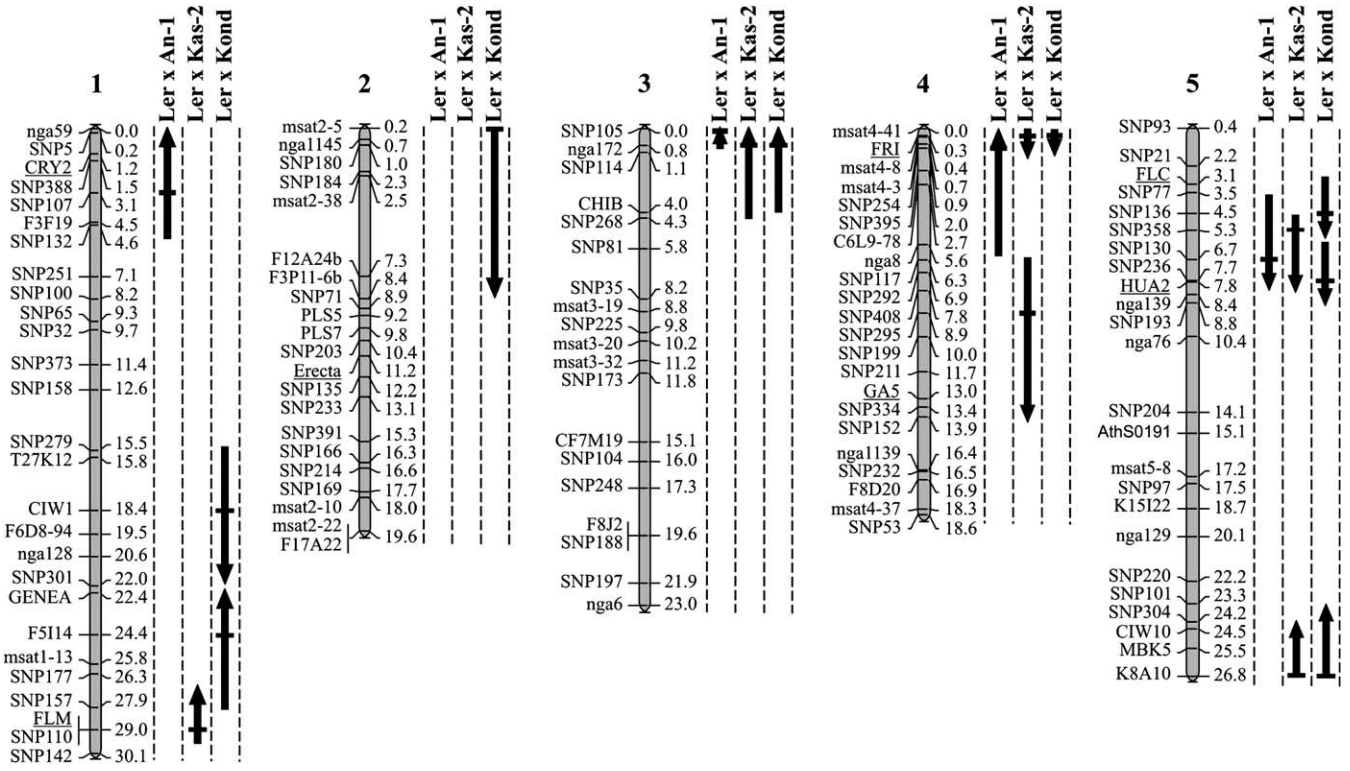

Figure 4.—Combined physical map of L*er* × An-1, L*er* × Kas-2, and L*er* × Kond containing markers that are used in this study in addition to several candidate genes (*CRY2*, *FLM*, *HUA2*). Flowering-time QTL are indicated by arrows along the chromosomes. Thick horizontal dashes in the arrows indicate the marker fixed during the MQM mapping analysis. The lengths of the arrows indicate the 2-LOD support intervals. The direction of the arrows indicates the allelic effect: upward, L*er* increasing the FT and the other allele decreasing; downward, the non-L*er* allele increasing and the L*er* decreasing.

TABLE 3

Characteristics of flowering-time QTL detected in the three populations

RILs	QTL at nearest marker	Map position[a]	LOD score	% of variance	Additive allele effect (days)[d]
L*er* × An-1				*68.3*[c]	
	SNP107	1, 7.9	2.8	4.0	0.6
	SNP105	3, 0.0	16.4	29.4	1.8
	SNP254	4, 6.2	3.3	4.9	0.8
	SNP130	5, 21.0	14.2	24.7	−1.6
	SNP105 × SNP130	$P = 0.000045$[b]	6.1		
L*er* × Kas-2				*78.8*[c]	
	SNP110	1, 95.5	7.8	6.5	3.0
	nga172	3, 0.0	3.5	2.4	2.0
	FRI	4, 0.0	23.8	24.0	−6.0
	SNP295	4, 30.0	3.8	2.8	−2.6
	SNP358	5, 20.2	8.8	7.2	−3.4
	MBK5	5, 89.6	16.8	15.0	4.8
	SNP110 × MBK5	$P = 0.006$[b]		4.7	
	SNP32 × SNP295	$P = 0.008$[b]		4.0	
L*er* × Kond				*84.8*[c]	
	CIW1	1, 59.2	4.2	2.6	−3.0
	F5I14	1, 78.4	6.2	4.2	3.6
	msat2-5	2, 0.0	5.1	3.4	−3.0
	nga172	3, 0.0	5.0	3.5	3.0
	FRI	4, 4.1	36.5	46.6	−11.4
	SNP136	5, 12.4	3.2	2.0	−2.8
	SNP236	5, 27.4	8.9	6.4	−5.0
	K8A10	5, 84.8	5.4	3.6	3.2
	FRI × SNP136	$P = 0.008$[b]		2.3	
	FRI × SNP236	$P = 0.009$[b]		2.2	

[a] Chromosome number is given, followed by the marker position in centimorgans.
[b] For interactions, the *P*-values are given instead of the LOD scores.
[c] Values in italics refer to the total variance explained by the additive effects of significant QTL and significant two-way interactions.
[d] Positive values indicate that L*er* alleles increase the trait value and negative values indicate that the non-L*er* alleles increase the trait value.

significant QTL in that region in L*er* × Kond (chromosome 1). When taking these suggestive QTL into account, the total number of QTL did not increase. In total we identified 12 different QTL for FT for which L*er* has alleles different from the alleles in one, two, or three of the other accessions.

DISCUSSION

In this study we describe three new recombinant inbred line populations for Arabidopsis having L*er* as a common parent. The SNPWave technique was applied to genotype these RIL populations on the basis of SNPs between Col-0 and L*er*. Of the SNPWave markers that are polymorphic between L*er* and Col-0, ~50% (37.6–62.5%) could be used to genotype populations made from crosses with L*er*. Some regions are not covered by the SNPWave markers, such as the upper part of the lower arm of chromosome 1 for all three populations, the middle part of chromosome 3 for L*er* × Kas-2, the top of chromosome 4 for all populations, and the middle part of chromosome 5 for L*er* × An-1. As far as this was due to insufficient coverage of the SNPWave markers in the three crosses, it implied that additional markers, such as the common PCR markers used here, were required to obtain genetic maps with equally distributed markers.

Since the maps contain many similar markers and, more importantly, all markers are linked to the physical map of Arabidopsis, a quantitative comparison of maps could be performed. This analysis showed that map lengths are quite similar and also in the same range as those published for other populations (Lister and Dean 1993; Alonso-Blanco *et al.* 1998b; Loudet *et al.* 2002; Clerkx *et al.* 2004). The two inverted pairs of markers that were detected in the Kas-2 population and the reduced recombination between the two markers on chromosome 2 might also be explained by structural chromosomal inversions between accessions. However, differences in local recombination rate as such may exist, as suggested by cytogenetic data (Sanchez-Moran *et al.*

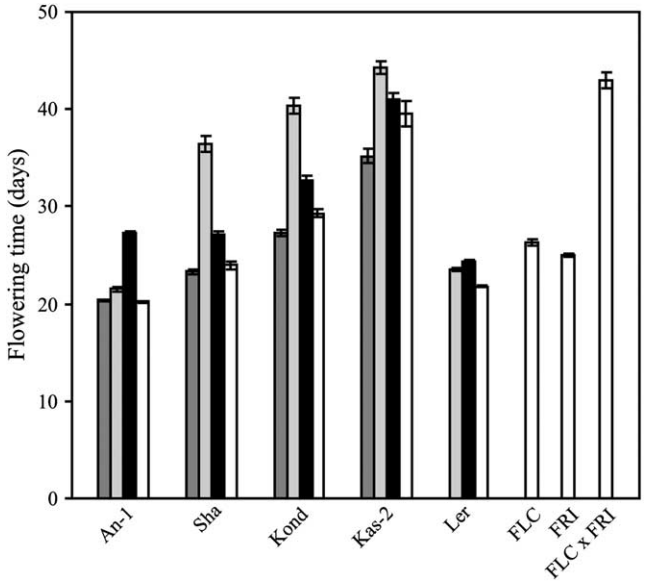

Figure 5.—Flowering time in hybrids of accessions with near-isogenic lines containing, respectively, *FLC–Col* and *FRI–M73* in a predominantly L*er* genetic background. Dark-shaded bars represent the cross with L*er*, light-shaded bars represent the cross with *FLC*–Col-0, solid bars represent the cross with *FRI*–M73, and open bars represent the accessions and pure lines.

2002). Structural chromosome variants between accessions have not been studied frequently but are not uncommon (reviewed in Koornneef *et al.* 2003). Suppression of recombination in specific regions makes map-based cloning in such regions difficult. In general, a solution for this problem is to perform mapping in a cross with another accession that does not show suppression of recombination.

RIL populations allow the identification of natural genetic variants for which the parents differ. Such populations facilitate the mapping of many traits in the same population (Koornneef *et al.* 2004). However, since the parents might not be different for a specific QTL for which variation is present within the germ plasm pool, additional mapping populations are being developed (http://www.inra.fr/qtlat/naturalvar/rilsummary.htm). The power of using multiple populations was recently demonstrated by Symonds *et al.* (2005), who identified nine QTL for trichome density in a total of four RIL populations, whereas individual population segregated for three to five QTL.

In this study similar results were obtained for FT, a frequently studied trait showing large natural variation in Arabidopsis. In this species extreme lateness is mainly due to the presence of dominant alleles at the *FRI* and *FLC* loci (Caicedo *et al.* 2004; Hagenblad *et al.* 2004; Koornneef *et al.* 2004). These large-effect loci mask the segregation of other minor-effect loci, which are easier to detect in mapping populations where these large-effect alleles do not segregate. This is well illustrated in the present example, where early and middle late accessions are combined and where, in total, 12 QTL could be detected. One of these is probably *FRI*, detected in the L*er* × Kas-2 and L*er* × Kond populations. Kas-2 and Kond have late alleles at the *FRI* locus, which is at least functional in Kond (Gazzani *et al.* 2003; Michaels *et al.* 2003; Hagenblad *et al.* 2004). Active *FRI* alleles may confer lateness without *FLC*, as was also found in Sha-derived populations (Loudet *et al.* 2002; El-Lithy *et al.* 2004). In the L*er* × An-1 population, a novel locus was also identified at the top of chromosome 4, for which An-1 accelerates FT.

The situation at the top of chromosome 5 is complex. As in the L*er* × Sha population (El-Lithy *et al.* 2004), we could not identify a putative *FLC* QTL in either the L*er* × Kas-2 or the L*er* × Kond population. This was confirmed by our hybrid data (Figure 5) and that of Michaels *et al.* (2003), which suggest that Kond has a weak *FLC* allele. This is in agreement with the fact that Kas-2, Kond, as well Sha all have a 1.2-kb insertion within the *FLC* gene, which might reduce *FLC* function (Hagenblad *et al.* 2004). However, we could identify a QTL at marker SNP136 close to the position of *FLC*, which was also used as a marker in the L*er* × Kond population. As explained above, we assume that this is not *FLC*, although the significant interaction between the QTL at the *FRI* locus and SNP136 (Table 3) is in agreement with the epistatic interaction described previously for *FRI* and *FLC*. However, its effect is relatively small and it cannot be excluded that other loci located below *FLC* are responsible for this interaction. In the L*er* × Cvi population (Alonso-Blanco *et al.* 1998a), two linked loci on chromosome 5 were found, both conferring lateness, but only when both alleles were derived from the non-L*er* parent. It has been suggested that the upper locus is *FLC* and that the lower locus (named *FLG* by Alonso-Blanco *et al.* 1998a) might encode *HUA2* (Doyle *et al.* 2005). For the An-1 cross, the 2-LOD interval includes the *FLC* locus but we could not separate this SNP130 QTL into two QTL. On the contrary, for total leaf number and rosette leaf number, traits that are known to be correlated to FT (Koornneef *et al.* 1991; Alonso-Blanco *et al.* 1998a), we could detect two distinct QTL (data not shown). Thus, probably An-1 contains an active, although not very strong, *FLC* allele that was confirmed by our hybrid data (Figure 5).

It appears that this region below *FLC* on chromosome 5 contains at least one and maybe two other QTL for FT for which L*er* has alleles different from the other parents. The observation that one or two loci are present in this region, depending on the cross, most likely can be explained by the difficulty to statistically separate linked QTL.

The L*er* × Kas-2 and L*er* × Kond populations have common QTL with the same allelic effects at nga172, at *FRI*, and at K8A10. At these positions also the Sha accession from Tadjikistan carries similar alleles (Loudet

et al. 2002; El-Lithy *et al.* 2004). Kas-2 and Sha may have similar alleles for the QTL at the bottom of chromosome 1 for which the *FLM* locus is a candidate gene (Werner *et al.* 2005).

This analysis shows that for a single trait additional genetic variation is detected when different populations are analyzed for the same trait. However, the accuracy of QTL mapping is such that colocation can also be due to two different closely linked QTL. Having available different sources for the same type of allelic variation allows the selection of the populations with the strongest alleles for future fine mapping and cloning. In addition, these genetic studies provide the basis of the correlation between function and molecular haplotype as has been described for the *FRI* and *FLC* loci (Gazzani *et al.* 2003; Michaels *et al.* 2003; Caicedo *et al.* 2004; Hagenblad *et al.* 2004). The new RIL populations show segregation for various other traits such as seed dormancy and plant performance (our unpublished data) that are currently being analyzed.

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