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Comparative mapping of QTLs determining the plant size of *Brassica oleracea*

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Abstract Quantitative trait loci (QTLs) influencing the size of leaves and stems were detected by restriction fragment length polymorphisms (RFLP) in three *Brassica oleracea* F_2 populations derived from crosses of rapid-cycling *Brassica* to three *B. oleracea* varieties, Cantanese, Pusa Katki and Bugh Kana. Morphological traits, including lamina length, lamina width, petiole length, stem length, stem width and node number were evaluated. A total of 47 QTLs were detected based on a LOD threshold of 2.5. Through comparative mapping we inferred that the 47 QTLs might reflect variation in as few as 35 different genetic loci, and 28 ancestral genes. For the trait of lamina length, we identified QTLs corresponding to five ancestral genes, which mapped near the locations corresponding to five known *Arabidopsis* mutations, *rev, axr1, axr3, axr4* and *as2*. For the trait of stem length, we identified QTLs corresponding to five ancestral genes, which mapped near the locations corresponding to nine known *Arabidopsis* mutations, *dw3, dw6, acl5, dw7, ga4, ga1, dw1, axr1* and *axr3*. The possibility of using *Arabidopsis/Brassica* as a model to extrapolate genetic information into other crops was examined.

Keywords *Arabidopsis* · Evolution · Genome analysis · Morphology · Quantitative trait loci

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Introduction

Plants are the foundation of our ecosystem, collecting solar energy by fixing atmospheric carbon into organic molecules. The organic carbon is then partitioned into various plant organs, which are the basic energy source of higher animals. The mechanisms controlling the process of carbon partitioning in plants are not fully understood. The species *Brassica oleracea* provides a unique opportunity to study how carbon is partitioned into different organs of a plant. *B. oleracea* includes many vegetables that carry distinct storage organs, including the enlarged inflorescence of cauliflower (*B. oleracea* subsp. *botrytis*) and broccoli (*B. oleracea* subsp. *italica*); the enlarged stem of kohlrabi (*B. oleracea* subsp. *gongylodes*) and marrow-stem kale (*B. oleracea* subsp. *medullosa*); the enlarged and twisted leaves of cabbage (*B. oleracea* subsp. *capitata*) and the enlarged apical buds of cabbage or the lateral buds of Brussels sprouts (*B. oleracea* subsp. *gemmifera*) (Kalloo and Bergh 1993), as well as the morphologically simple ''rapid-cycling'' genotypes with life cycles of 6–8 weeks (Williams and Hill 1986). The fact that many members of *B. oleracea* are inter-crossable, and able to generate fertile progenies, provides an expedient route to investigate the genetic basis of carbon partitioning and the formation of specialized storage organs.

B. oleracea has a center of diversity in the eastern Mediterranean area, and appears to have been subjected to intensive selection pressure during the past century (Prakash and Hinata 1980). Formal genetic studies of *B. oleracea* started in the 1920s (Kristofferson 1924; Detjen 1926; Pease 1926). The use of DNA markers to dissect the complex inheritance of *B. oleracea* has been reported (Kennard et al. 1994). Marker-trait associations were found, but the results were limited due to the small population size. The genus *Brassica* (tribe *Brassiceae*) is in the same taxonomic family as *Arabidopsis thaliana*, an ephemeral which contains the first plant genome to be completely sequenced. Such a close relationship suggests that crop plants of the genus *Brassica* will be among the earliest beneficiaries of the *Arabidopsis*

sequence. Their close relationship also provides an efficient way for *Brassica* scientists to utilize the extensive toolboxes, such as YAC/BAC contigs, ESTs and genetic/physical maps, that are available in *Arabidopsis*. A significant effort was invested in the construction of detailed *Brassica-Arabidopsis* comparative maps in recent years (Kowalski et al. 1994; Lagercrantz et al. 1996; Osborn et al. 1997). Such maps provide a direct way to align *Brassica* genes and QTLs to the corresponding chromosomal segments in *Arabidopsis*. Further, the model of *Brassica/Arabidopsis* may be extended to plants beyond the same family (Paterson et al. 1996).

To better understand the genetic control of *Brassica* morphology, we made three *Brassica* F_2 populations which shared one common parent, rapid-cycling *B. oleracea* (RCB). By growing the three populations under the same environment, genetic differences detected among the populations are inferred to come from the parent that is *not* common to the three populations. To survey the genetic divergence that has accumulated during the course of evolution in the *Brassica* genus, we chose genotypes originating from different geographic areas, and exhibiting different morphologies, to cross to RCB. These were *B. oleracea* var. Cantanese (origin Italy), var. Pusa Katki (origin India) and var. Bugh Kana (origin Thailand). By comparative QTL mapping among these three populations, we investigated the genetic control underlying common traits in the different populations. The present work, based on three *B. oleracea* F_2 populations and six leaf/stem-related traits, provides a detailed report of QTL-phenotype associations in *B. oleracea*, and a comparative analysis of possible orthologous mutations in *Arabidopsis.*

Materials and methods

Plant materials

Three *B. oleracea* F_2 populations were used for QTL analysis: rapid-cycling *B. oleracea* (RCB, self-compatible) x *B. oleracea* var. Cantanese (USDA accession No. PI462224, CAN), RCB × *B. oleracea* var. Pusa Katki (USDA accession No. PI274783, PK), and RCB × *B. oleracea* var. Bugh Kana (USDA accession No. PI249556, BK), composed of 247, 250 and 246 individuals respectively. One *B. oleracea* F_2 population, RCB × *B. oleracea* var. Green Comet (USDA accession No. G30771, GC), containing 56 individuals, was used to facilitate the construction of a composite linkage map. Rapid-cycling *Brassica* was from the Crucifer Genetics Cooperative, Madison, Wis. A single-plant selection derived by selfing a randomly chosen rapid-cycling plant was used. Seed and/or pollen of other *B. oleracea* varieties were generously provided by Dr. J. McFerson, and Dr. S. Kresovich, USDA/ARS, Geneva, N.Y. To minimize the environmental effect among populations, all the plants used for QTL analysis were grown in the same experimental field of the Texas Agricultural Experiment Station at the College Station, Texas, over the same period of time (from October 1994 to April 1995). A completely randomized design was used for field planting. Spacing between rows, and spacing of plants within rows, are 1 meter. Two parental RCB, one of the other parent, and one F_1 were planted in every row at randomly determined positions within the row. *Brassica* seedlings were nursed in the growth chamber for 2 weeks before transplanting to the field. Fertilization, irrigation and insecticide application were consistent with commercial production conditions.

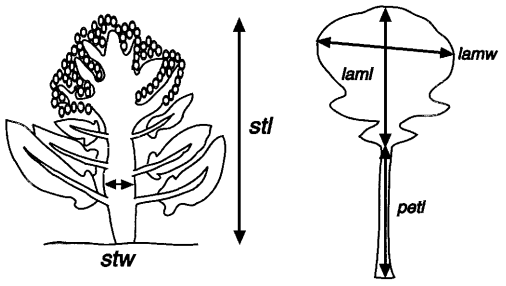


Fig. 1 Illustration of the plant size-related traits studied

Phenotyping

The following traits were measured (Fig. 1):

(1) Lamina length (*laml*): the length of the lamina on the longest leaf.
(2) Lamina width (*lamw*): the width of the lamina on the longest leaf.
(3) Petiole length (*petl*): the length of the petiole on the longest leaf.
(4) Stem length (*stl*): the length of the stem from the apical meristem to the ground.
(5) Stem width (*stw*): the diameter of the main stem at the widest point.
(6) Node number (*non*): the number of nodes on the main stem.

All the traits were measured on the date of appearance of the first flower. Leaf traits (*laml, Lamw, petl*) were measured in the BK and CAN populations only. Stem traits (*stl, stw, non*) were measured in the BK, CAN and PK populations.

Genotyping

DNA extraction, electrophoresis, Southern blotting and autoradiography were as previously described (Kowalski et al. 1994). A total of 113 *Brassica Pst*I genomic clones (''EW'', ''WG'' and ''WR'', from Pioneer HiBred), 35 *Arabidopsis* genomic clones (''M'', from Dr. E. Meyerowitz, Caltech), 23 *Arabidopsis* anonymous cDNA clones (''AC'', ''ATEX'' and ''TCH''), 4 cloned RAPD-PCR products (''R'', unpublished data), 198 *Arabidopsis* EST clones (''EST'', from Dr. R. L. Scholl, the *Arabidopsis* Biological Resources Center, Ohio State University) and 19 putatively embryo-specific *Arabidopsis* EST clones (''AHD'', ''AKJ'', AKN'', ''Cla'', ''d2P'', ''FLS'', ''HD'', ''HMG'', ''K'', ''S'' and ''Seed'', from Dr. Terry L. Thomas, Texas A&M University) were used in this study. The assembly of the *Brassica* composite linkage map was described elsewhere (Lan et al. 2000).

Data analysis

Trait means, correlations, and histograms were calculated by using SAS and Microsoft Excel. Broad-sense heritabilities were estimated from variances of the F_2 and F_1 (Falconer 1989). RFLP linkage maps were constructed by using Lander et al. (1987) and the Kosambi centiMorgan (cM) function. QTL likelihood maps, the % variance explained and gene actions were determined by using interval mapping in individual and multiple QTL models by MapMaker/QTL (Lander and Botstein 1989). A threshold of LOD (logarithm of odds) = 2.5 was used, appropriate for the marker density and the number and length of the *B. oleracea* chromosomes. For each phenotype, the ''free genetics mode'' was used to ''scan'' the whole genome and locate QTLs. Then the QTL was ''fixed'', and the genome re-scanned to seek QTLs that were masked by the largest QTL. If a new QTL was found, then the second largest QTL was also fixed, and the genome was scanned again until no further QTLs were found. For each QTL, LOD 1 and 2 support intervals were plotted.

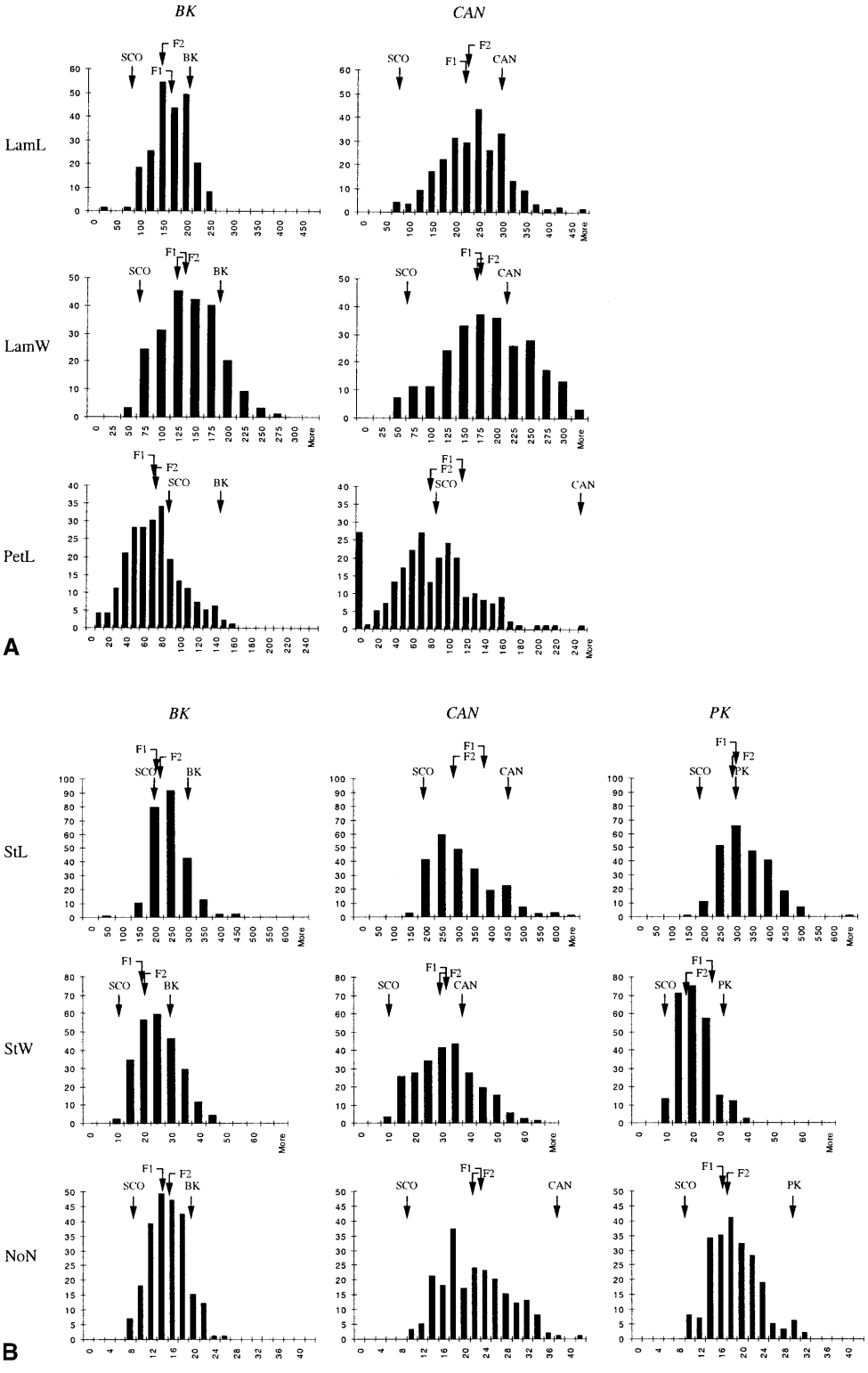


Fig. 2 Histograms of the plant size-related traits measured. The *arrows* indicate the average values of parents and F2

Results

Phenotypic variation

Figure 2 illustrates the phenotypic distribution, parental means and population means. For *petl and stl*, log transformation was used to improve normality. Potential transgression was observed in the trait of *petl* (for both BK and CAN populations).

Table 1 summarizes the broad-sense heritability for each trait investigated. Generally, for the same trait, broad-sense heritability varied greatly between

Table 1 Broad-sense herbitability for traits affecting leaf and stem morphology

Population	*laml*	*petl*	*lamw*	*stl*	*stw*	*non*
BK	0.58	0.80	0.27	0.29	0.32	0.87
CAN	0.78	0.86	0.88	0.36	0.56	0.49
PK	–	–	–	0.79	0.16	0.74
Average	0.68	0.83	0.57	0.48	0.35	0.70

Table 2 Correlation among traits affect leaf and stem morphology

Population	Trait	laml	petl	lamw	stl	stw
BK	petl	0.56**	1.00			
	lamw	0.80**	0.66**	1.00		
	stl	0.10	0.13	0.13	1.00	
	stw	0.58**	0.47**	0.57**	–0.03	1.00
	non	0.35**	0.24**	0.36**	–0.04	0.42**
CAN	petl	0.42**	1.00			
	lamw	0.80**	0.62**	1.00		
	stl	0.44**	0.37**	0.49**	1.00	
	stw	0.70**	0.56**	0.73**	0.40**	1.00
	non	0.44**	0.44**	0.48**	0.47**	0.51**
PK	stw	–	–	–	0.26**	1.00
	non	–	–	–	0.35**	0.43**

* a ≤ 0.1, ** a ≤ 0.01

Table 3 Biometrical parameters of QTLs associated with lamina length

Population	Chromosome	POS[b]	Weight	d/a	%VAR	LOG-LIKE	Gene action	LOD at 2′ site	LOD at 3′ site	LOD at BK site	LOD at Can site	Possible *Arabidopsis* mutations
BK	C4	EW4D04w+43	–20.54	–0.47	15.0%	2.68	DRA	1.63	0.08	–	3.60	*rev*
BK	C7	EW8C11a+0	–30.59	–0.32	33.0%	17.21	A	1.70	1.13	–	13.27	*axr1, axr3*
BK[a]	C6	EW2C08a+14	–5.90	–2.72	4.5%	2.74	R	1.33	0.02	–	0.20	
BK[a]	C9	EST131a+0	–11.12	–0.10	3.6%	2.65	RA	1.21	0.32	–	0.01	
CAN	C4	WG3F04b+4	–34.32	–0.11	13.7%	5.57	RA	1.01	1.41	2.30	–	*rev*
CAN	C7	EW8C11a+4	–51.59	–0.50	30.3%	14.80	RA	1.10	2.38	15.79	–	
CAN	C1	EW6F02+1	–15.24	1.19	4.0%	3.38	DA	0.74	1.08	0.98	–	*axr4, as2*

[a] QTL detected after fixing the ''largest'' QTL. The variance explained is the portion of residual variance remaining after the major QTL is fixed, so is not directly comparable to %VAR for the QTLs detected prior to fixing the largest QTL. The variance explained is from the original model in which none of the QTLs are fixed, in order to make the variance of each QTL directly comparable to one another

[b] POS: position of the QTL; Weight: additive component of the QTL effect; d/a: the ratio of the dominant effect to additive effect; %VAR: percentage of the total variance explained by the QTL; LOG-LIKE: Log-likelihood score of the QTL; Gene action: when the effect of the QTL allele followed a Mendelian model, it could either be dominant (D), recessive (R), or additive (A); LOD at 2′ site: Log-likelihood score at the second homoeologous location of the *Brassica* genome; LOD at 3 ′ site: Log-likelihood score at the third homoeologous location of the *Brassica* genome; LOD at BK site: Log-likelihood score at the same location of the BK genome; LOD at CAN site: Log-likelihood score at the same location of the CAN genome

populations with the exception of *petl* which was very high (0.80, 0.86). Such variations suggest different genetic control of the traits under study in these populations.

Correlations among traits

Many leaf traits were correlated with each other (Table 2). Specifically, *laml* and *lamw* were highly correlated (r = 0.8) in the BK and CAN populations. For stem traits, *stl* appeared to be correlated with leaf traits (*laml, lamw and petl*) in the CAN population, but not in the BK population. Similarly, *stl* appears to be correlated with *stw* and *non* in the CAN and PK populations, but not in the BK population.

QTLs detected for each trait

Figure 3 illustrates, and Tables 3–8 summarize, the QTLs detected for each trait:

Lamina length (laml).

In the BK population, four QTLs were found on chromosomes 4, 6, 7 and 9. A full model containing these QTLs

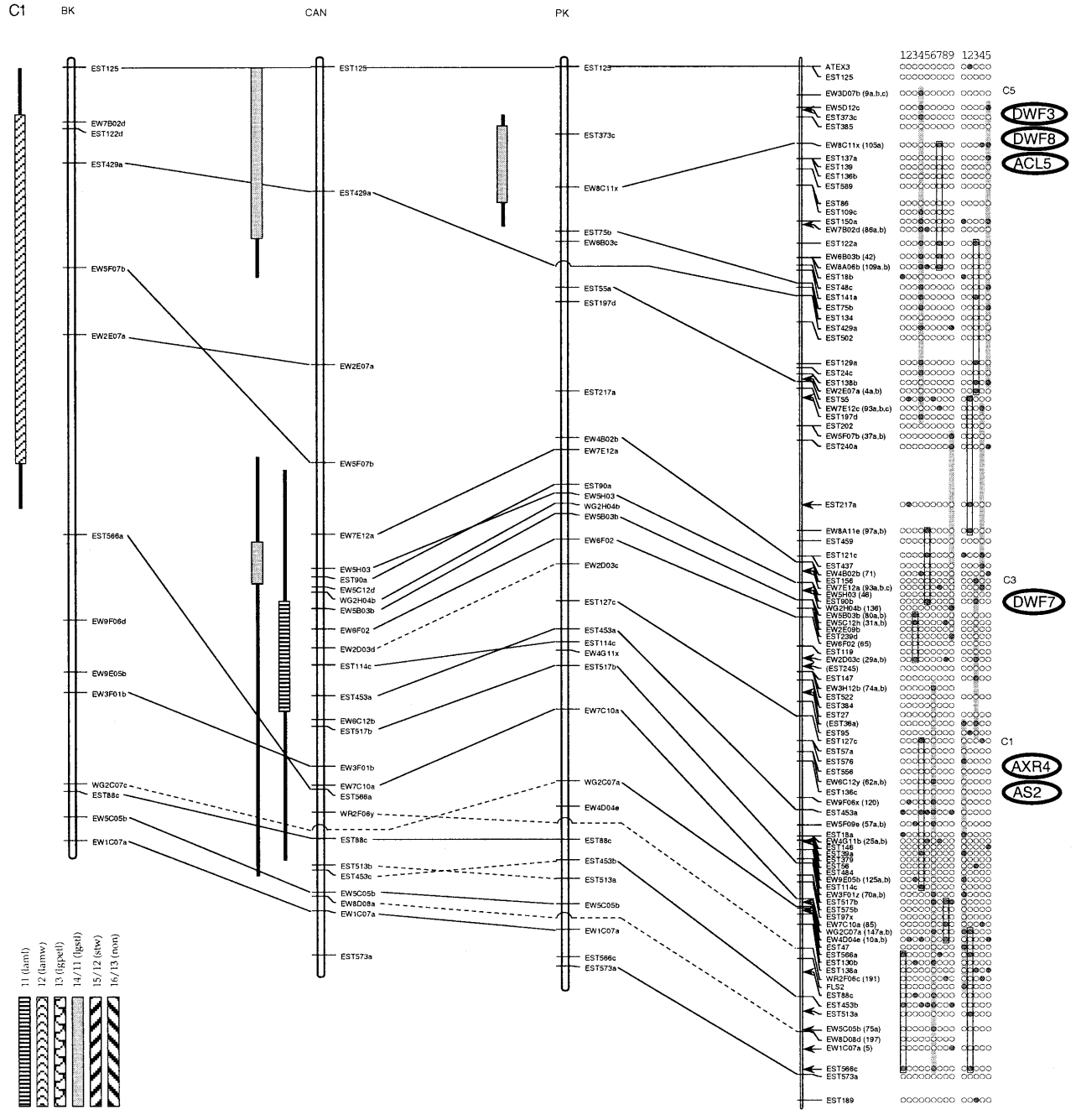


Fig. 3 QTL maps of the plant size-related traits. LOD 1 and 2 support intervals are indicated by *bars* and *whiskers* respectively

explained 43.4% of the phenotypic variance, with individual QTL models explaining 15.0–38.2% of the variance. The allele effects were all consistent with the difference between parents, as all of the BK alleles increase lamina length. BK alleles were mostly recessive or additive to RCB alleles, with the one exception being an allele of small effect for which gene action could not be resolved.

In the CAN population, three QTLs were found on chromosomes 1, 4 and 7. A full model containing these QTLs explained 51.2% of the phenotypic variance, with individual QTL models explaining 13.7–35.0% of the variance. The allele effects were all consistent with the difference between parents, as all of the CAN alleles increase lamina length. CAN alleles were largely additive or recessive to RCB alleles, the one exception being largely dominant.

Across two *Brassica* populations, the total of seven significant marker-trait associations may reflect variation at as few as five different genetic loci. The BK QTL on chromosome 4 corresponded closely to a CAN QTL; and the BK QTL on chromosome 7 corresponded closely to a CAN QTL.

No cases of possible homoeology were found, indicating that the five different genetic loci may be derived

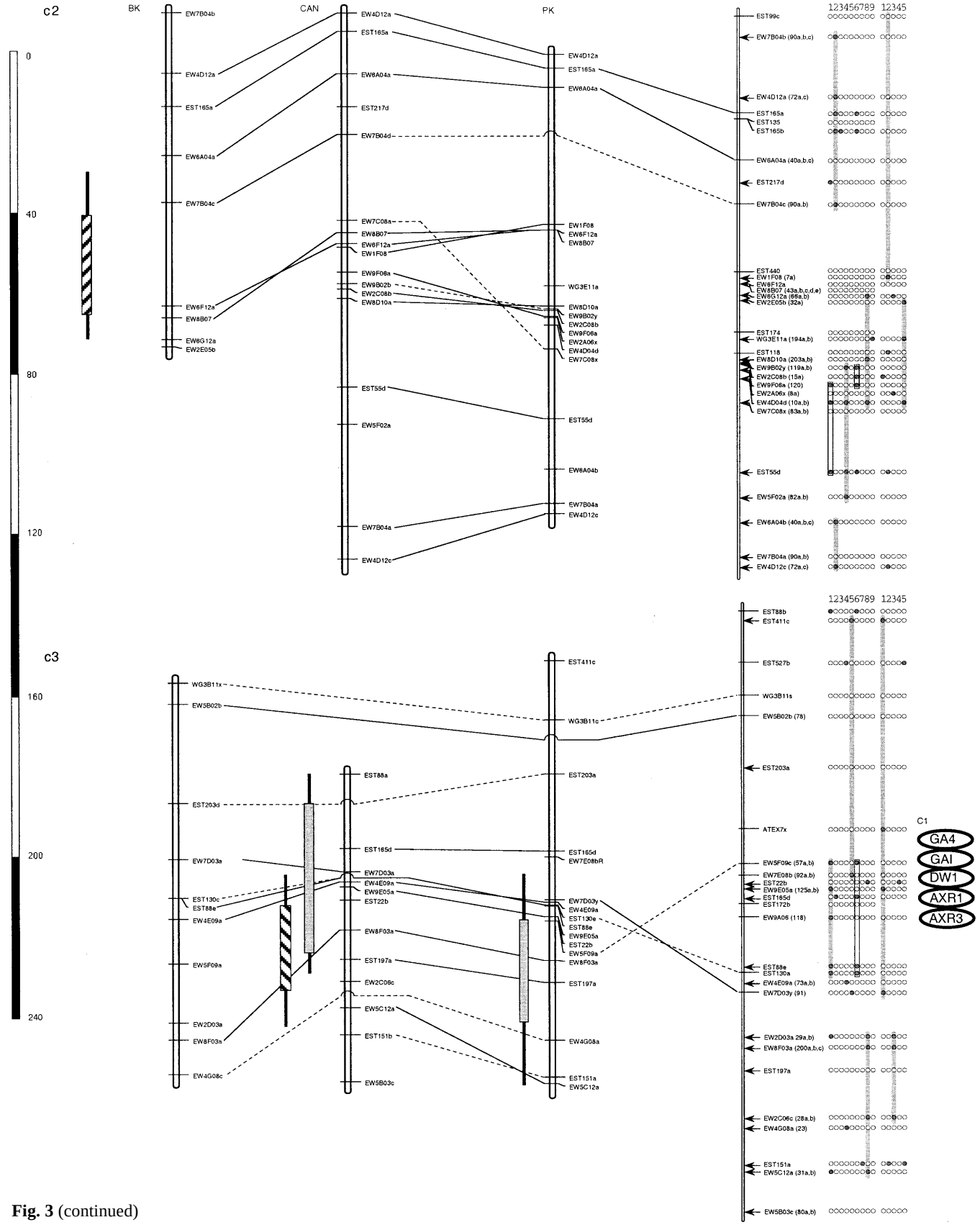
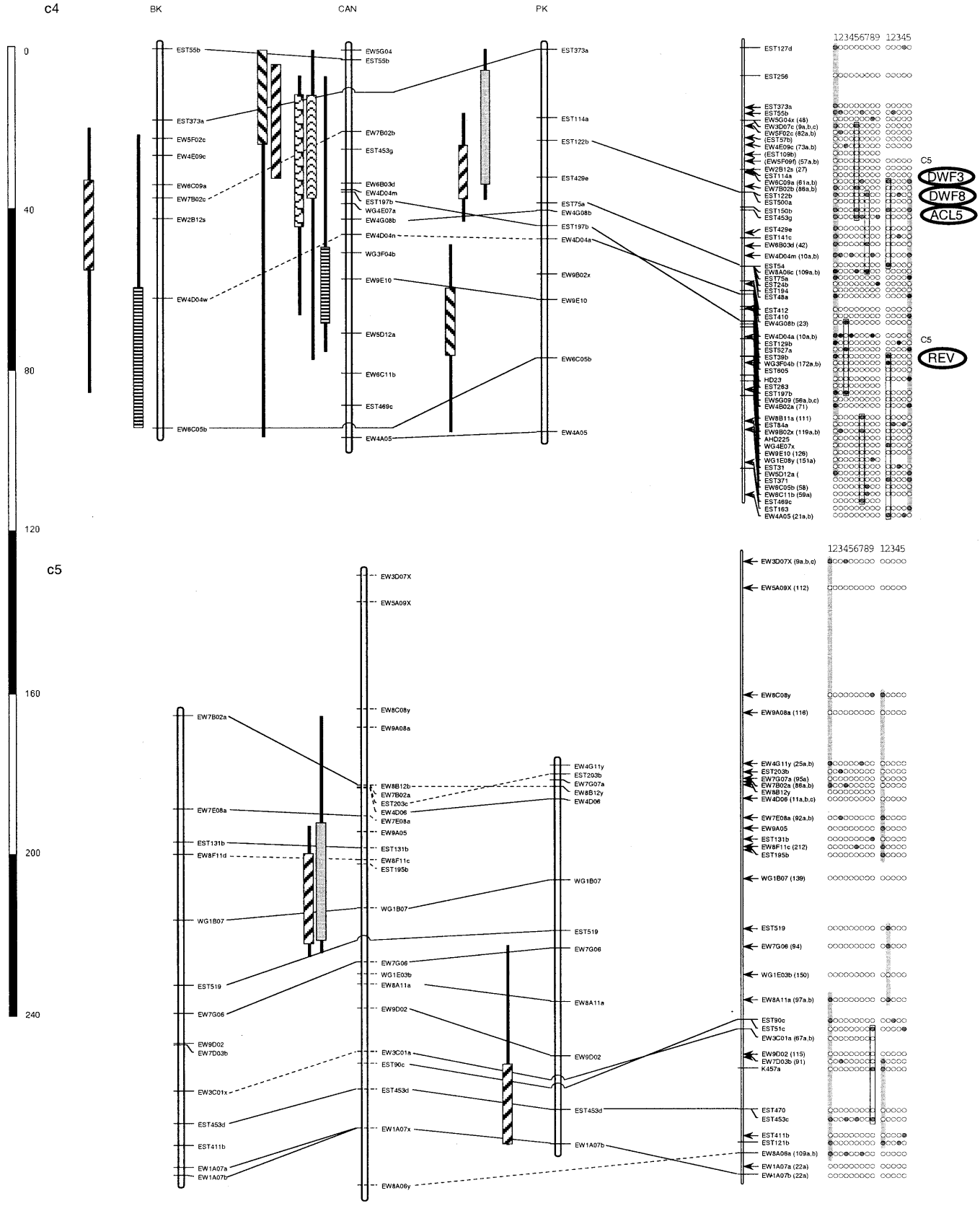


Fig. 3 (continued)



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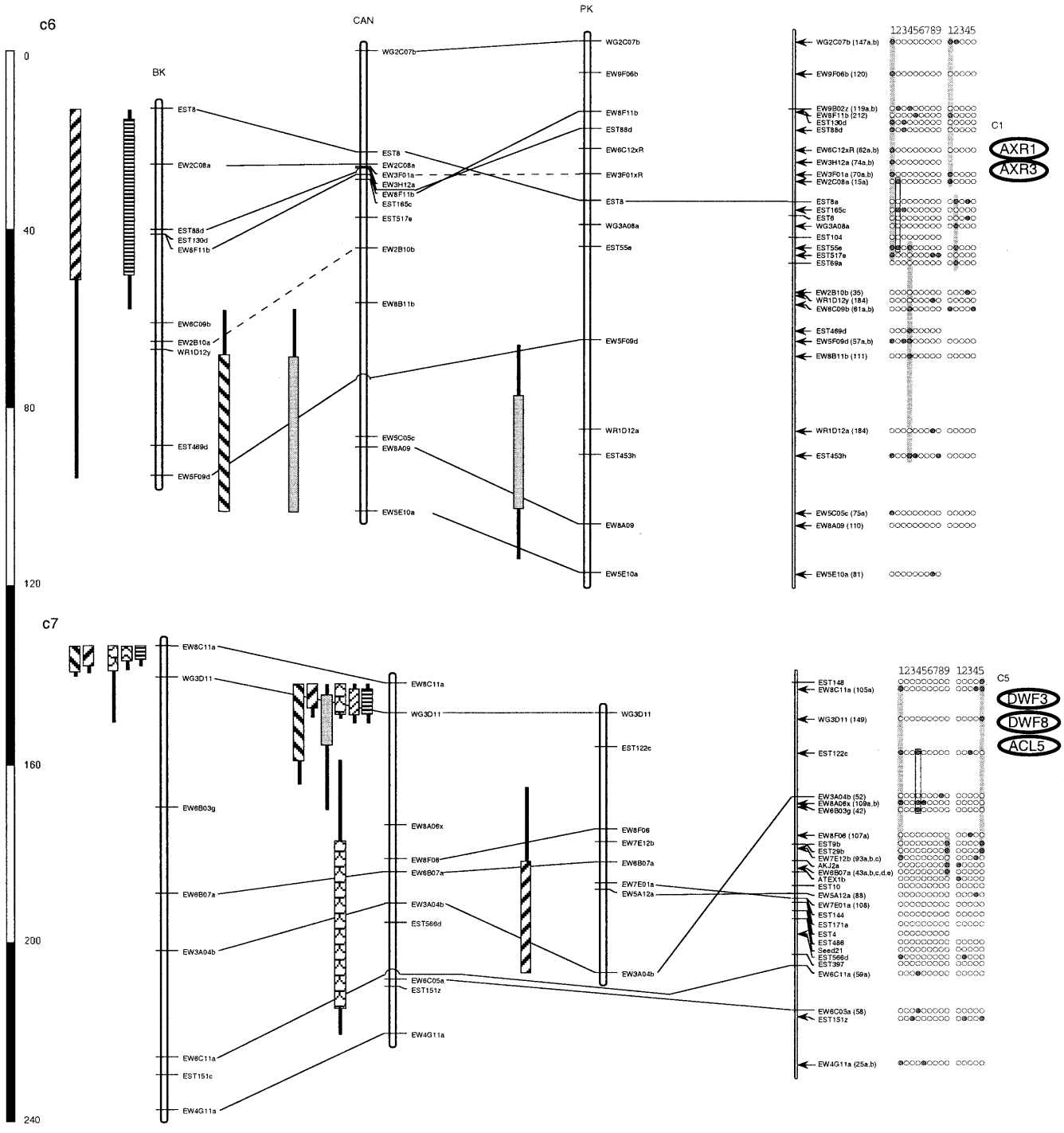

Fig. 3 (continued)

from five ancestral genes. These five ancestral genes correspond approximately to the locations of at least five mutants in *Arabidopsis* that are implicated in influencing stem length (Table 3).

Lamina width (lamw).

In the BK population, two QTLs were found on chromosomes 1 and 7. A full model containing these QTLs explained 44.7% of the phenotypic variance, with individual QTL models explaining 20.0–32.4% of the variance. Allele effects were consistent with the difference between parents, as both of the BK alleles increase lamina width. One BK allele was largely dominant to the RCB allele, and the other was largely recessive.

In the CAN population, three QTLs were found on chromosomes 4, 7 and 8. A full model containing these QTLs explained 54.7% of the phenotypic variance, with

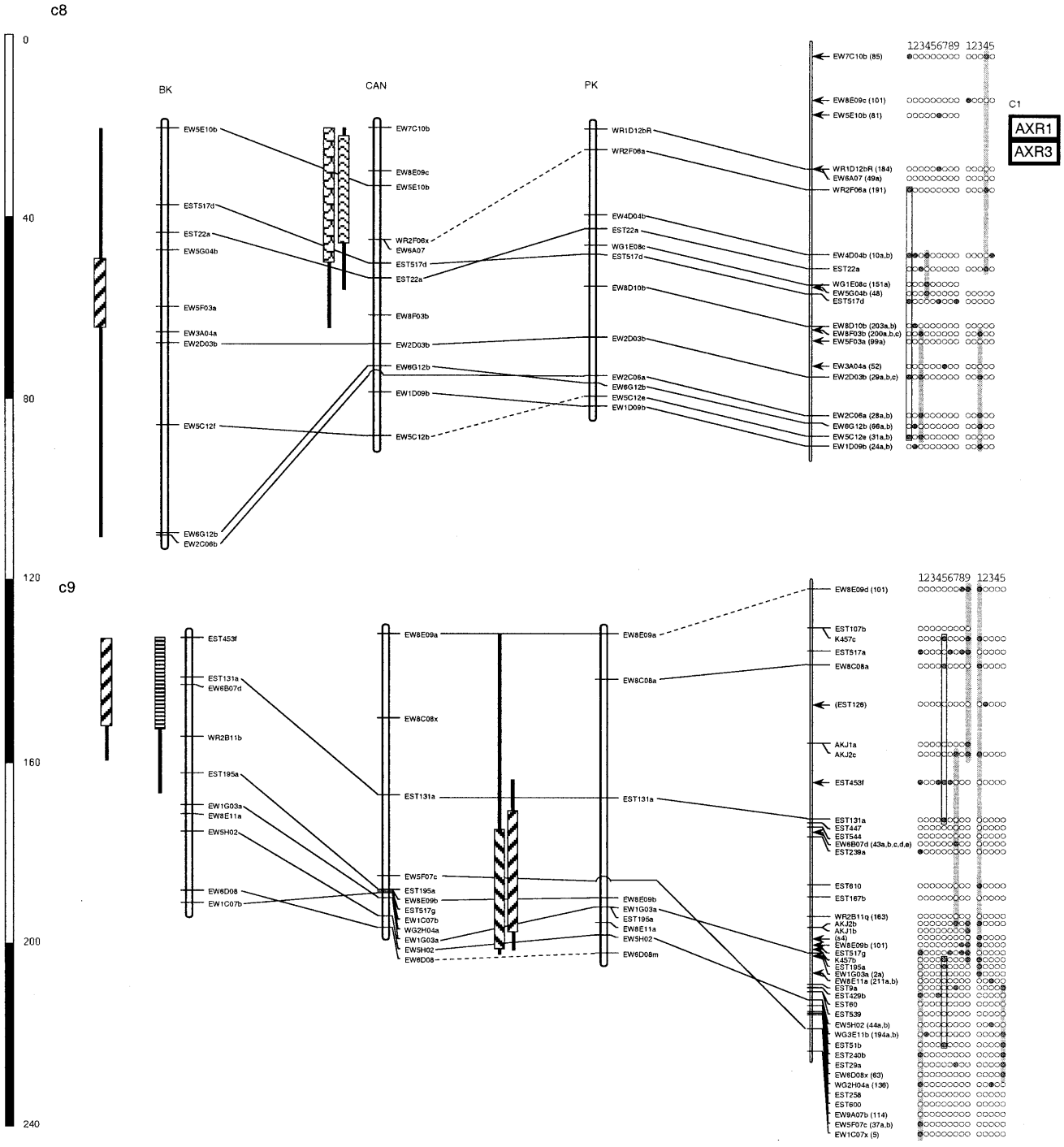

Fig. 3 (continued)

individual QTL models explaining 9.3–36.7% of the variance. Allele effects were consistent with the difference between parents, as all of the CAN alleles increase lamina width. The CAN alleles were mostly additive or recessive to the RCB alleles.

Across the two *Brassica* populations, the total of five significant marker-trait associations may reflect variation at as few as four different genetic loci. The BK QTL on chromosome 7 corresponded closely to a CAN QTL.

Further, the four different genetic loci may be derived from as few as three ancestral genes. The chromosome-4 QTL (CAN: EST453g+2) falls in a region that corresponds to the chromosome-7 QTLs (BK: EW8C11a+0, CAN: EW8C11a+4).

We found no candidate genes for this phenotype in the corresponding locations of *Arabidopsis*.

Table 4 Biometrical parameters of QTLs associated with lamina width

Population	Chromosome	POS	Weight	d/a	%VAR	LOG-LIKE	Gene action	LOD at 2′ site	LOD at 3′ site	LOD at BK site	LOD at Can site
BK	C1	EW2E07a+16	–24.87	0.74	20.0%	3.10	DA	1.08	0.67	–	0.03
BK	C7	EW8C11a+0	–34.03	–0.41	32.4%	17.58	RA	2.31	1.23	–	15.00
CAN	C4	EST453g+2	–26.66	–0.12	9.6%	4.40	RA	0.48	13.78	0.99	–
CAN	C7	EW8C11a+4	–54.69	–0.33	36.7%	17.71	A	0.64	2.94	16.83	–
CAN	C8	EW809c+0	–19.95	–1.16	9.3%	3.29	R	0.65	0.03	(0.25)	–

Table 5 Biometrical parameters of QTLs associated with petiole length

Population	Chromosome	POS	Weight	d/a	%VAR	LOG-LIKE	Gene action	LOD at 2′ site	LOD at 3′ site	LOD at BK site	LOD at Can site
BK	C7	EW8C11a+2	–0.15	0.03	19.5%	9.21	A	0.38	0.71	–	0.97
CAN	C4	EW7B02b+2	–0.11	–0.13	12.9%	5.67	A	1.19	7.53	0.76	–
CAN	C7	EW8C11a+2	–0.17	–0.44	25.7%	10.97	RA	3.19	0.65	9.21	–
CAN[a]	C7	EW3A04b+3	0.07	0.29	1.5%	2.82	DRA	0.67	4.28	0.05	–
CAN[a]	C8	WR2F06x+7	–0.08	–0.28	7.3%	2.88	DRA	1.02	1.63	(1.24)	–

Table 6 Biometrical parameters of QTLs associated with stem length

Population	Chromosome	POS	Weight	d/a	% VAR	LOG-LIKE	Gene action	LOD at 2′ site	LOD at 3′ site	LOD at BK site	LOD at Can site	LOD at PK site	Possible *Arabidopsis* mutations
CAN	C1	EST125+9	–0.05	0.96	11.1%	3.15	DA	0.11	3.47	0.09	–	2.98	*dwf3, dwf8, acl5*
CAN	C1	EW5H03+0	–0.05	0.20	6.8%	3.60	DA	1.82	0.08	0.08	–	0.54	*dwf7*
CAN	C3	EST165d+0	–0.05	0.55	7.0%	3.10	DA	1.90	1.15	0.07	–	0.59	*ga1, ga4, dw1, axr1, axr3*
CAN	C5	EST195b+8	–0.05	0.75	10.8%	5.05	DA	0.87	–	0.85	–	0.58	
CAN	C6	EW8A09+6	–0.06	–0.25	9.7%	3.61	RA	0.14	–	(0.13)	–	3.33	
CAN	C7	WG3D11+12	–0.07	1.24	22.1%	5.25	D	0.75	0.37	0.96	–	0.03	*dwf3, dwf8, acl1*
PK	C1	EST373c+4	–0.06	0.34	14.3%	5.09	DA	0.02	3.57	0.02	2.77	–	*dwf3, dwf8, acl1*
PK	C3	EW5F09a+6	–0.03	0.44	5.9%	2.77	DA	0.69	0.44	0.02	3.02	–	*ga1, ga4, dw1, axr1, axr3*
PK	C4	EST122b+2	–0.05	–0.20	11.3%	5.03	A	4.78	0.08	1.07	0.71	–	*dwf3, dwf8, acl5*
PK	C6	WR1D12a+0	–0.05	–0.34	9.5%	4.90	RA	3.02	0.07	0.13	2.93	–	

Petiole length (petl).

In the BK population, one QTL was found on chromosome 7. A full model containing this QTL explained 19.5% of the phenotypic variance. The allele effect was consistent with the difference between parents, as the BK allele increased petiole length. The BK allele was additive to the RCB allele.

In the CAN population, four QTLs were found on chromosomes 4, 7(2) and 8. A full model containing these QTLs explained 49.1% of the phenotypic variance, with individual QTL models explaining 12.9–32.7% of the variance. Allele effects were generally consistent with the difference between parents, as three of the CAN alleles increase petiole length, while one of the RCB alleles increases petiole length. CAN alleles were largely additive to RCB alleles, the two exceptions being alleles of small effect for which the gene action could not be clearly resolved.

Across the two *Brassica* populations, the total of five significant marker-trait associations may reflect variation at as few as four different genetic loci. The BK QTL on chromosome 7 corresponded closely to one CAN QTL.

Further, the four different genetic loci may be derived from as few as three ancestral genes. The chromosome-4 QTL (CAN: EW7B02b+2) falls in a region that corresponds to the chromosome-7 QTLs (BK: EW8C11a+2, CAN: EW8C11a+2).

We found no candidate genes for this phenotype in the corresponding locations of *Arabidopsis*.

Stem length (stl).

No QTL was found in the BK population. In the CAN population, six QTLs were found on chromosomes 1 (2), 3, 5, 6 and 7. A full model containing these QTLs ex-

Table 7 Biometrical parameters of QTLs associated with stem with

Population	Chromosome	POS	Weight	d/a	%VAR	LOG-LIKE	Gene action	LOD at 2′ site	LOD at 3′ site	LOD at BK site	LOD at Can site	LOD at PK site
BK	C2	EW7B04c+16	0.88	6.83	18.7%	3.65	D	1.91	2.40	–	0.17	0.95
BK	C4	EW2B12s+0	–2.48	–0.77	7.1%	2.89	DA	0.59	14.16	–	3.69	15.06
BK	C7	EW8C11a+2	–5.72	–0.01	32.2%	17.12	A	0.37	1.10	–	16.69	(1.60)
BK	C8	EW5G04b+13	–2.33	–0.04	5.6%	2.69	DRA	1.72	3.12	–	0.23	0.35
BK	C9	EW6B07d+0	–2.66	0.01	6.9%	3.38	A	2.15	0.61	–	0.68	2.99
BK[a]	C6	EWc2C08a+2	–2.29	0.11	5.7%	2.64	DRA	1.81	1.37	–	0.23	0.08
CAN	C4	EST55b+14	–5.18	0.18	11.7%	4.57	DA	0.84	15.42	1.30	–	14.54
CAN	C5	EST195b+10	2.28	2.64	11.0%	4.87	D	0.34	–	0.11	–	0.17
CAN	C7	Ew8C11a+2	–8.99	–0.24	33.3%	16.69	A	1.12	4.52	17.12	–	(1.60)
PK	C4	EST122b+7	–4.94	0.06	32.5%	16.48	A	0.86	2.19	2.56	3.85	–
PK	C7	EW5A12a+0	–2.51	–0.37	8.0%	4.12	RA	0.69	0.08	0.63	1.41	–
PK	C9	EST131a+15	–3.16	0.57	12.4%	4.06	DA	0.75	2.90	2.69	0.73	–
PK[a]	C5	EST453d+0	–1.87	–0.50	5.3%	2.71	RA	0.45	1.72	0.54	0.39	–

[a] QTL detected after fixing the ‘‘largest’’ TL. The variance explained is the portion of residual variance remaining after the major QTL is fixed, so is not directly comparable to %VAR for the QTLs detected prior to fixing the largest QTL. The variance explained is from the original model in which none of the QTLs are fixed, in order to make the variances of each QTL directly comparable to one another

Table 8 Biometrical parameters of QTLs associated with node number

Population	Chromosome	POS	Weight	d/a	%VAR	LOG-LIKE	Gene action	LOD at 2′ site	LOD at 3′ site	LOD at BK site	LOD at Can site	LOD at PK site
BK	C7	EW8C11a+0	–1.71	–0.39	13.7%	6.95	RA	0.50	0.93	–	6.87	(0.41)
CAN	C3	EW8F03a+3	–3.20	0.46	13.7%	5.69	DA	0.78	0.42	0.70	–	0.28
CAN	C7	EW8C11a+4	–3.75	–0.31	16.5%	7.19	RA	1.83	1.93	6.39	–	(0.41)
CAN[a]	C4	EST55b+6	–2.13	–0.70	5.8%	2.90	RA	2.19	6.95	1.13	–	(3.55)
CAN[a]	C6	EW5C05c+0	–1.97	0.55	4.3%	2.70	DRA	0.64	1.48	–	–	1.06
PK	C4	EW9E10+4	–2.71	0.37	17.7%	6.24	DA	0.35	0.33	0.16	3.63	–
PK	C9	EW8E11a+0	–1.56	0.25	6.1%	2.53	DA	0.31	1.4	0.16	0.09	–

[a] QTL detected after fixing the ‘‘largest’’ TL. The variance explained is the portion of residual variance remaining after the major QTL is fixed, so is not directly comparable to %VAR for the QTLs detected prior to fixing the largest QTL. The variance explained is from the original model in which none of the QTLs are fixed, in order to make the variances of each QTL directly comparable to one another

plained 58.9% of the phenotypic variance, with individual QTL models explaining 6.8–22.1% of the variance. Allele effects were generally consistent with the difference between parents, as five of the CAN alleles increase stem length, while one of the RCB alleles increases stem length. CAN alleles were mostly additive or dominant to the RCB alleles, the one exception being largely recessive.

In the PK population, four QTLs were found on chromosomes 1, 3, 4 and 6. A full model containing these QTLs explained 37.2% of the phenotypic variance, with individual QTL models explaining 5.9–14.3% of the variance. Allele effects were generally consistent with the difference between parents, as all of the PK alleles increase stem length. PK alleles were mostly dominant or additive to RCB alleles, the one exception being largely recessive.

Across the three *Brassica* populations, the total of ten significant marker-trait associations may reflect variation at as few as seven different genetic loci. CAN QTLs on chromosomes 1, 3 and 6 corresponded closely to PK QTLs.

Further, the seven different genetic loci may be derived from as few as five ancestral genes. The chromosome-1 QTL (CAN: EST125+9, PK: EST373C+4) falls in a region that corresponds to the chromosome-4 (PK: EST122b+2) and chromosome-7 (CAN: WG3D11+12) QTLs.

Finally, these five ancestral genes correspond approximately to the locations of at least nine mutants in *Arabidopsis* that are implicated in influencing stem length (Table 6).

Stem width (stw).

In the BK population, six QTLs were found on chromosomes 2, 4, 6, 7, 8 and 9. A full model containing these QTLs explained 54.5% of the phenotypic variance, with individual QTL models explaining 5.6–37.2% of the variance. Allele effects were generally consistent with the difference between parents, as five of the BK alleles increase stem width, while one of the RCB alleles in-

creases stem width. BK alleles were mostly dominant or additive to RCB alleles, the two exceptions being alleles of small effect for which no mode of gene action could be resolved.

In the CAN population, three QTLs were found on chromosomes 4, 5 and 7. A full model containing these QTLs explained 52.7% of the phenotypic variance, with individual QTL models explaining 11.0–33.3% of the variance. Allele effects were generally consistent with the difference between parents, as two of the CAN alleles increase stem width while one of the RCB alleles increases stem width. CAN alleles were mostly dominant or additive to RCB alleles.

In the PK population, four QTLs were found on chromosomes 4, 5, 7 and 9. A full model containing these QTLs explained 56.2% of the phenotypic variance, with individual QTL models explaining 8.0–37.0% of the variance. Allele effects were consistent with the difference between parents, as all of the PK alleles increase stem width. PK alleles were mostly additive or recessive to RCB alleles, the one exception being largely dominant.

Across the three *Brassica* populations, the total of 13 significant marker-trait associations may reflect variation at as few as nine different genetic loci. BK QTLs on chromosome 4 corresponded closely to CAN and PK QTLs, BK QTLs on chromosome 7 corresponded closely to the CAN QTL, and BK QTLs on chromosome 9 corresponded closely to the PK QTL.

Further, the nine different genetic loci may be derived from as few as seven ancestral genes. The chromosome-4 QTL (BK: EW2B12s+0, CAN: EST55b+0, PK: EST122b+7) falls in a region that corresponds to the chromosome-7 QTL (BK: EW8C11a+2, CAN: EW8C11a+2); and the chromosome-7 QTL (PK: EW5A12a+0) corresponds to those on chromosome-9 (BK: EW6B07d+0, PK: EST131a+15).

Node number (non).

In the BK population, one QTL was found on chromosome 7, which explained 13.7% of the phenotypic variance. The allele effect was consistent with the difference between parents. The BK allele of this QTL increased the node number, and was recessive or additive to the RCB allele.

In the CAN population, four QTLs were found on chromosomes 3, 4, 6 and 7. A full model containing these QTLs explained 42.0% of the phenotypic variance, with individual QTL models explaining 13.7–23.5% of the variance. Allele effects were generally consistent with the difference between parents, as all of the CAN alleles increase the number of nodes. CAN alleles were mostly recessive or additive to the RCB alleles, the one exception being largely dominant, while one was unable to rule out any effect.

In the PK population, two QTLs were found on chromosomes 4 and 9. A full model containing these QTLs explained 23.8% of the phenotypic variance, with individual QTL models explaining 6.1–17.7% of the variance. Allele effects were consistent with the difference between parents, as both of the PK alleles increase the number of nodes. PK alleles were mostly dominant or additive to RCB alleles.

Across the three *Brassica* populations, the total of seven significant marker-trait associations may reflect variation at as few as six different genetic loci. The BK QTL on chromosome 7 corresponded closely to the CAN QTL.

Further, the six different genetic loci may be derived from as few as five ancestral genes. The chromosome-4 QTL (CAN: EST55b+6) falls in a region that corresponds to the chromosome-7 QTL (BK: EW8C11a+0, CAN: EW8C11a+4).

We found no candidate genes for this phenotype in the corresponding locations of *Arabidopsis*.

Discussion

Brassica evolution appears to have included a number of 'morphological excursions' that resulted in profound changes in the size, shape, and timing of development of most plant organs. The fact that such morphological divergence has occurred among taxa that can be intercrossed to generate fertile progenies makes *Brassica* a fascinating subject for genetic analysis. This study dissects variation in leaf and stem traits that differentiate between the ephemeral habit of some wild *Brassicas*, and the robust architecture of *Brassica* cultivated for curd (enlarged inflorescence) production.

The genetic control of leaf and stem architecture in *Brassica* is complex. By a comparison of the QTL maps constructed from three different F_2 populations, we have identified a total of 47 QTLs based on a LOD threshold of 2.5. The three crosses shared one common parent, an ephemeral *rapid-cycling Brassica* strain of relatively small stature and dimensions consistent with its short life cycle. Among the three different parents, *B. oleracea* var. Bugh Kana (BK), which generates small curd compared to varieties CAN and PK, is believed to be the progenitor of broccoli and cauliflower (Song and Osborn 1992). CAN and PK, from Italy and India respectively, represent robust curd-forming types of diverse morphology and geographical origin. Studies of other traits (Lan and Paterson submitted) have tended to support the notion that BK may represent a progenitor of types such as CAN and PK, in that the BK × RCB population showed simpler genetic control of most traits**.** However, the results of this study were equivocal in this regard. Some traits such as node number and petiole length showed very simple genetic control in the BK population; however, other traits such as lamina length and stem width showed a remarkable amount of variation.

Arabidopsis candidate genes were identified for the *Brassica* QTLs based on their corresponding chromosomal location in the *Arabidopsis* genome. Among the

candidate genes, *AXR1* encodes a protein related to the amino-terminal half of the ubiquitin-activating enzyme E1 (Leyser et. al. 1993), and *AXR3* encodes *IAA17* (Rouse et. al. 1998) of the *AUX/IAA* gene family. All of the aux mutants are isolated under an auxin-resistant environment, and are involved in the auxin-signaling pathway. *GA1* encodes the enzyme ent-kaurene synthase A (Silverstone et. al. 1997), and *GA4* encode 3-beta-hydroxylase (Chiang et. al. 1995); both enzymes involved in the biosynthetic pathway of gibberellin acid (GA). *Arabidopsis* mutant *dw1* accumulates 24-methylenecholesterol (Klahre et. al. 1998) and mutant *dw7* (Choe et. al. 1999) is defective in the delta-7-sterol-C-5 de-saturation step. Both enzymes belong to the biosynthetic pathway of brassinosteroids (BRs). Auxin, GA and BRs are plant hormones, controlling various plant development and differentiation processes. These processes, including cell division and cell elongation, will directly influence the traits lamina length and shoot length, which we investigated here.

Among the 47 QTLs detected, six displayed dominance/additive(d/a) absolute values larger than 1, suggesting overdominance. This is much less evidence of overdominance than was found for curd-related traits (29 of 114 QTLS with d/a >1). These loci include QTLs for the traits *laml* (BK: EW2C08a+14, CAN: EW6F02+1), *lamw* (CAN: EW8E09c+0), *stml* (CAN: WG3D11+12) and *stmw* (BK: EW7B04c+16, CAN: EST195b+10). Two of the six QTLs show a negative d/a value, including EW2C08a+14 (BK) and EW8E09c+0 (CAN) which control *laml* and *lamw* (leaf size) respectively. QTL EW7B04c+16 has the greatest d/a value of 6.83. It has been observed that crosses between parents of different origins generally exhibit greater heterosis than crosses between parents of similar origins in *Brassica* (Grant and Beversdorf 1985; Lefort-Buson et al. 1987; Brandle and McVetty 1990; Ali et al. 1995). Our results show that four QTLs with an overdominance effect were mapped in the CAN population, and two were mapped in the BK population, which tend to support the previous observation that rapid-cycling *Brassica* exhibits a closer genetic distance to *B. oleracea* var. Bugh Kana than to the other varieties (Song and Osborn 1992).

By the comparison of three diverse *B. oleracea* genotypes, we sought to investigate the extent to which apparently similar phenotypes were under common genetic control. Although this experiment was designed to investigate the comparative evolution of the inflorescence, the study of other aspects of plant morphology and their relationship (if any) to curd-related traits may provide a more detailed understanding of the suite of changes needed to transform a small ephemeral plant into cauliflower. Through comparative mapping we inferred that the 47 QTLs might reflect variation in as few as 35 different genetic loci, illustrating that most QTLs differed between populations. Further, the comparison of ostensibly homoeologous locations of duplicated *Brassica* chromosomal regions showed that the 35 putatively different genetic loci might be accounted for by 28 ancestral genes, i.e., about 1/5 of the QTLs had possible homoeologs. These data reinforce the picture obtained based on QTLs for curd-related traits (Lan and Paterson 2000), and also previously-published data (Kowalski et al. 1994) that the *Brassica* genome may be relatively rapidly evolving, perhaps facilitated by a high level of duplication (Lan et al. 2000). A few striking cases do suggest that some *Brassica* QTLs may represent independent mutations at corresponding duplicated (homoeologous) loci. For example, for the trait *stl*, the chromosome-1 QTLs (CAN: EST125+9, PK: EST373C+4) appear to fall in a region that corresponds to the chromosome-4 QTL (PK: EST122b+2) and the chromosome-7 QTL (CAN: WG3D11+12). However, the gene action shows that QTL EST122b+2 (PK) is additive, and QTL WG3D11+12 (CAN) is dominant, suggesting that, even though their corresponding locations are the same, they are more likely to be different QTLs.

Both leaf and stem traits showed evidence for the coordinate control of (nominally) different traits. Among seven *laml* QTLs, five *lamw* QTLs and five *petl* QTLs, six QTLs mapped to a homologous region involving the top of *B. oleracea* chromosome 7 in both the BK and CAN populations. Four QTLs controlling *lamw* and *petl* mapped to the top of chromosomes 4 and 8 in the CAN population. These results suggest that either single QTLs controlling pleiotropic effect in these regions, or clusters of tightly linked genes, may be involved. We identified ten *stl* QTLs, 13 *stw* QTLs and seven *non* QTLs; five QTLs controlling *stl*, *stw* and *non* mapped to the top of *B. oleracea* chromosome 7 in both BK and CAN, but not in PK populations. *Stw* and *non* QTLs also mapped to the same location at the top of chromosomes 4 (CAN population) and 9 (PK population). The fact that *stl* QTLs mapped to the top of chromosome 7 in the CAN population, but not in the BK population, further suggested there were at least two different genes controlling *stl*. A pleiotropic effect could still be involved in *stw* and *non* in this region. To resolve the above issue would require a high-resolution study, such as ''substitution mapping'' (Paterson et al. 1990).

Brassica scientists will be among the first beneficiaries of a complete genomic sequence of *A. thaliana*, another member of the crucifer family; already, it is evident that comparative data can point to possible candidate genes from *Arabidopsis* that may help to identify transcripts accounting for important *Brassica* phenotypes. For trait *laml*, a region of *Brassica* chromosome 1 corresponds to a region of *Arabidopsis* chromosome 1 containing the leaf mutations *axr4* and *as2*; a homologous region of *Brassica* chromosome 4 corresponds to a region of *Arabidopsis* chromosome 5 containing the leaf mutation *rev*; and a region of *Brassica* chromosome 6 corresponds to a region of *Arabidopsis* chromosome 1 containing the leaf mutations *axrl* and *axr3*. For trait *stl*, a homologous region of *Brassica* chromosome 1 (CAN: EST125+9, EST373c+4), chromosome 4 (PK: EST122b+2) and chromosome 7 (WG3D11+12) correspond to a region of *Arabidopsis* chromosome 5 containing the leaf mutations *dwf3*, *dwf8* and *acl5*; a homolo-

gous region of *Brassica* chromosome 1 (CAN: EW5H03+0) corresponds to a region of *Arabidopsis* chromosome 3 containing the leaf mutation *dwf7*; and a homologous region of *Brassica* chromosome 3 corresponds to a region of *Arabidopsis* chromosome 1 containing the leaf mutation *ga4, ga1, dw1, axr1,*and *axr3*. No *Arabidopsis* mutations were found to correspond to the *Brassica* chromosome-7 QTL for *laml*, and no *Arabidopsis* mutations were found to correspond to the *Brassica* chromosome 6 QTL for *stl*.

Of special importance to plant biology may be the 'specialized' phenotypes of *Brassica*, for which *Arabidopsis* counterparts have not been found, such as *stw* (stem width). The trait *stw* provides a chance to examine the control mechanism of carbon partitioning while comparing to trait *rk1* (first-rank branching, the number of branches within the curd that originated from the main stem which were measured on the first flowering day), which was considered to be the most important component of curd size (Lan and Paterson 2000). Comparing the 13 QTLs regulating *stw* to the 13 QTLs controlling *rk1*, most fall at similar locations with three exceptions. First, *rk1* detected QTLs located on chromosomes 1 and 3, while *stw* did not. Second, *stw* detected a QTL located on chromosome 2, while *rk1* did not. These QTLs might be a good starting point to investigate carbon partitioning in *Brassica*. A fascinating future study will be to investigate the *stw* QTLs detected here in crosses made between kohlrabi (*B. oleracea* subsp. *gongylodes*) and rapid cycling *Brassica*, as well as marrow stem kale (*B. oleracea* subsp. *medullosa*). Both kohlrabi and marrow stem kale have a much larger (wider) stem width than the genotypes we investigated here. Further, a similar approach can be extended to more distant crosses such as *turnip (B. compestris L.*), radish (*Raphanus sativus L.)* or swede (*Brassica napus L*. var. napobrassica) which represent another storage organ, the root. The latter will add a new dimension to the comparative genomics of *Brassica* in that it is a polyploid of recent origin, and may add new dimensions to our understanding of the consequences of polyploid formation and subgenomic divergence for quantitative inheritance (Jiang et al. 1998; Wright et al. 1998).

The *Brassica* genus, and *B. oleracea* in particular, illustrates the 'specialized' features that distinguish major crops from *A. thaliana*, a botanical model for molecular genetic studies. The prospect of a complete sequence for *Arabidopsis* in the near future will open new doors into many aspects of plant biology by using comparative approaches, with special importance for its close relatives such as *Brassica*. With the morphological diversity of *Brassica* and the abundance of *Arabidopsis* resources, we believe that establishing the Cruciferae as a model for dissecting crop development will soon bear fruit.

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