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ORIGINAL PAPER

Development of public immortal mapping populations, molecular markers and linkage maps for rapid cycling *Brassica rapa* and *B. oleracea*

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Abstract Publicly available genomic tools help researchers integrate information and make new discoveries. In this paper, we describe the development of immortal mapping populations of rapid cycling, self-compatible lines, molecular markers, and linkage maps for *Brassica rapa* and *B. oleracea* and make the data and germplasm available to the *Brassica* research community. The *B. rapa* population consists of 160 recombinant inbred (RI) lines derived from the cross of highly inbred lines of rapid cycling and yellow sarson *B. rapa*. The *B. oleracea* population consists of 155 double haploid (DH) lines derived from an F1 cross between two DH lines, rapid cycling and broccoli. A total of 120 RFLP probes, 146 SSR markers, and one phenotypic trait (flower color) were used to construct genetic linkage maps for both species. The *B. rapa* map consists of 224 molecular markers distributed along 10 linkage groups (A1–A10) with a total distance of 1125.3 cM and a marker density of 5.7 cM/marker. The *B. oleracea* genetic map consists of 279 molecular markers and one phenotypic marker distributed along nine linkage groups (C1–C9) with a total distance of 891.4 cM and a marker density of 3.2 cM/marker. A syntenic analysis with *Arabidopsis thaliana* identified collinear genomic blocks that are in agreement with previous studies, reinforcing the idea of conserved chromosomal regions across the Brassicaceae.

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Introduction

Brassica rapa L. ($n = 10$) and *B. oleracea* L. ($n = 9$) belong to the Brassicaceae family and include vegetables, oilseeds, condiments, and forages of economical importance. Genetic linkage maps for *B. rapa* and *B. oleracea* have been developed and widely utilized. To date, there have been seven maps reported for *B. rapa* (Song et al. 1991; Chyi et al. 1992; Teutonico and Osborn 1994; Kole et al. 1997; Suwabe et al. 2006; Kim et al. 2006; Choi et al. 2007) and 12 for *B. oleracea* (Slocum et al. 1990; Landry et al. 1992; Kianian and Quiros 1992; Ramsay et al. 1996; Bohuon et al. 1996; Camargo et al. 1997; Hu et al. 1998; Sebastian et al. 2000; Lan and Paterson 2000; Li et al. 2003; Babula et al. 2003; Gao et al. 2007). These linkage

maps have been used, in part, to understand and exploit the genetic control of morphological diversity within the genus.

The absence of a common set of genetic tools, however, has hindered the ability to compare and integrate the results obtained from different mapping studies. Therefore, *Brassica* researchers refer to a variety of genetic maps with different marker types, loci, and nomenclatures for homologous linkage groups. Attempts to unify some of the maps generated by independent groups have been made (see, e.g., Hu et al. 1998); however, the lack of common sets of lines and molecular markers has made this task difficult. Thus, there is also a need for accessible sets of immortal mapping populations, since the vast majority of mapping studies in the diploid *Brassica* crops has been conducted using F2 populations. Recently, there have been three reported immortal mapping populations, a recombinant inbred line set for *B. rapa* (Kole et al. 1997), and two double haploid sets for *B. oleracea* (Bohuon et al. 1996; Sebastian et al. 2000). These populations are difficult to maintain due to self-incompatibility and the lack of common molecular markers between the two species making them difficult to compare. *Brassica* researchers are in need of a common set of tools in order to compare and relate findings from different studies.

The close sequence identity of coding regions (~87%) between the genomes of *Brassica* species and *Arabidopsis thaliana* (Cavell et al. 1998), allows for detailed comparative analysis (Lan and Paterson 2000; Lagercrantz 1998; Lagercrantz and Lydiate 1996; Lukens et al. 2003; O'Neill and Bancroft 2000; Osborn et al. 1997; Parkin et al. 2002, 2005; Paterson et al. 2001). For instance, comparative mapping studies have allowed for the assignment of orthologous segments in *Arabidopsis* and *Brassica* species, allowing the identification of candidate genes that may directly account for *Brassica* quantitative and qualitative trait loci (Paterson et al. 2001).

The objectives of the research presented here were to develop sets of immortal, public mapping populations for *B. rapa* and *B. oleracea*; to generate linkage maps using previously mapped and publicly available RFLP probes and SSR primer pairs from different research groups; to map newly developed and publicly available SSR primer pairs; and to align the maps with the *Arabidopsis* genome. The use of previously mapped RFLP and SSR markers and common molecular markers between both populations will aid in the unification of different genetic maps for these crops.

Materials and methods

Population development and isolation of genomic DNA

The *B. rapa* mapping population (hereafter refer to as BraIRRi) was derived by crossing a highly inbred rapid cycling *B. rapa* (IMB211) and a highly inbred annual yellow sarson (R500). An F_1 plant was self-pollinated and recombinant inbred lines (RILs) were obtained by single seed descent (through selfing for five generations) as described in Poehlman and Sleper (1995). This population is composed of 160 RILs. The *B. oleracea* mapping population (hereafter refer to as BolTBDH) was created from an F_1 individual that was derived by crossing a double haploid (DH) broccoli line ("Early Big") and a DH rapid cycling (TO1000DH3) line. The F_1 generation was sent to the USDA/ARS facility in Charleston, South Carolina where over 200 double haploid plants were obtained by anther culture. These plants were self-pollinated to create a population size of 155 DH lines. Seeds for all lines were increased in the greenhouse and will be available for public distribution from the corresponding author and from the Adding Value to the UK Brassica Crop Science Community (AdVaB) project in the UK (http://www.brassica.info/CropStore/maps.php). All lines are self-compatible, facilitating seed increase without the need for bud pollination.

Flower buds were harvested and bulked from 10 to 15 plants of the parents and lines of each population. The collected tissue was lyophilized and DNA of each line from each population was isolated from 300 to 500 mg samples of lyophilized bud tissue. DNA isolation was conducted following the CTAB procedure described by Kidwell and Osborn (1992). The isolated DNA was used to generate Southern blots for RFLP marker genotyping and as PCR templates for SSR genotyping.

Source of RFLP probes and SSR primer pairs for mapping

All of the RFLP probes, including DNA clones of *FLC* (*BrFLC1*, *BrFLC2*, *BrFLC3* and *BrFLC5*) (Schranz et al. 2002), EZ3, and *CHS28* that were mapped in the BraIRRIL and BolTBDH populations were previously used in other mapping studies and their origin, development and utilization were described in detail by Udall et al. (2005). The nomenclature adopted by Parkin et al. (1995) and Sharpe et al. (1995) (prefixes pW and pX) was used to name RFLP marker loci in order to facilitate cross-reference with other studies that used this nomenclature. All the pW and pX RFLP probes have been sequenced and their sequences were deposited in the NCBI probe database (e.g. accession number CZ906485 corresponding for probe pW191 and DT469171 for probe pX138). The majority of SSR primer pairs used in this study (prefix "fito") were developed using genomic sequence derived from the TO1000DH3 parental line (Iniguez-Luy, 2008). A smaller set of SSR primer pairs surveyed from the literature was used as potential anchor markers to facilitate cross-reference with other mapping studies. These were identified by their original designation, B.n., BRMS, PMR and Na/Ol as described

in Szewc-McFadden et al. (1996); Suwabe et al. (2002); Uzunova and Ecke (1999) and the UK CROPNET *Brassica* database (http://ukcrop.net/perl/ace/search/BrassicaDB), respectively.

The probes (94 pWs, 30 pXs and six DNA cloned genes) were screened by hybridization to blots containing genomic DNA from the parents and seven progeny lines from each of the mapping populations. Genomic DNAs (parents and progeny) were digested using four restriction enzymes (RE), *Hin*dIII, *Eco*RI, *Xba*I, and *Dra*I. This strategy of screening the parents and a small set of progeny (minimum of seven individuals) for each enzyme allowed us to identify the maximum number of useful polymorphic loci per probe and the best enzymes for genotyping the population. When a probe was polymorphic with more than two of the enzymes used, samples were digested with the enzyme that offered the clearest polymorphic pattern and, if possible, had been used previously by Parkin et al. (2005) and/or Udall et al. (2005). The fito SSRs (587 primer pairs) and the literature surveyed SSRs (144 primer pairs) were screened as described in (Iniguez-Luy, 2008).

Southern blot hybridization and PCR reactions

Five µg of DNA from parental lines and the segregating populations were digested with *Hin*dIII, *Eco*RI, *Xba*I, and *Dra*I in separate reactions using 8 units/µg of DNA. Digests of the parental lines and segregating populations were electrophoresed in 0.8% agarose gels (1X TAE), run for 14 h at 24 V and then transferred onto Hybond-XL membranes (Amersham, NJ) using an alkaline transfer method. The DNA was fixed to the membrane by UV crosslinking followed by 2 h in a 90°C vacuum oven. Probe labeling and hybridization was conducted as described by Teutonico and Osborn (1994) with minor modifications. Probes were amplified by PCR using universal primers T3 and T7. PCR products were purified using the GFX™ PCR DNA and gel band purification kit (GE Healthcare Bioscience, NJ). PCR for SSR detection were conducted as described in Iniguez-Luy et al. (2008). Each of the selected and informative primer pairs was run on parental lines and six segregating lines from the BraIRRi and BolTBDH populations.

Segregation and linkage analysis

Marker locus names for RFLP probes and SSR primer pairs followed the methodology adopted by Udall et al. (2005). Deviations of marker locus frequencies from expected segregation ratios for an F5 generation RIL of 1:1 with 6% residual heterozygosity and for a Double Haploid Line (DHL) of 1:1 were calculated by χ^2 analysis using the program JoinMap® v3.0 (Van Ooijen and Voorrips 2001). A scoring matrix was created using MSExcel and the data was transformed into JoinMap® using a Microsoft® Visual macro Recomb_addin.xla v0.5 developed by Udall (2003) (contact information: Dr. Josh Udall, Brigham Young Univeristy, jaudall@byu.edu).

Linkage analysis and map construction were conducted separately for each population using JoinMap® v3.0. Linked loci were grouped using a LOD threshold of 5–8 and a maximum recombination fraction of 0.4. Grouped RFLP and SSR marker loci were arranged into a scoring matrix using MSExcel. Data were inspected using the Microsoft® Visual macro Recomb_addin.xla v0.5 developed by Udall (2003). Double crossovers were checked and the data were corrected whenever appropriate. After the original scores were rechecked, a final linkage map was constructed for each population. Map distances in centi-Morgans (cM) were calculated using the Kosambi mapping function. Finally, marker position and order were checked with RECORD (Van Os et al. 2005). The linkage maps were also named as BraIRRi for the *B. rapa* population and BolTBDH for the *B. oleracea*. The maps were drawn for presentation using PowerPoint.

The identification of homoeologous loci in both maps (BraIRRi and BolTBDH) and their designation to corresponding linkage groups (LGs) (under the international nomenclature) was conducted as follows: (1) For the BolTBDH, the mapped loci position where corroborated by examining segregating data and linkage designation derived from Udall et al. (2005) which contained, in one of their populations, the parental line TO1000DH3. In addition, locus position for the RFLP probes and SSR primer pairs that were used in Lukens et al. (2003), Parkin et al. (2002), and Parkin et al. (2005) was inferred only when appropriate (e.g. sharing collinearity and/or position well established like those for Flowering Locus C). (2) For the BraIRRi, the mapped loci position where corroborated by examining segregating data derived from Teutonico and Osborn (1994) which contained, in one of their populations, the parental line R500. Conversion of the old LG designation from Teutonico and Osborn (1994) to the new international nomenclature was conducted in our lab and adopted by the current study. In addition, mapped loci position where inferred from single locus SSR used in common with the map developed Suwabe et al. (2006).

BLAST analysis and map alignment with the *Arabidopsis* genome

Sequences of the *Brassica* cDNA and genomic DNA fragments used as RFLP probes or *Brassica* genome sequences flanking the SSR marker motifs were used with the program BLASTN (http://www.ncbi.nlm.nih.gov/) was used to identify *A. thaliana* homologous sequences. Default

BLASTN parameters were used for the analysis, and two sequences were considered putatively homologous if the *E* value of their alignment was <0.01. One note of caution should be stated on the fact that we based our alignment analysis using a very high *E* value (0.01). However, based on a previous study conducted by Lukens et al. (2003) it was observed that such *E* value was acceptable to make genomic comparison between *Arabidopsis* and *B. oleracea*. *Brassica* sequences were compared with each of the five *A. thaliana* chromosomes as represented in the NCBI refseq records NC_003070.5, NC_003071.3, NC_003074.4, NC_003075.3 and NC003076.4. Bioperl scripts executed a BLAST search for each *Brassica* sequence and parsed the output (available upon request). The length, nucleotide position, *E* value and percent identity for each significant high scoring pairwise alignment (HSP) were recorded. A perl script merged the *Brassica* marker genetic map locations and *A. thaliana* genome locations into one file. All 226 probes and primer pairs used to construct the genetic maps (BraIRRi and BolTBDH) were derived from *Brassica* sequences. Synteny between the two diploid *Brassica* maps and the *A. thaliana* genomes were visualized by illustrating the portion of collinear runs along each of the 10 and 9 LGs for both maps, respectively.

Results

Screening of polymorphic markers using a small set of progeny lines and segregation analysis

The 131 RFLP probes used in this study were screened for useful polymorphisms using four RE and genomic DNA extracted from parental mapping lines and seven progeny lines from each mapping population (BraIRRi and BolTBDH). Of these probes, 113 (86%) were polymorphic in the BraIRRi population and 116 (88%) were polymorphic in the BolTBDH population using at least one RE (Table 1). For the BraIRRi mapping population, 37% of the polymorphic loci were scored using *Hin*dIII digests, 28% using *Eco*RI, 28% using *Xba*I, and 8% using *Dra*I. For the BolTBDH mapping population, 34% of the polymorphic loci were scored using *Hin*dIII digests, 29% using *Eco*RI, 28% using *Xba*I and 9% using *Dra*I.

The use of multiple RE coupled with the inclusion of a small set of segregating progeny, increased the number of scorable loci by 22 and 27% in the BraIRRi and BolTBDH populations, respectively, over the number possible using the single most informative enzyme. In some cases, the same locus was identified by multiple RE for the same probe. For instance, the RFLP probe pW125 identified three DNA fragments corresponding to one segregating locus (pW125aH) using *Hin*dIII and four DNA fragments corresponding to two segregating loci (pW125aE and pW125dE) using *Eco*RI in the BolTBDH population. By pre-screening seven progeny, we determined that locus pW125aE cosegregated with locus pW125aH, indicating they were the same locus, and mapping to C1. Therefore, only one of these loci (pW125aH) was used for linkage analysis in the full population. Locus pW125dE, however, was detected to be a different segregating locus for the same RFLP probe, and thus, was included in the linkage analysis (mapping to C2). The screening analysis using multiple RE and seven progeny lines detected 188 informative RFLP

Table 1 Characteristics of the *Brassica rapa* (BraIRRi) and *B. oleracea* (BolTBDH) mapping populations, and of the RFLP, SSR and phenotypic markers screened and mapped in the populations

Characteristics	BraIRRi	BolTBDH
Immortal population type	Recombinant inbred lines	Double haploid lines
No. of lines	160	155
No. of RFLP probes tested	131	131
No. of uninformative RFLP probe	18	15
No. of polymorphic RFLP probes	113	116
No. of RFLP Prb. detected as 1 P. loci	59	56
No. of RFLP Prb. detected as 2 P. loci	36	40
No. of RFLP Prb. detected as 3 P. loci	15	16
No. of RFLP Prb. detected as 4 P. loci	3	4
Total No. of RFLP loci detected[a]	188	200
Average polymorphic loci/RFLP probe	1.4	1.5
No. of RFLP probes used on full map pop.	102	115
Total No. of RFLP loci mapped	131	155
No. of SSR primer pairs tested	731	731
No. of uninformative SSR primer pairs[b]	491	474
No. of polymorphic SSR primer pairs	240	257
No of SSR PP. detected as 1 P. loci	224	237
No of SSR PP. detected as 2 P. loci	12	13
No of SSR PP. detected as 3 P. loci	4	6
No of SSR PP. detected as 5 P. loci	–	1
Total No of SSR loci detected[a]	260	286
Average polymorphic loci/SSR primer pair	1.1	1.1
No. of SSR primer pairs used on full map pop.	64	91
Total No. of SSR loci mapped	93	122
No. of phenotypic markers	0	1

Prb. RFLP probe, *P.* polymorphic, *PP* primer pairs

[a] The parental lines were screened together with seven progeny lines chosen from each mapping populations. Enzymes used in the screening of RFLPs were *Hin*dIII, *Eco*RI, *Xba*I and *Dra*I

[b] This number includes monomorphic and fail to amplify target DNA fragment SSR primer pairs

loci (1.4 loci per probe) in the BraIRRi population and 200 loci (1.5 loci per probe) in the BolTBDH population (Table 1).

The 731 SSR markers included in this study were screened for polymorphisms using the parental mapping lines and six progeny lines for each population. Although the inclusion of a small set of progeny lines facilitated the visualization of segregating SSR markers it did not influence the number of all possible informative SSR markers as in the previous case for RFLP markers. From the polymorphic screening 240 SSR markers (33%) were polymorphic in the BraIRRi population and 257 (35%) were polymorphic in the BolTBDH population (Table 1). About 50–55% of the 731 SSR markers tested amplified the correct PCR product size (bp) based on predictions set by design parameters. These fragment sizes were, however, indistinguishable between the mapping parents. The remainder of the screened SSR markers (~20–30%) did not produce PCR fragments or the fragment produced did not meet the predicted size.

The informative SSR markers detected 260 segregating SSR loci (1.1 loci per primer pair) in the BraIRRi population and 286 loci (1.1 loci per primer pair) in the BolTBDH population (Table 1).

The majority of the RFLP and SSR markers detected in the two mapping populations were scored as codominant markers, only 24 (6 RFLP and 18 SSR) and 27 (9 RFLP and 17 SSR) loci were scored as dominant markers in the BraIRRi and BolTBDH populations, respectively.

Linkage analysis

A portion of the informative marker loci identified by the polymorphism screening (225 of 448 loci for BraIRRi and 279 of 486 loci for BolTBDH) were selected based on ease of scoring, availability of sequence information and use in previous maps, and these markers were used to screen the entire mapping populations and build the two genetic linkage maps. Flower color (white TO1000DH3 and yellow Early Big broccoli) was a phenotypic trait segregating in the BolTBDH. This trait was scored and included in the linkage analysis (mapped to the bottom of C3). The number of LGs obtained were equivalent to the haploid chromosome number of *B. rapa* (A genome $n = 10$) and *B. oleracea* (C genome $n = 9$). There were 17 and 19 unlinked loci in the BraIRRi and BolTBDH mapping populations, respectively. The average numbers of crossover per individual per linkage group were 1.2 and 1.0 for the BraIRRi and BolTBDH mapping populations, respectively.

The use of previously mapped RFLP and SSR markers allowed us to designate all the LGs following the international nomenclature established for the A (A1–A10) and C (C1–C9) diploid genomes, formerly referred to as R and O groups (Parkin et al. 1995; Lowe et al. 2004; Suwabe et al. 2002; Udall et al. 2005). The total genetic lengths of the final BraIRRi and BolTBDH linkage maps were 1113.6 and 891.8 cM, respectively (Figs. 1, 2; Table 2; Supplementary Materials 1 and 2). The largest LG in the BraIRRi population corresponded to A3 followed by A2 and A6. A1, A4, A5, A7, A9 and A10 were of similar size (Table 2). The smallest LG was A8. The largest LG in the BolTBDH population corresponded to C6. C1, C2, C3, C4, C5 and C7 were of similar sizes (Table 2). The smallest LG for the BolTBDH population was C9. The BraIRRi population had an average spacing between loci of 5.8 cM, and the BolTBDH was slightly denser with an average spacing between loci of 4.2 cM. The density varied across LGs for both maps (Table 2). Based on estimated DNA contents of 529 Mbp for *B. rapa* and 696 Mbp for *B. oleracea* (Johnston et al. 2005) the estimated physical average spacing between markers is 2.7 and 3.2 Mbp for the BraIRRi and BolTBDH populations, respectively. The RFLP and SSR markers used to build the linkage maps were distributed evenly across the two populations studied (Figs. 1, 2; Supplementary Materials 1 and 2). In addition, at least two SSR markers were mapped to each LG of both maps. Because of the versatility of SSR markers, single locus SSRs can be used as potential markers to anchor these maps to existing *B. rapa* and *B. oleracea* maps.

Segregation distortion

Deviations from the expected Mendelian segregation ratio (RIL 1:1-with 6% residual heterozygosity for an F5- and DHL of 1:1) were observed in both populations. The percentages of loci with significant levels ($P < 0.01$) of allelic imbalance were 25% (57/225) and 49% (139/279) for the BraIRRi and BolTBDH populations, respectively. In both populations, the marker loci exhibiting distorted segregation were not randomly distributed throughout the genome (Figs. 1, 2). The majority of the loci (50) in the BraIRRi showed an excess of alleles from the rapid cycling parent IMB211. These marker loci clustered within A2, A3, A4, A5, A6, A7 and A9. Marker loci having an excess of R500 marker loci (7 loci) were clustered in LG A2. There was no significant ($P > 0.01$) deviation from the expected portion of residual heterozygote loci (6%) in the BraIRRi population. In the BolTBDH population, alleles at loci showing segregation distortion were more evenly distributed among the two parental lines. Most of the loci exhibiting excess of Early Big alleles (77 loci) were clustered on LGs C1, C2, C4, C5 and C7; those with an excess of TO1000DH3 alleles (62 loci) were on LGs C3 (in its entirety), C6 and C8. As expected, there were no heterozygous loci detected in the BolTBDH population.

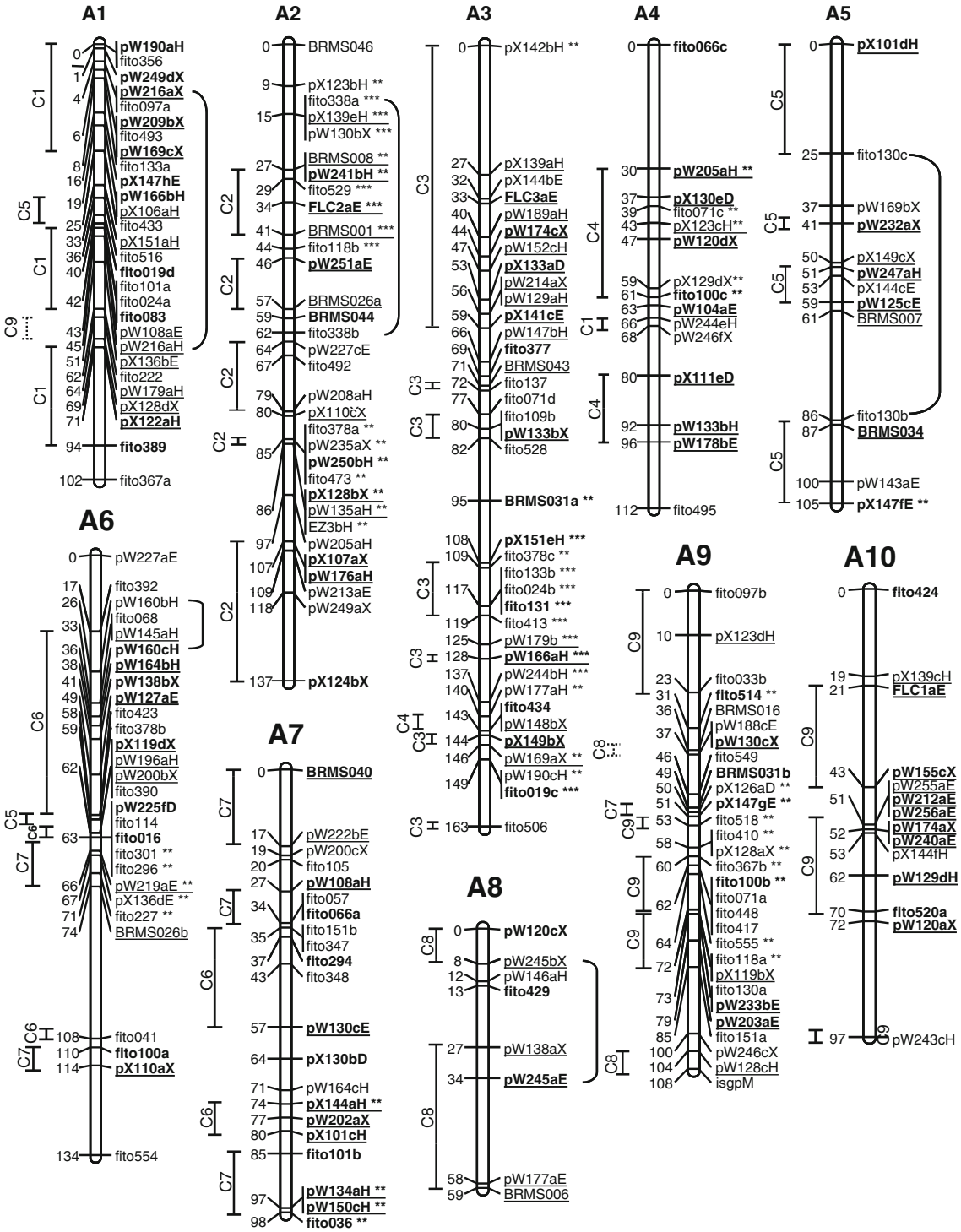

Fig. 1 *Brassica rapa* linkage map based on a population of recombinant inbred lines (BraIRRi). *Vertical bars* represent linkage groups A1–A10 (international nomenclature). Marker locus names and distances (cM) are located to the *left* and *right* of the *vertical bars*, respectively. *Bold* marker loci represent common loci between the BraIRRi and BolTBDH maps. *Underlined* marker loci represent common markers with previous mapping studies (Parkin et al. 2005; Udall et al. 2005). *Brackets* to the *right* of each linkage group represent duplicated loci within the same chromosome. The degree of segregation distortion (**$P < 0.01$ and ***$P < 0.001$) is represented by an *asterisk* next to marker locus names. *Vertical lines* next to distances (cM) illustrate putative homeologous regions between the A (BraIRRi) and C (BolTBDH) genomes. RFLP loci are labeled by the prefixes pW and pX (FLC and EZ3 were also used as RFLP probes). SSR loci are labeled by the prefixes fito and BRMS

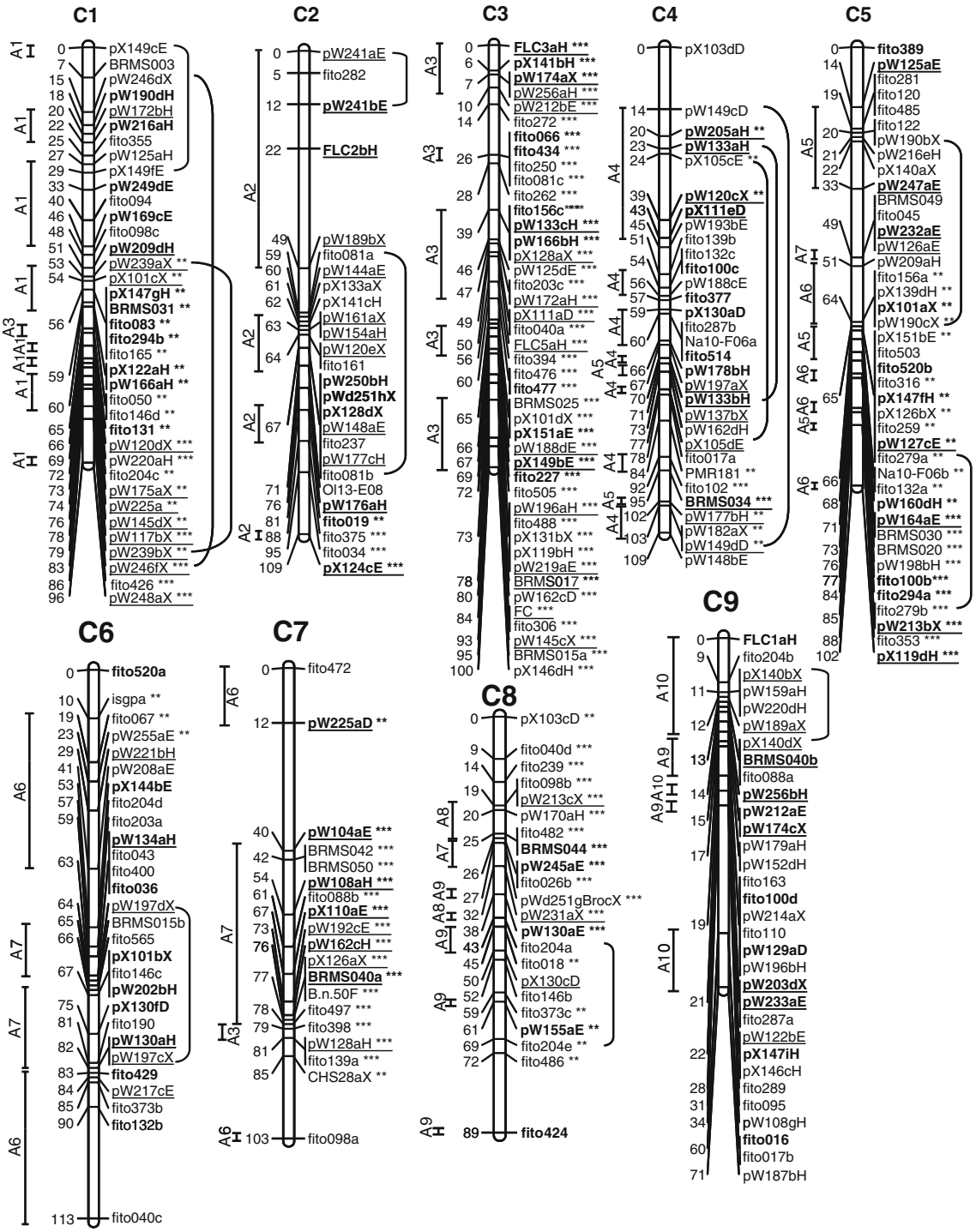

Fig. 2 *Brassica oleracea* linkage map based on a population of double haploid lines (BolTBDH). *Vertical bars* represent linkage groups C1–C9 (international nomenclature). Marker locus names and distances (cM) are located to the *left* and *right* of the *vertical bars*, respectively. *Bold* marker loci represent common loci between the BolTBDH and BraIRRi maps. *Underlined* marker loci represent common markers with previous mapping studies (Parkin et al. 2005; Udall et al. 2005). *Brackets* to the *right* of each linkage group represent duplicated loci within the same chromosome. The degree of segregation distortion (**$P < 0.01$ and ***$P < 0.001$) is represented by *asterisk* next to marker locus names. *Vertical lines* next to distances (cM) illustrate putative homeologous regions between the C (BolTBDH) and A (BraIRRi) genomes. RFLP loci are labeled by the prefixes pW and pX (FLC and CHS28 were also used as RFLP probes). SSR loci are labeled by the prefixes fito, BRMS, Ol, Na, B.n. and PMR. FC stands for flower color

Table 2 Linkage group size, number of marker loci, average marker interval per linkage group, and map characteristics for the two mapping populations

Brassica rapa RIL map (BraIRRi)				*Brassica oleracea* DH map (BolTBDH)			
LG[a]	Size (cM)	Number of loci	Average marker interval (cM)	LG[a]	Size (cM)	Number of loci	Average marker interval (cM)
A1	101.5	28	3.6	C1	95.7	37	2.6
A2	136.8	32	4.3	C2	109.0	26	4.2
A3	162.9	37	4.4	C3	100.0	43	2.3
A4	111.7	15	7.4	C4	109.4	31	3.5
A5	105.1	13	8.1	C5	101.8	41	2.5
A6	133.6	28	4.8	C6	113.2	28	4.0
A7	97.6	21	4.6	C7	102.8	19	5.4
A8	59.1	8	7.4	C8	88.8	22	4.0
A9	107.9	29	3.7	C9	71.0	32	2.2
A10	97.4	14	7.0	–	–	–	–
Total	1113.6	225	4.9	–	891.8	279	3.2

[a] Linkage Group designation using internationally recognized nomenclature (Parkin et al., 1995; Lowe et al., 2004; Suwabe et al., 2002 and Udall et al., 2005). These references refer to the R and O nomenclature that has now been converted to the A and C nomenclature

Replicated marker loci and the relationship between *B. rapa* and *B. oleracea*

About 70% of the polymorphic RFLP loci detected multiple loci (Table 1) in both populations (129 and 144 RFLP loci in the BraIRRi and BolTBDH populations, respectively). In contrast, only 15% of the polymorphic SSR loci detected multiple loci (Table 1) in both populations (36 and 49 SSR loci in the BraIRRi and BolTBDH populations, respectively). The frequency of probes and primer pairs together detecting more than one locus (hereafter refer to as replicated loci) within the A genome (BraIRRi population) was approximately 50%. Replicated loci located within the C genome (BolTBDH population) had a similar frequency as the A genome (48%). Out of these frequencies, 5% (in the BraIRRi population) and 19% (in the BolTBDH population) were found to be duplicated within a given LG (Figs. 1, 2). For example, the RFLP probe pW239 detected two loci on C1 (Fig. 2). In both populations, replicated loci tended to cover the entire A and C genomes, although there was a concentration of replicated loci in A1, A2, A3, C1, C3, C5 and C9 (Figs. 1, 2).

The utilization of common markers to construct both linkage maps, and prior information regarding genomic location of these markers (see "Discussion") in other mapping studies (Parkin et al. 2005; Udall et al. 2005), allowed us to establish relationships between the LGs of these diploid species. Different levels of marker conservation were visually examined between the A and C genomes (Figs. 1, 2). Segments detecting more than five homeologous markers between the BraIRRi and BolTBDH populations resided in A1/C1, A2/C2, A3/C3, A4/C4, A6/C6, A7/C7 and A10/C9. There were also instances when homeologous segments (e.g. top of C6/A6) were interrupted with segments from other LG (e.g. middle of C6/A7). The most heterogeneous LGs, in terms of containing different homeologous segments, were A6, A7, A9, C5, C8 and C9.

Map alignment of the two mapping populations to *A. thaliana*

The alignment of the two genetic maps with the *Arabidopsis* genome was conducted using a total of 109 sequenced RFLP probes and 119 sequenced regions flanking SSR motifs. Of the 540 (including the 36 unlinked loci, two arbitrary PCR-markers and one phenotypic marker) loci mapped on both genetic maps, 497 had putative *A. thaliana* homologs. Ninety-three percent (122/131) and 90% (140/155) of the RFLP loci detected in the BraIRRi and BolTBDH populations, respectively, had putative *Arabidopsis* homologs. The percentage of loci with homology to the *Arabidopsis* genome was lower for SSR loci, with 78% (73/93) and 63% (77/122) for the BraIRRi and BolTBDH populations, respectively. About 30% of the RFLP loci were homologous to a single *Arabidopsis* chromosomal region and 60% were homologous to more than one of the five *Arabidopsis* chromosomes (the remaining 10% had no homology to *Arabidopsis*). For SSR loci, a higher percentage were homologous to one *Arabidopsis* chromosomal region (43 and 46% of all loci for the BraIRRi and BolTBDH populations, respectively) than were homologous to multiple regions (35 and 20% for the BraIRRi and BolTBDH populations, respectively). The average number of *Arabidopsis* chromosomal hits for RFLP and SSR loci

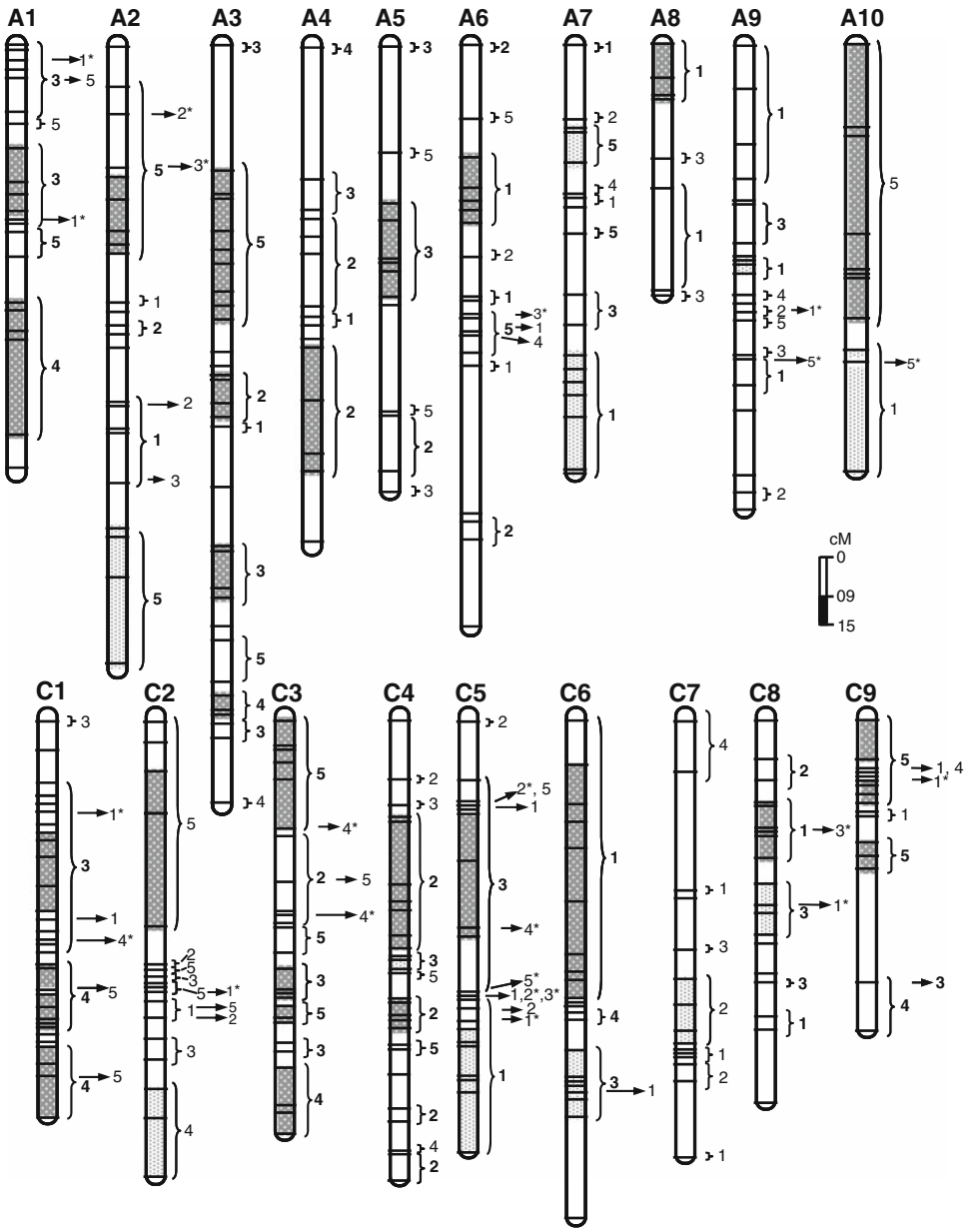

Fig. 3 Alignment of conserved areas between the BraIRRi (A1–A10) and BolTBDH (C1–C9) maps with the *Arabidopsis* genome. Putative collinear runs that were detected in previous studies are denoted within a bracket. *Numbers* to the *right* of each LG indicate the *Arabidopsis* chromosome to which the segment is collinear. *Arrows* indicate the presence of a marker, at the same position or within a putative collinear segment, sharing homology to a different *Arabidopsis* chromosome. *Asterisks* next to a *number* highlight the presence of an SSR locus interrupting a collinear region. *Shaded areas* within LGs represent the most conserved runs based on marker position and physical distance estimated from the *Arabidopsis* genome. *Dark shades* illustrate putative areas of similarity between the A (BraIRRi) and C (BolTBDH) genome. *Light shades* illustrate collinear segments that appear to be A and/or C genome specific

combined, was 1.9 and 1.8 for the BraIRRi and BolTBDH populations, respectively.

The alignment of our two maps with the *Arabidopsis* genome identified several putative collinear regions among the three genomes examined (Fig. 3; Supplementary Material 3). The five *Arabidopsis* chromosomes were scattered into short segments throughout the A (BraIRRi) and C (BolTBDH) genomes. However, there were regions where collinearity was interrupted by the presence of markers showing hits to multiples regions of the *Arabidopsis* genome. For instance, the middle of A6 had markers with sequence similarity to *Arabidopsis* chromosomes 1, 2 3, 4 and 5; and the lower half of C2 had markers showing homology with *Arabidopsis* chromosomes 1, 2, 3 and 5 (Fig. 3). Using a criterium of three or more uninterrupted collinear markers, we identified 17 and 22 putative regions in the BraIRRi and BolTBDH maps, respectively, with significant homology to the *Arabidopsis* genome (Fig. 3). The average number of loci per collinear segment was 4.6 for the BraIRRi and 4.9 for the BolTBDH. The total length of collinear segments covering each of the two maps was 367 cM (33%) for BraIRRi and 407 cM (46%) for the BolTBDH. This coverage reflected an average length of 20 cM per collinear region for the A and C genomes. The longest putative collinear regions were located in LGs A10 (89 cM) and C5 (71 cM) and were composed of eight loci each (Fig. 3). The smallest putative collinear region (4 cM in length and spanning four loci) was identified in LG R9 (Fig. 3). Approximately 56% of each of the A and C genome segments identified as syntenic with *A. thaliana*

Table 3 Number of SSR loci mapped in both *Brassica rapa* and *B. oleracea* mapping populations according to the type of sequence flanking the SSR motifs

SSR loci mapped per primer pair	Flanking sequence type						Sub-total	No. info. available	Total
	Coding region[a]	Non-coding region	Unknown	TE	Pseudo-gene	Plastid genome			
1	50	8	4	–	1	1	64	39	105
2	4	8	–	4	–	–	16	10	24
3	5	1	–	–	–	–	6	3	9
4	–	–	–	–	–	–		–	–
5	–	1	–	–	–	–	1	–	1

TE transposable elements

[a] Coding region included genes and hypothetical proteins/express sequences

were also identified as putative common regions between our *B. rapa* and *B. oleracea* maps (Figs. 1, 2, 3).

There were differences in the degree of conservation between SSR and RFLP loci. For example, more than half (151/286) of the total mapped RFLP loci (in both populations) were detected as collinear throughout the genome, while only 38% (85/221) of the total mapped SSR loci were detected in collinear regions across the genome. In addition, non homologous SSR loci were found to interrupt collinear regions for about 55% of the collinear segments in both populations (e.g. A1, A2, A6, A9, C1, C2, C3, C5, C8 and C9; Fig. 3). Moreover, only 19 of a possible 153 SSR loci were mapped in both A and C genomes; and of these, 5 (26%) mapped to similar conserved regions of both genomes. For RFLP markers, 68% (43/63) of the loci that mapped to both populations landed in conserved genomic regions. SSR markers also differed between the type of sequence flanking SSR motifs and the number of SSR loci mapped in both populations (Table 3). Most SSR markers that mapped as single loci showed homology to a single *Arabidopsis* chromosome and were associated (76% of the time) with coding regions of the genome (genes and hypothetical proteins/express sequences). SSRs that mapped to more than one locus were associated with coding as well as non-coding regions and transposable elements.

Discussion

Genetic maps characteristics

The linkage maps presented in this study are in general agreement (cM coverage, similar probe/marker positions, similar number of LGs, etc.) with the 18 previously reported maps for both species (see "Introduction"). As in other *Brassica* mapping studies, we found a large number of loci with deviations from the expected Mendelian ratios in the BraIRRi (25%) and BolTBDH (49%) populations. Intra- and inter-specific crosses have been shown to be associated with segregation distortion in *Brassica* (Slocum et al. 1990; Chyi et al. 1992; Teutonico and Osborn 1994) and other plant crops (Havey and Muehlbauer 1989; Paterson et al. 1990), possibly due to gametic selection accompanied with chromosomal rearrangements. The process of generating double haploid lines could also influence segregation ratios (Foisset and Delourme 1996; Parkin and Lydiate 1997). One explanation for the deviation of Mendelian ratios in androgenic populations such as BolTBDH could be selection pressure during in vitro androgenesis. Another cause for the observed skewed ratios could be an unintentional selection for seed from lines that flower earlier and/or showed better fertility than others. The distribution and load of the distorted ratios observed are comparable to other studies using the same parental lines (R500 and TO1000DH3). In the case of the R500, Chyi et al. (1992) reported a bias against loci from this line which clustered in six regions of the genome. Teutonico and Osborn (1994) found 24% skewed loci when using R500 as one of the parental lines in an F2 mapping population. A recombinant inbred mapping population derived from Teutonico and Osborn (1994) F2 mapping population (Kole et al. 1997) also found similar levels of segregation distortion. The genomic distributions and frequencies of the skewed loci in the BolTBDH population are in agreement with those of Udall et al. (2005) who used an inbred line of TO1000 to generate a synthetic *B. napus* line.

In the present linkage maps (BraIRRi and BolTBDH) there were elevated levels (>50%) of replicated marker loci. Past and present mapping studies in *B. rapa* and *B. oleracea* (see "Introduction" for references), have highlighted the presence of extensive replicated marker loci in these genome. The maintenance of duplicated loci at high frequencies appears to be a prevalent feature of plant genomes (Moore and Purugganan 2005). In the *Brassica* genome, the preservation of duplicated loci may provide novel genetic variation upon which selection can act.

This may help to explain the large morphological variation exhibited by the *Brassica* crop species (Lukens et al. 2004).

Marker conservation between the A and C genome was observed (Figs. 1, 2). However, there were few shared marker loci detected between the BraIRRi and BolTBDH genetic maps. This could be due to a lack of shared polymorphism between both populations at homeologous regions, sequence divergence (since these species split from a common ancestor about four MYA; Inaba and Nishio 2002), or by conservative scoring of segregating loci that may have omitted possible markers in common between BraIRRi and BolTBDH. Nevertheless, we could identify additional markers in conserved homeologous regions by following segregation patterns of a common parental line (TO1000DH3) from a mapping study conducted by Udall et al. (2005) and by assuming putative locus positions, for the same RFLP probe, with other studies (Lagercrantz and Lydiate 1996; Parkin et al. 2005). This allowed us to accurately assign identical loci between all genetic maps (bolded and underlined marker loci in Figs. 1, 2). Implications of genome conservation during the evolution of diploid *Brassica* crops (e.g. duplications and chromosomal rearrangements) and possible breeding effects (e.g. hybrid vigor) have been discussed by other authors (Lagercrantz and Lydiate 1996; Lagercrantz et al. 1996; Parkin et al. 2005; Udall et al. 2005).

Alignment of the BraIRRi and BolTBDH maps with *A. thaliana*

The *Brassica* and *Arabidopsis* genomes share about 87% sequence identity in coding regions (Cavell et al. 1998). This feature has been extensively exploited and has resulted in a prolific number of comparative mapping studies between *Brassica* crops and *Arabidopsis* (for examples refer to Lukens et al. 2004; Parkin et al. 2005). These efforts not only led to the identification of genomic regions that explained some of the observed morphological variation (flowering time, leaf morphology, etc.) but also yielded valuable insights on the genome structure and evolution of *Brassica* crops. Such results have highlighted the complexity of the *Brassica* genome with its extensive replicated nature, as well as frequent appearance of chromosomal rearrangements (Cheung et al. 1997; Lagercrantz 1998; Osborn and Lukens 2003; Ryder et al. 2001; Parkin et al. 2005; Udall et al. 2005).

The collinear segments (Fig. 3) obtained from our comparative analysis are consistent with those reported by Parkin et al. (2005) for the A and C genomes of *B. napus* and with Lukens et al. (2003) for the C genome of *B. oleracea*. This reinforces the idea that conserved regions, at the macro-collinear level, have been maintained during the evolution of *Brassica* crops. The maintenance of these conserved segments appears to persist across *Brassica* species and may be dependent on their origin (whole genome replication and/or segmental or local duplication) and fate (rearrangements within the *Brassica* genome).

There were regions for which we could not detect previously reported conserved chromosomal segments due to the lack of molecular markers. In addition, there was a different degree of conservation between the types of molecular markers used: RFLP loci were more conserved than SSR loci (Table 3). This may be due to hypervariability at SSR motifs (Li et al. 2002; Li et al. 2004). We envision that further addition of markers to create denser maps will improve our insights on the genetics and evolution of *Brassica* genomes through comparisons with the *Arabidopsis* genome.

Public distribution of reference mapping populations, molecular markers and segregation data

Here we present two immortal public mapping populations for the base diploid *B. rapa* and *B. oleracea* species, sets of molecular markers and their corresponding linkage maps. Our intention is to offer these populations and genetic maps to the *Brassica* research community as genetic reference tools. *Brassica* researchers are in need of reference linkage maps in order to relate the findings from different studies. An improvement towards relating studies on *Brassica* crops has been achieved by the agreement of several laboratories on the usage of a common international nomenclature to name the LGs from different *Brassica* mapping studies (Parkin et al. 1995; Lowe et al. 2004; Suwabe et al. 2002; Udall et al. 2005). However, unifying results within the same genetic background could expedite the knowledge gain from different aspects of *Brassica* genetics. We anticipate that by releasing the information provided by this publication, we will be contributing to the unification of the *Brassica* research community. These two populations have already been distributed to several *Brassica* researchers in the public and private sectors who are working on an array of different aspects of *Brassica* breeding, genetics, ecology and evolution, and they are available to other on request to the corresponding author or through the AdVaB UK project.

To our knowledge, these populations will not segregate for traits of commercial interest; however, the lines do segregate for a number of morphological traits (e.g. flower color, height, leaf shape and silique length, among others), which are of biological interest. All lines are self-compatible and annual, flowering relatively early. Moreover, all the parents used to develop these two mapping populations have been either studied previously or are the subject of current studies. Briefly, a sister line of IMB211 (IMB218) was and is being used to study genome changes upon

polyploid formation (Lukens et al. 2005; Gaeta et al. 2007). R500 was used as a mapping parent in our laboratory and others (Chyi et al. 1992; Teutonico and Osborn 1994; Kole et al. 1997). The broccoli line "Early Big" was used to generate segregating populations to study the inheritance of genes involved in the synthesis of aliphatic glucosinolates (Li et al. 2003). TO1000DH3 was and is being used in several projects including the development of linkage maps in *B. napus* (Udall et al. 2005), the development of the set of mutants (EMS) for research and education (Himelblau, personal communication) and the formation of synthetic polyploidy lines (Lukens et al. 2005; Gaeta et al. 2007). This line was also used in a sequencing effort undertaken by the Cold Spring Harbor Laboratory (CSHL) and The Institute for Genome Research (TIGR) to aid in *Arabidopsis* gene annotations (Ayele et al. 2005; Katari et al. 2005).

Therefore, the fact that the parental lines have been used in previous studies make these populations valuable as reference tools for relating the findings of current and past *Brassica* research efforts.

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