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ORIGINAL PAPER

Structural and functional comparative mapping between the *Brassica* A genomes in allotetraploid *Brassica napus* and diploid *Brassica rapa*

Congcong Jiang · Nirala Ramchiary · Yongbiao Ma · Mina Jin · Ji Feng · Ruiyuan Li · Hao Wang · Yan Long · Su Ryun Choi · Chunyu Zhang · Wallace A. Cowling · Beom Seok Park · Yong Pyo Lim · Jinling Meng

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Abstract *Brassica napus* (AACC genome) is an important oilseed crop that was formed by the fusion of the diploids *B. rapa* (AA) and *B. oleracea* (CC). The complete genomic sequence of the *Brassica* A genome will be available soon from the *B. rapa* genome sequencing project, but it is not clear how informative the A genome sequence in *B. rapa* (A^r) will be for predicting the structure and function of the A subgenome in the allotetraploid *Brassica* species *B. napus* (A^n). In this paper, we report the results of structural and functional comparative mapping between the A subgenomes of *B. napus* and *B. rapa* based on genetic maps that were anchored with bacterial artificial chromosomes (BACs)-sequence of *B. rapa*. We identified segmental conservation that represented by syntenic blocks in over one third of the A genome; meanwhile, comparative mapping of quantitative trait loci for seed quality traits identified a dozen homologous regions with conserved function in the A genome of the two species. However, several genomic rearrangement events, such as inversions, intra- and inter-chromosomal translocations, were also observed, covering totally at least 5% of the A genome, between allotetraploid *B. napus* and diploid *B. rapa*. Based on these results, the A genomes of *B. rapa* and *B. napus* are mostly functionally conserved, but caution will be necessary in applying the full sequence data from *B. rapa* to the *B. napus* as a result of genomic rearrangements in the A genome between the two species.

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C. Jiang and N. Ramchiary contributed equally to this work.

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C. Jiang · Y. Ma · J. Feng · R. Li · Y. Long · C. Zhang · J. Meng (✉)
National Key Laboratory of Crop Genetic Improvement, Huazhong Agricultural University, Wuhan 430070, China
e-mail: jmeng@mail.hzau.edu.cn

N. Ramchiary · S. R. Choi · Y. P. Lim (✉)
Molecular Genetics and Genomics Lab, Department of Horticulture, Chungnam National University, Daejeon 305-764, Republic of Korea
e-mail: yplim@cnu.ac.kr

M. Jin · B. S. Park
Genomics Division, National Academy of Agricultural Science, Rural Development Administration, Suwon 441-707, Republic of Korea

H. Wang
Hybrid Rapeseed Research Center of Shaanxi, Dali 715105, China

W. A. Cowling
The UWA Institute of Agriculture, c/o School of Plant Biology M084, The University of Western Australia, Crawley, WA 6009, Australia

Introduction

The genus *Brassica* is the closest crop-plant relative of the model plant *Arabidopsis thaliana* and some similarities at the sequence level are expected since their progenitors diverged approximately 20 million years ago (Koch et al. 2000; Yang et al. 1999). In addition, *Brassica* is an ideal genus to study genome evolution and diversification because it contains diploid (*B. rapa*, genome AA, $n = 10$; *B. nigra*, BB, $n = 8$; *B. oleracea*, CC, $n = 9$) and allotetraploid (*B. juncea*, AABB, $n = 18$; *B. napus*, AACC, $n = 19$; *B. carinata* BBCC, $n = 17$) species that are related in an interesting manner represented by "U's triangle" (U 1935).

Polyploidization is a common feature of plant evolution, and involves extensive genomic disturbing events, such as the "polyploid ratchet" described by Gaeta and Pires (2010), or the transposon activation caused by genome shock (Parisod et al. 2010). Owing to the fact that the allotetraploid *B. napus* was formed via a polyploidization process, and its two ancestral diploid genomes, A and C, are homoeologous and diverged only several million years before their fusion into the tetraploid form (Inaba and Nishio 2002), large-scale homoeologous recombination between the A and C genomes accompanied by genomic rearrangements may have occurred in the genome of early *B. napus* (Song et al. 1995; Gaeta and Pires 2010). The tetraploid genome might have become stable as a result of the evolution and selection of a locus that could control the pairing of the homoeologous chromosomes (Jenczewski et al. 2003). Furthermore, various exotic resources from *B. rapa* and other species were introgressed into the *B. napus* during modern breeding practices in order to improve desirable traits (Li et al. 2004; Qian et al. 2005, 2006; Zou et al. 2010). Therefore, it is possible that the A genomes of modern tetraploid *B. napus* and diploid *B. rapa*, defined as subgenomes A^n and A^r, respectively (Li et al. 2004), have significantly diverged from each other at the structural or functional level.

Many comparative mapping studies between Arabidopsis and Brassicas have revealed the complex organization of the *Brassica* genomes, which has arisen due to extensive segmental duplication and rearrangements (Kowalski et al. 1994; Lagercrantz 1998; Lan et al. 2000; Lukens et al. 2003; Panjabi et al. 2008; Parkin et al. 2005; Sadowski et al. 1996; Teutonico and Osborn 1994). Some recent studies have urged caution in the transfer of candidate genes and their flanking sequence information from Arabidopsis to *Brassica* species because of divergence of gene content and relative positions as well as discontinuous collinearity (Park et al. 2005; Town et al. 2006; Trick et al. 2009). Therefore, a model species within the *Brassica* genus, rather than Arabidopsis, is required in order to study the genomic complexity and facilitate the genetic improvement of *Brassica* crops, especially the tetraploid *B. napus* and *B. juncea*, which are important resources for both edible oil and renewable energy.

Thus, among the three *Brassica* genomes, namely, A, B, and C, the A genome from *B. rapa* ssp. *pekinensis* ($n = 10$) was selected as the representative model for genome sequencing by the Multinational *B. rapa* Genome Sequencing Project (BrGSP, http://www.brassica.info/) in 2003 based on the Chinese cabbage inbred line Chiifu-401. At present, hundreds of sequence-informative markers have been developed on the basis of *B. rapa* bacterial artificial chromosomes (BACs) sequencing, and the reference genetic map of *B. rapa* has been improved from the simple genetic linkage map (Choi et al. 2007; Kim et al. 2006) to the BAC anchored map (Kim et al. 2009; Mun et al. 2008).

Sequence alignments revealed that the A genomes of the diploid *B. rapa* and tetraploid *B. napus* have diversified at the microstructural level (Cheung et al. 2009; Cho et al. 2009; Rana et al. 2004). The first chromosome in *B. rapa* to be sequenced (A3) (Mun et al. 2010) will be followed soon by the entire genome sequence of *B. rapa*. The more complex genome of *B. napus* is being shotgun-sequenced with various strategies applying next-generation sequencers (http://www.brassica.info/). In order to make better use of the A-genome sequence of *B. rapa* for assembly of the A genome sequence in *B. napus*, it will be necessary to determine the genomic segmental divergence at the macrostructural level between the A genomes in each species. Previous studies revealed general conservation between *B. rapa* and *B. napus* A genomes at the chromosomal level, but also a few apparent inversions (Suwabe et al. 2008; Xu et al. 2010). In this study, we evaluated the degree of structural divergence or conservation between the A^r and A^n subgenomes of representative *B. rapa* and *B. napus* lines, respectively, via comparative mapping based on BAC anchored genetic maps of high density; and we also examined the effect of genomic structural changes on their functional expression by comparing QTL underlying economically important traits.

Materials and methods

Genetic linkage maps

The TN genetic linkage map of *B. napus*, which was introduced by Long et al. (2007), was updated in the present study using 188 doubled-haploid (DH) lines derived from a cross between the divergent lines Tapidor and Ningyou7. A total of 291 new primer pairs, including simple sequence repeat (SSR) and intron-based polymorphism (IBP), which were developed from *B. rapa* BAC ("KBr") sequences (National Institute of Agricultural Biotechnology, Korea) (Mun et al. 2009), were used for mapping and to improve the TN genetic map. JoinMap4.0 software (Van Ooijen 2006) was used to construct the linkage map. LOD values that ranged from 8 to 19 and Kosambi mapping function were used to assign markers into linkage groups and to construct the map.

The new version of the CK genetic map of *B. rapa*, which was developed using BAC anchored markers (Kim et al. 2009), was used in the present study for comparative mapping with *B. napus*. The CK DH population consists of 78 DH lines and is derived from a cross between the divergent Chinese cabbage inbred lines Chiifu-401 and Kenshin.

Dissection of Brassicaceae building blocks on the CK and TN genetic maps

The building blocks of Brassicaceae genomes that were proposed by Schranz et al. (2006) were identified on both the CK and TN genetic maps. These blocks are represented by 24 distinct segments of the Arabidopsis genome (separated by their physical positions) and are considered the conserved units for the organization of Brassicaceae genomes. All available sequence-informative markers (such as the public SSRs, RFLPs/STS, and SNPs), including the *B. rapa* BAC-sequence-derived markers on both *B. napus* and *B. rapa* genetic maps (Table 1), were subjected to BLASTN analysis in order to identify their physical positions on Arabidopsis chromosomes. Subsequently, the Brassicaceae building blocks that corresponded to each informative marker were identified. For each linkage group of these two genetic maps, the length of a particular block was demarcated from the first to the last marker in the same block that was undisrupted by sequences from other blocks. Each isolated informative marker was denoted as a single label for a particular block (Online Resource 1). Blocks with genetic length over 5 cM were defined as major blocks; blocks characterized by single informative marker were defined as isolated blocks.

Comparative mapping and definition of probable genome rearrangement events

Owing to the fact that the informative markers on the two genetic maps were derived from two different sequence resources (*B. rapa* and *A. thaliana* BACs; Table 1), the names of all the informative markers on the maps were substituted by the names of *B. rapa* BACs (prefix "KBr") or *A. thaliana* BACs (prefix "At") to facilitate comparison (Online Resource 1). Markers that were derived from the same BAC were defined as "common markers" between the two maps, whereas those that were derived from different BACs but whose physical positions were quite close to each other and within the same Brassicaceae building block, were taken as "secondary common markers" (Online Resource 2).

The homologous linkage groups from the *B. napus* TN and *B. rapa* CK genetic maps were aligned using the common and secondary common markers, and then the possible genome conservation or rearrangement events were defined as follows:

1. Syntenic conservation—consistent relative positions of several sequential markers between the homologous linkage groups of *B. napus* and *B. rapa* indicated a syntenically conserved fragment;
2. Inversion—two adjacent markers, or more consecutive markers, whose relative position was inverted between the homologous linkage groups of *B. napus* and *B. rapa* indicated a putative inversion;
3. Intra-chromosome translocation—within a region of syntenic conservation, a marker whose position on the linkage group of one species did not agree with the syntenic conservation but shifted with two or more adjacent markers relative to its position on the homologous linkage group of the other species indicated a putative intra-chromosomal translocation;
4. Inter-chromosomal translocation—common markers that were located on non-homologous linkage groups

Table 1 Resources of the sequence-informative markers used for comparative mapping between the A subgenomes of *B. rapa* and *B. napus*

Sequence resources	Marker resources	*B. rapa* CK map		*B. napus* TN map (A subgenome)	
		Mapped	Informative[a]	Mapped	Informative
"KBr" BAC	SSR from CNU	133	133	53	53
	SSR/IBP from NIAB	66	66	101	100
	Others	1	1	56	51
	Sub-total	200	200	210	204
"At" BAC	Public RFLP probe	5	5	34	32
	SNP from BBSRC	–	–	56	54
	SSR from BBSRC	36	28	63	47
	SSR from AAFC	145	96	23	5
	Others	42	35	9	9
	Sub-total	228	164	185	147
Total		428	364 (2.9 cM)[b]	395	351 (3.2 cM)

CNU Chungnam National University, Korea, *NIAB* National Institute of Agricultural Biotechnology, Korea, *BBSRC* Biotechnology and Biological Sciences Research Council, UK, *AAFC* Agriculture and Agri-Food Canada

[a] Some markers were not assigned definitively to a specific BAC

[b] The values in the brackets indicate the average intervals of the informative markers distributed over the A subgenomes

between *B. napus* and *B. rapa* and were in regions that were not duplicated within the A genome as proposed by Schranz et al. (2006), indicated a putative inter-chromosomal translocation in one of the species.

Field experiments and measurement of seed quality traits

The TN DH population, that had been planted following a randomized complete-block design in three replications in consecutive years at different locations in China as described earlier by Shi et al. (2009), was used for phenotypic measurement of seed quality traits by near-infrared reflectance spectroscopy (NIRS). Total seed glucosinolate content (μmol/g) and oil content (% of seed dry weight) was measured from the seeds harvested in four experiments:

03N, 04N—in 2003 and 2004, respectively, at Weinan, North China; and
03S, 04S—in 2003 and 2004, respectively, at Wuhan, South China;

Whereas the seed protein content (% of seed dry weight) was determined from the seeds harvested in three experiments:

N6—in 2006 at Weinan, North China; and
S5, S6—in 2005 and 2006, respectively, at Wuhan, South China.

The CK DH population, along with the parental lines Chiifu-401 and Kenshin, was grown and harvested in two consecutive years at different locations:

S7, S8—in 2007 and 2008, respectively, at Wuhan, South China;
N8—in 2008 at Weinan, North China; and
K8—in 2008 at Daejoen, Korea.

Open-pollinated seeds were pool-harvested for each line in each replicate, and the total glucosinolate, oil, and protein contents were analyzed by NIRS.

QTL mapping

WinQTL Cartographer 2.5 software (Wang et al. 2001–2005) was used to map QTL that controlled seed quality traits in both the *B. napus* and *B. rapa* populations. QTL were identified with composite interval mapping (CIM) method performed at 2 cM intervals and 10 cM window. For declaring the presence of a QTL, genome wide threshold values ($p \leq 0.5$) were estimated from 1,000 permutations of each trait data across all genetic intervals (Churchill and Doerge 1994) and 2 LOD support limit was taken to demarcate the QTL region.

QTL that were identified in both *B. napus* and *B. rapa* in the corresponding regions of homologous linkage groups and flanked by same markers or found in the same blocks controlling the same traits in the two species, were defined as conserved QTL.

Results

Improving genetic maps of *Brassica* with *B. rapa* BAC-sequence-derived markers

After the first effort of mapping *B. rapa* BAC-sequence-derived SSR markers, which were developed by Chungnam National University (CNU) and National Institute of Agricultural Biotechnology (NIAB), onto the *B. napus* TN genetic map (Long et al. 2007), we mapped more available SSR and IBP markers generated by the ongoing *B. rapa* genome sequencing project in the present study. A total of 121 out of the 291 new primer pairs (approximately 40%) showed clear polymorphism between the parental lines, Tapidor and Ningyou7. Of these polymorphic primers, we mapped 108 new markers, which represented 104 distinct BACs of *B. rapa*, onto the *B. napus* TN genetic map.

With the previously mapped "cnu-", "niab-", and other independently developed markers, the TN genetic map was updated with a total of 265 markers representing 208 distinct BACs of *B. rapa*. A total of 179 distinct BACs have been mapped on the A subgenome chromosomes of TN map (A^n), most of which were represented by single locus. However, 16 of these BACs were each mapped to 2–3 loci within same linkage group, and another 5 BACs were each mapped to 2–3 loci on different linkage groups.

The additional sequence-informative markers besides *B. rapa* BACs were public markers of *Brassica* that had been developed previously (Table 1). The updated TN genetic map of *B. napus* contained 351 sequence-informative markers that were distributed among the 10 linkage groups of the A^n subgenome, and covered a total length of 1117 cM with an average interval of 3.2 cM.

The second generation *B. rapa* CK genetic map that was reported previously by Kim et al. (2009) was used in this comparative study. The map contains a total of 719 markers, of which 364 are sequence-informative and derived from the same marker resources as the *B. napus* TN genetic map shown in Table 1.

Structural conservation and differentiation between the two A subgenomes of *B. napus* and *B. rapa* as represented by general organization of the Brassicaceae genomes building blocks

The sequence-informative markers on both the *B. rapa* CK and *B. napus* TN maps were used to align the Brassicaceae genomes building blocks, which are represented by 24

distinct segments of the Arabidopsis genome (separated by their physical positions), with the genetic maps. For the entire A subgenomes, 133 and 138 separate blocks were identified on the CK and TN genetic maps, respectively (Online Resource 1), among which, 31 blocks on the CK map and 27 blocks on the TN map had genetic lengths of over 5 cM and were considered to be major blocks. On the other hand, approximately 40% (47/133, 52/138) of the blocks in both species were isolated blocks that were identified from a single informative marker and were dispersed separately or internally within other blocks (Fig. 1).

Fifty-four pairs of common blocks were identified in corresponding regions between the homologous linkage groups of *B. rapa* and *B. napus*. Seventeen of these pairs were major blocks and represented the conservation of one-third of the A genome (Table 2). For nearly one-half of the major blocks in either species, their counterparts in the other species were disrupted by smaller or isolated blocks (Fig. 1). For example, the major block U at the bottom of linkage group A^r3 in *B. rapa* was intact but isolated block G was inserted into the corresponding region on A^n3 in *B. napus*. Furthermore, a significant proportion of blocks in both species, usually isolated ones, had no apparent counterpart within the homologous linkage group of the other species. For example, the isolated blocks S and J on linkage group A^n6 of *B. napus* were not identified in *B. rapa* within the corresponding regions of A^r6, whereas the blocks R, X, and F on A^r6 were not identified on A^n6.

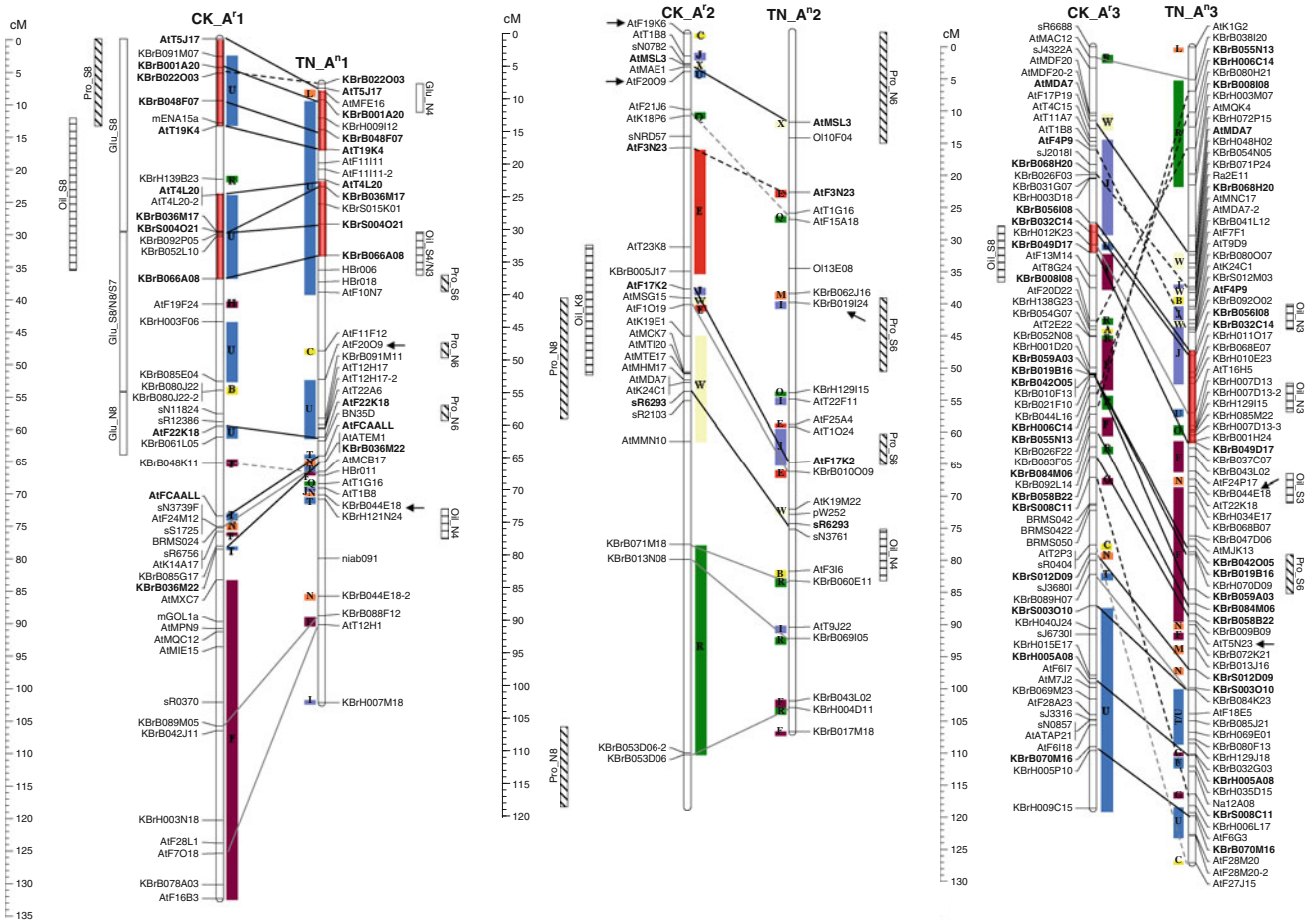

Fig. 1 Comparative mapping of homologous linkage groups between the A subgenomes of *B. rapa* (A^r) and *B. napus* (A^n). For each linkage group of the two species, the Brassicaceae building blocks (shown by attached *grey bars* with different darkness) were identified. The markers that were common between the homologous linkage groups are indicated by *bold letters* and connected with *black lines*; the secondary common markers, which were derived from different BACs but had physical positions that were located quite close together and assigned to the same block, are connected with *grey lines*. The syntenic conservation between A^r and A^n subgenomes were indicated by *solid line* connections between common markers, while the inconsistent locations of common markers between the homologous linkage groups of the two subgenomes were indicated by *dashed line* connections, which includes rearrangement events such as inversions and intra-chromosomal translocations. Inter-chromosomal translocations are indicated by *arrows* that point to particular markers. QTL that were found to control the three seed quality traits in multiple experiments are indicated by *rectangular bars* with various patterns; they were identified on each linkage group of the two species. QTL regions that were conserved between homologous linkage groups are indicated by *rectangular shadow* on the linkage groups

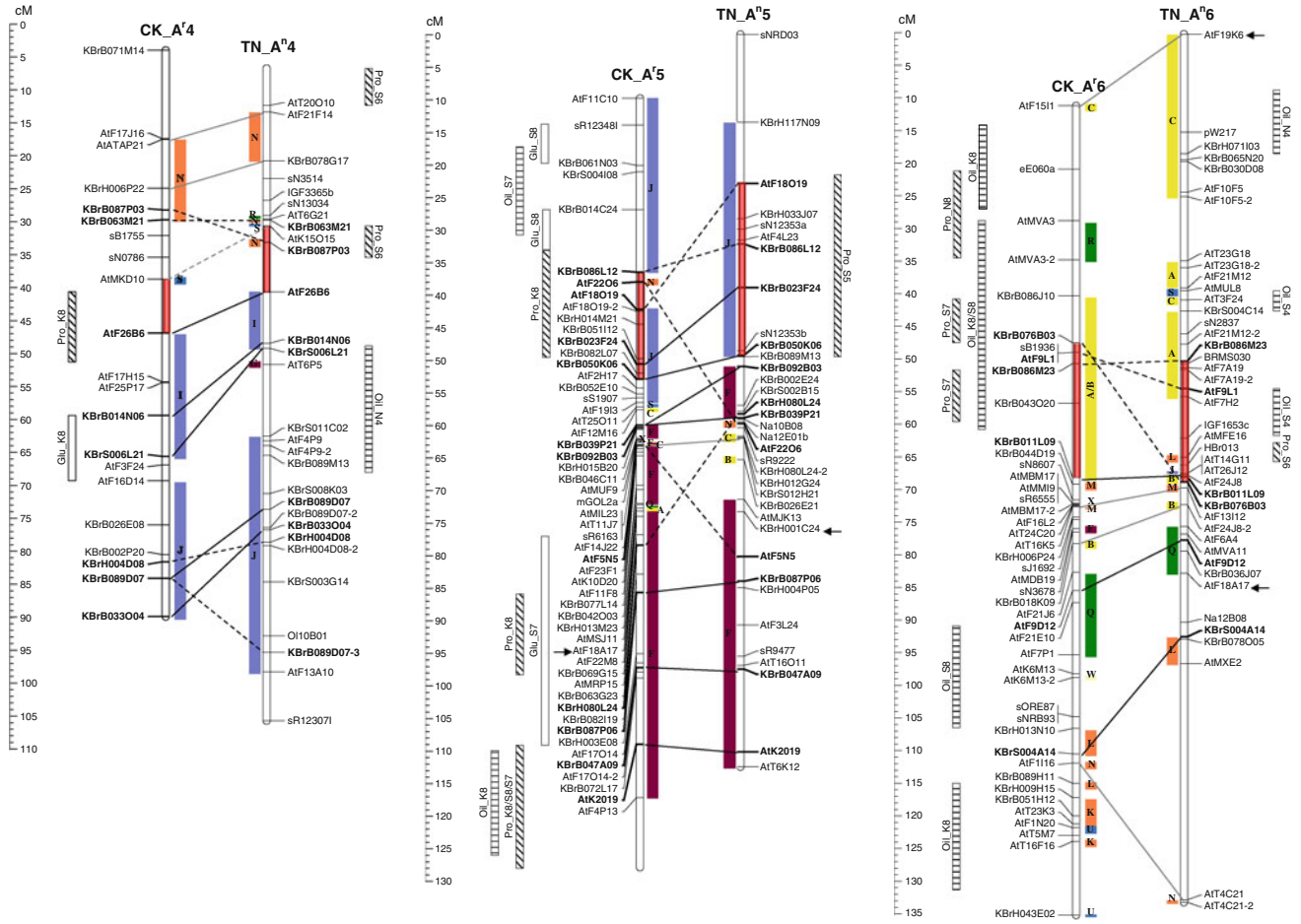

Fig. 1 continued

Comparative mapping based on sequence-informative markers reveals genomic rearrangements in addition to conservation

Primers giving more than one markers were correctly identified for their size and their mapping locations along with other markers that belong to the homologous linkage groups in the other species before designating the common marker. Through the comparison of homologous linkage groups between the A^r and A^n subgenomes, a total of 98 pairs of common markers (derived from the same BACs, of which 72 were from *B. rapa* BACs, 24 were from *A. thaliana* BACs, and two were public SSR markers) and 31 pairs of secondary common markers (derived from different BACs but with physical positions located quite close together and assigned to the same Brassicaceae building blocks; Online Resource 2) were identified and found to be distributed on all the 10 linkage groups of A subgenome.

Among the total 129 pairs of common and secondary common markers, the relative positions of 70% (93/129) pairs within particular linkage groups were consistent between A^r and A^n subgenomes, which reflected extensive conservation (Fig. 1). However, the relative positions of the other common and secondary common markers were inconsistent between the two A subgenomes, which might have resulted from either the presence of genomic rearrangement events such as inversions and intra-chromosomal translocations or the discrepancies related to the different population size used for mapping in the two species. To avoid the later problem, we have taken two sequential markers showing rearrangement to designate intra-chromosomal translocations, namely on linkage group A3 and A4, while three sequential markers showing rearrangement to designate inversions, namely on linkage group A3 and A6 (Table 3). The inversions covered 6.9 and 13.8% of the length of their referred A^n linkage group, respectively; the intra-chromosomal tranlocations covered 8.3% and 15.4% of the length of their referred A^n linkage group, respectively. In total, these two types of rearrangement event, inversion and intra-chromosomal translocation, corresponded to a genetic length of 54.3 cM, which represents at least 5% of the *Brassica* A genome.

It is notable that, in addition to the common markers that mapped onto homologous linkage groups between A^n and

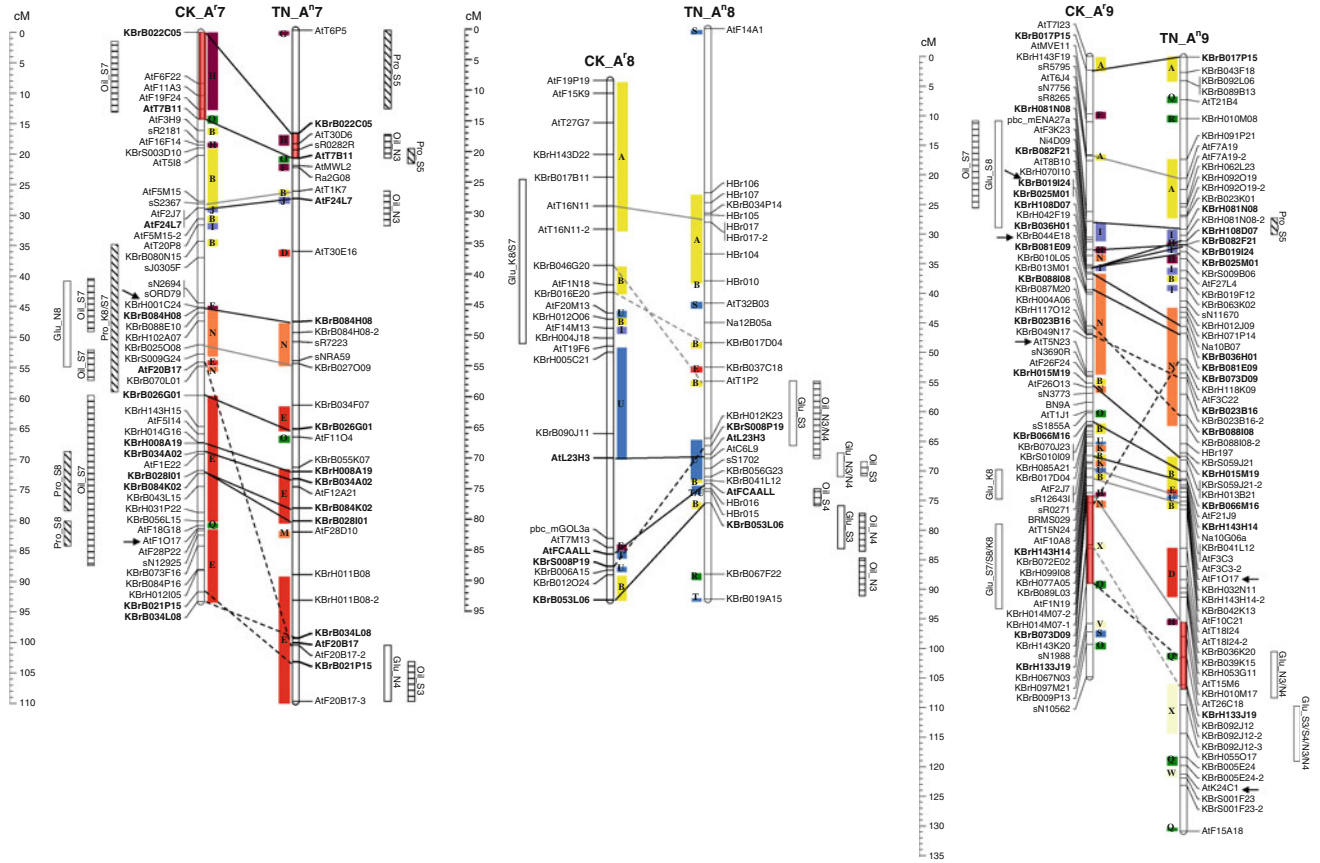

Fig. 1 continued

A^r subgenomes, there were 13 pairs of common markers mapped on linkage groups that were non-homologous between these two subgenomes. According to the reported segmental duplication within *Brassica* A genomes (Schranz et al. 2006), we found four of the 13 pairs of common markers were located within duplicated Brassicaceae building blocks (Fig. 2a) on linkage groups that were non-homologous between the A^n and A^r subgenomes (namely, AtMDA7, block W, A^r2/A^n3; AtF4P9, block J, A^r3/A^n4; KBrH012K23, block U, A^r3/A^n8; KBrB017D04, block B, A^r9/A^n8), whereas the other nine pairs of common markers on non-homologous linkage groups were not located within the duplicated building blocks proposed by Schranz et al. (2006) (Fig. 2b). In other words, it is apparent that these nine pairs of divergent marker locations resulted from inter-chromosomal translocation events rather than from altered polymorphisms between duplicated segments (Table 3).

The A^n3 of TN DH linkage map was further compared with the physical map of A^r3 in *B. rapa* cv. Chiifu (Mun et al. 2010) via 40 common BAC clones of *B. rapa* (Fig. 3). Three quarters of the chromosome constitution represented by these BACs showed synteny between both A subgenomes. Of the rearrangement events identified above, the intra-chromosomal translocation which associated with BAC KBrS008C11 (Fig. 3, solid triangle), was detected again. There were also some other common BACs exhibited inconsistent locations between the A^r and A^n subgenomes during this comparison.

Functional conservation between the A subgenomes of *B. napus* and *B. rapa* as revealed by QTL comparison for seed quality traits

The parental lines of the TN DH population, Tapidor and Ningyou7, differed greatly in seed total glucosinolate content compared with the parental lines of the *B. rapa* CK DH population, Chiifu-401 and Kenshin; while the seed oil and protein content differed moderately between parental lines of both the CK and TN population. The seed oil content in the *B. napus* TN population was a little higher than that in the *B. rapa* CK population (Table 4). Continuous distributions were observed in both populations for all the traits that were analysed. A total of 41 and 42 QTL were detected for the three seed quality traits in the A subgenomes of *B. napus* and *B. rapa*, respectively (overlapping QTL for the same trait detected from different experiments were considered to be a single QTL) (Table 5; Online Resource 3).

Comparison of QTL between homologous linkage groups between the two A subgenomes revealed that all the

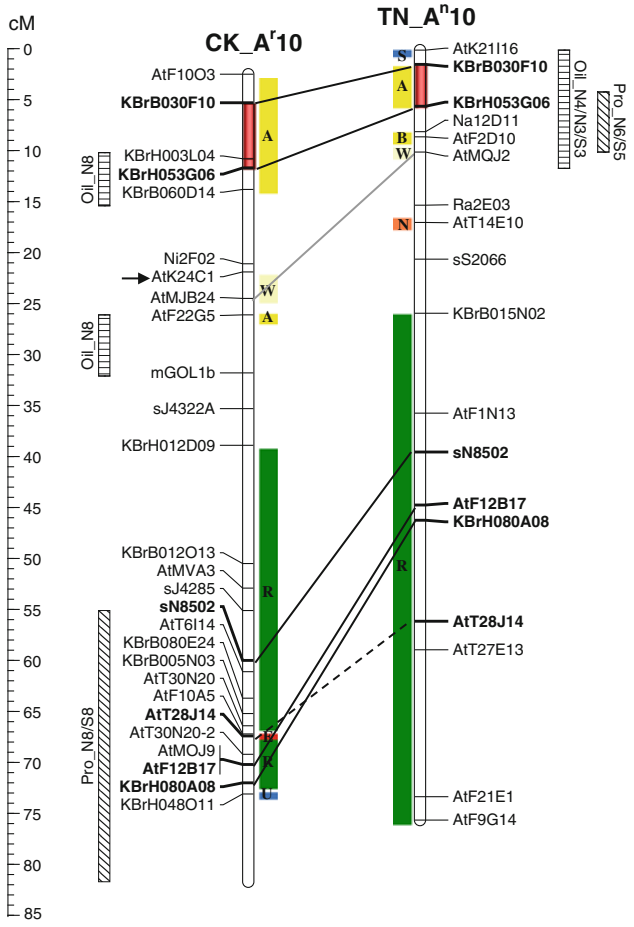

Fig. 1 continued

Table 2 Conserved major blocks identified on both CK and TN genetic maps

Linkage group	Conserved block	Genetic interval of the block (cM)[a]	
		B. rapa CK map	*B. napus* TN map
A1	U	2.7–61.3	9.7–61.6
A3	J	14.1–28.8	36.8–52.3
	F	32–60.1	61.3–89.4
	U	87.1–118.6	99.8–122.8
A4	N	17.4–29.7	13.1–33.4
	I	46.9–65.6	40.4–49
	J	69.3–89.9	62.3–98.1
A5	J	9.7–56.4	13.5–49.5
	F	59.9–117.1	51–112.5
A6	A	40.3–68.2	34.7–55.5
	Q	82.7–95.3	75.4–82.6
A7	N	45.3–55.3	47.8–54.7
	E	59.5–93.3	61.4–110
A8	A/B	8.4–43	26.8–41.2
	U	51.8–70	66.8–73.1
A9	N	36.4–53.3	42.3–62.2
A10	R	38.9–72	25.8–75.5
Sum	17	Total length	
		475.5 cM	454.5 cM

[a] The interval for some blocks include several adjacent ones of the same although they were separated when at least one block was a major block and syntenic on both genetic maps

homologous linkage groups contained QTL that controlled at least one identical trait in both A^n and A^r (Table 5). For example, QTL for all three traits were identified on linkage group A1 and A7 in both species, whereas QTL for two traits (except total glucosinolate content) were identified on linkage group A2, A6 and A10 in both species. Among these many QTL, 9 pairs were detected at corresponding locations on homologous linkage groups that flanked by common markers and controlled the same trait(s) in both *B. napus* and *B. rapa*, which demonstrates the functional conservation of these corresponding regions between the A subgenomes of the two species (Table 5; Fig. 1). These 9 QTL were taken to be conserved QTL, and they were related to all the traits that we examined. However, some of the conserved QTLs located in the regions where the syntenic structure between the two A subgenomes were disturbed. For example, the conserved QTL intervals close to the middle of the linkage group A6 and the bottom of A9, were identified in the regions of putative fragmental inversions between the A^n and A^r subgenomes, respectively.

There was also a considerable number of QTL (other than the 9 conserved QTL) in both species for which no counterparts that controlled the same trait(s) could be detected at their corresponding regions in the other species. It was noted that there were some coincidences between locations of these QTL and the presence of genomic rearrangements we identified in Table 3. For example, the QTL for protein content at the top of TN linkage group A^n2 was not detected at the corresponding region in *B. rapa* (Fig. 1); since the implicated two Arabidopsis BACs at the corresponding region of A^r2 were mapped on conserved blocks (block U on A^n1 and block C on A^n6) in the *B. napus* genome, inter-chromosomal translocations were considered to be occurred and resulted in insertions of blocks C and U on the top of A^r2 where QTL was not correspondingly identified.

Discussion

The *B. rapa* (AA) genome sequencing project, in addition to the model plant Arabidopsis, will shed new light on the genomic dissection of more complex *Brassica* crops, especially the tetraploids *B. napus* (AACC) and *B. juncea* (AABB), which contain the A subgenome. However, it

Table 3 Genome rearrangement events revealed by common and secondary common markers between the A subgenomes of *B. rapa* (A^r) and *B. napus* (A^n)

Linkage group	Inversion		Intra-chromosomal translocation		Inter-chromosomal translocation			
	Markers	Interval (cM)[a]	Markers	Interval (cM)[a]	Markers	Block	*B. rapa*	*B. napus*
A2					AtF19K6	C	A^r2	A^n6
					AtF20O9	U	A^r2	A^n1
A3	KBrB055N13-KBrB008I08	6.8–15.7	KBrS008C11-AtF27J15	116.3–127				
A4			KBrH004D08-KBrB089D07-3	78.9–95.1				
A5					AtF18A17	Q	A^r5	A^n6
A6	KBrB086M23-KBrB076B03	50.1–68.6						
A7					AtF1O17	E	A^r7	A^n9
					KBrH001C24	F	A^r7	A^n5
A9					AtT5N23	N	A^r9	A^n3
					KBrB019I24	I	A^r9	A^n2
					KBrB044E18	N	A^r9	A^n1, A^n3
A10					AtK24C1	W	A^r10	A^n9
	Total length (cM)	27.4		26.9				

[a] The intervals are counted as locations of the inversions or intra-chromosomal translocations spanned markers in the A^n subgenome

remains to be clearly discerned how much structural and functional conservation occurs between the A subgenomes of the simple diploid *B. rapa* and its related polyploid *B. napus*. Through comparative mapping of both genomic structure and function between representative lines of the allotetraploid *B. napus* and diploid *B. rapa*, we have uncovered a certain amount of genomic structural divergence, in addition to general conservation, between the two A subgenomes, and also have characterized the genomic functional conservation on QTL locations.

In terms of the general organization of the Brassicaceae building blocks that we identified in the A subgenomes of *B. rapa* and *B. napus*, structural conservation was evident in more than 400 cM, which represents over one-third of the A genome (Table 2). However, a large number of blocks did not show equivalence between the two A subgenomes in occurrence or size. Some conserved blocks were observed to be disrupted by markers that were assigned to different blocks in other studies of particular *Brassica* genomes (Panjabi et al. 2008; Parkin et al. 2005), and we found that the nature of such disruptions varied between the two A subgenomes. For example, the conserved block U, which occupied almost one-half of linkage group A1, was disrupted by isolated blocks R, H, and B in *B. rapa* (A^r1) and by isolated blocks L and C in *B. napus* (A^n1) (Fig. 1). It was similar for the disruption of block A/B on the upper part of linkage group A6 that we introduced above. This differentiation probably resulted from either particular polymorphism in the two patterns of A genome, or the genomic rearrangements during the polyploidization process that led to the formation of the allotetraploid *B. napus* from two closely related diploids, *B. rapa* and *B. oleracea*. As these disruptions exhibited like insertion of small pieces into major blocks, we presumed the differentiations were resulted from specific insertions rather than diverged polymorphisms in each of the two A genomes. However, mapping of more sequence-informative markers is necessary in *B. napus* to improve the density of the genetic map so that the intactness and the boundaries of the conserved blocks may be confirmed with a higher degree of accuracy.

It is notable that there was about 10% of the mapped BACs of *B. rapa* had 2–3 locations each on the A^n subgenome of *B. napus* TN genetic map, either within a same linkage group or among different ones. In our analysis, we took the contiguous loci that derived from same BAC as the same locus physically, and it was seen with most of the BACs which were mapped at several locations on the same linkage group. However, segmental duplications may also result in mapping a BAC of *B. rapa* on several discontiguous loci of *B. napus* genome, such as those separated by a certain distance on the same linkage group or assigned to different linkage groups. There was quite an example at the bottom of linkage group A4 (Fig. 1); the BAC KBrB089D07 has been mapped to three distinct loci on the A^n4, while two of the loci were contiguous but the third was distant away. Compared to the location of this BAC on A^r4 and its surrounding organization, we identified an intra-chromosomal translocation of the segment that bounded

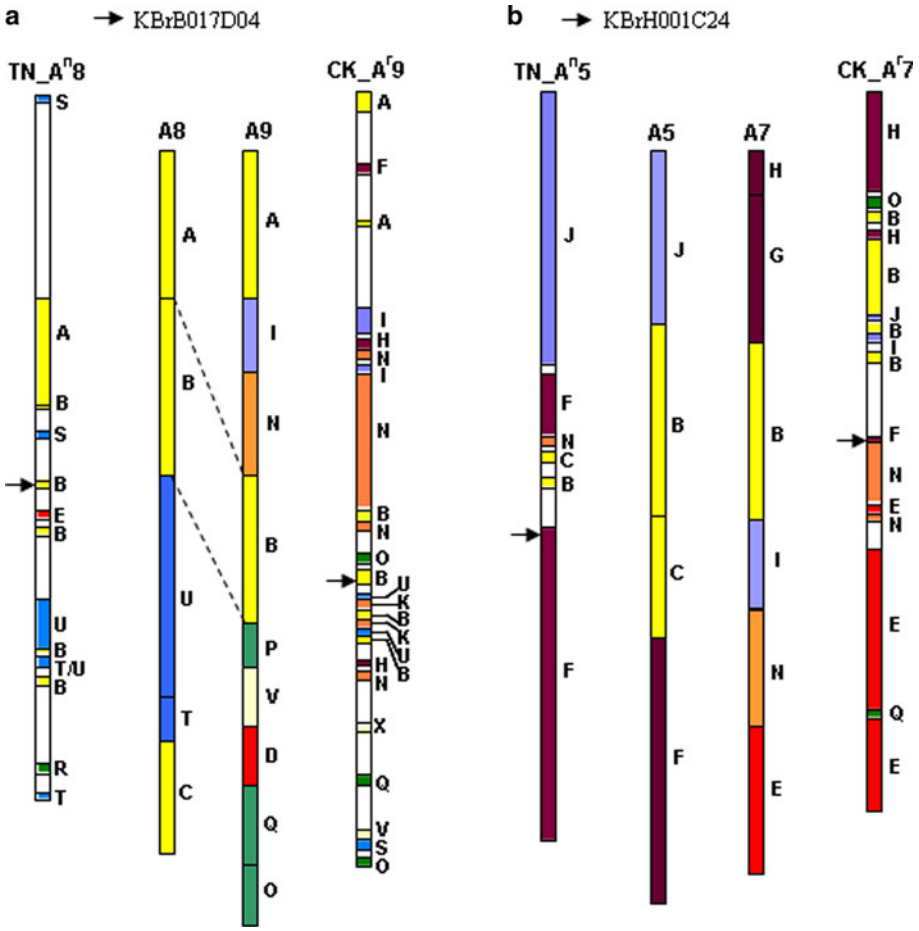

Fig. 2 Divergent chromosomal assigning of common markers between the A subgenomes of *B. napus* (A^n) and *B. rapa* (A^r). Common markers that were derived from the same BAC but located (*black arrows*) on non-homologous linkage groups (A^n8/A^r9 and A^n5/A^r7) of *B. napus* and *B. rapa* arose from two different circumstances. The *grey bars* with different darkness indicate the organization of the Brassicaceae building blocks on particular linkage groups. The two linkage groups in the middle of each panel show the general organization of conserved blocks on the *Brassica* A genome chromosomes that Schranz et al. (2006) reported, and were used to infer whether the different locations of common markers resulted from segmental duplication within the genome (**a**) or inter-chromosomal translocation (**b**)

with the BAC KBrB089D07 and KBrH004D08, and which might probably have resulted from a local duplication.

The inter-chromosomal translocations were reported widely existed in *B. napus* genomes, due to the homeologous recombination between the A and C subgenomes during the tetraploid species formation (Osborn et al. 2003; Udall et al. 2005). In this study, we identified several inter-chromosomal translocations within the A subgenome instead of between the A and C subgenomes. The translocations in A^n subgenome relative to A^r may result from either the genomic fusion of A and C subgenomes or later the inter-specific introgression of A^r to A^n subgenome that happened to one of the *B. napus* parental line we used, Ningyou7 (Qiu et al. 2006). However, there were some inter-chromosomal translocations seemed occurred in A^r subgenome relative to A^n, because the surrounding blocks organization was more integrated in A^n but shattered in A^r, such as the BAC AtF19 and AtF20O9 at the top of A^r2 and the BAC KBrH001C24 in the middle of A^r7 (Fig. 1). It has been illustrated that both *B. rapa* and *B. napus* have polyphyletic origins (Gómez-Campo C, Prakash S, 1999; Song and Osborn 1992); and the diploid Brassicas also experienced polyploidization and then diploidization in their evolutionary history, probably via an ancient of hexaploid (Lysak et al. 2005). Therefore, we propose that the divergence between the A^r and A^n subgenome might be either due to genomic rearrangements during allopolyploidization process from which formed *B. napus*, or the genomic divergence in ancestral A-lines contributing to modern *B. rapa* and *B. napus* separately.

When we compared the *B. napus* linkage group A^n3 with the physical constitution of the same chromosome in *B. rapa* cv. Chiifu, an inversion event, which was identified from comparison between both linkage groups A3 of TN and CK genetic map, was not identified again (Fig. 3, empty triangle). Such deviations between the genetic and physical locations may be the result of the relatively small population size which formed the CK genetic map, or interference between paralogous loci. On the other hand, if there was genomic divergence between the two parental lines that were used for genetic mapping, this would also interfere with the genetic locations around the divergent regions. In this case, we suspect that the deviation between the genetic and physical locations around the BAC KBrB055N13 to KBrB008I08 region on chromosome A^r3 in *B. rapa* is the result of genomic divergence between the parental lines Chiifu and Kenshin; the genomic organization in the sequenced parent Chiifu was similar to Tapidor

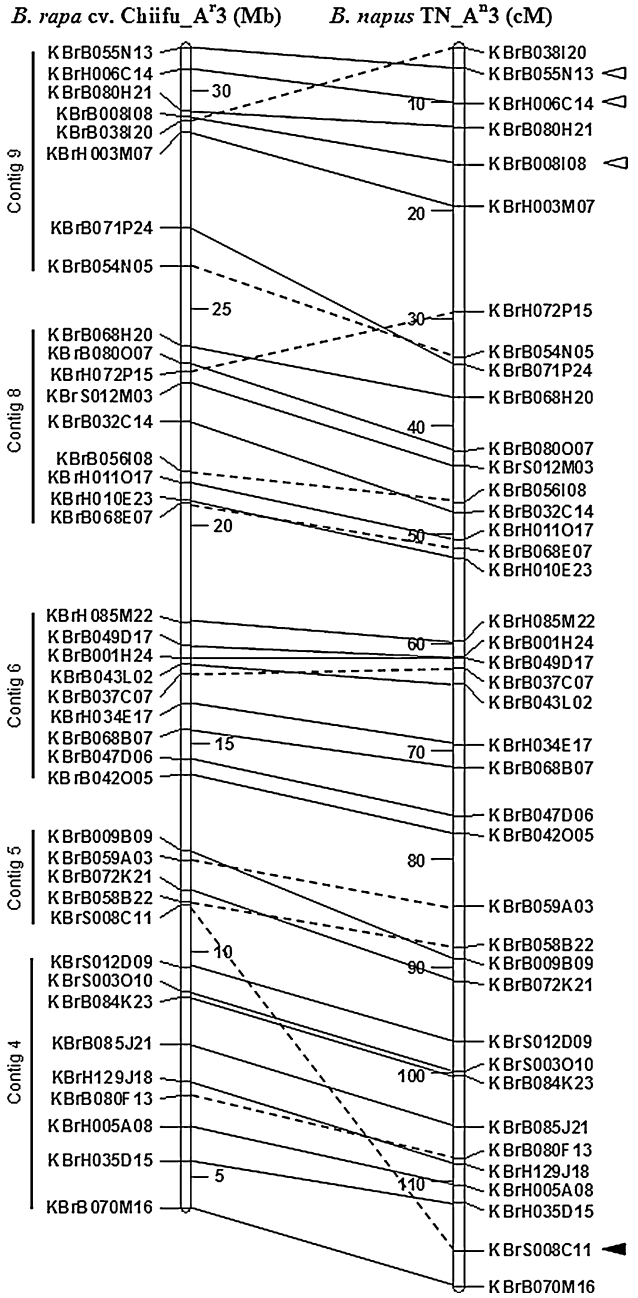

Fig. 3 Comparison of A3 chromosome between the *B. napus* linkage group and the *B. rapa* physical map. The *left panel* is the BACs forming contigs on *B. rapa* cv. Chiifu chromosome A^r3; the *right panel* is the location of these common BACs on linkage group A^n3 of *B. napus* TN genetic map. The syntenic conservation is indicated by the *solid connections* between common BACs, while the breaking of synteny is indicated by the *dashed connections*. The BACs showing rearrangement events consistently on comparisons between *B. napus* genetic map and both genetic and physical map of *B. rapa*, are pointed by *solid triangle*; the BACs showing rearrangement on comparison between the genetic maps of *B. napus* and *B. rapa*, but not physical map, are pointed by *empty triangle*

and Ningyou7 in this region and demonstrates syntenic organization between the Chiifu physical map and TN genetic linkage map.

Table 4 Phenotypic variation of seed quality traits in *B. rapa* and *B. napus* mapping populations

Trait (content in seeds)	Phenotypic value in *B. rapa* population					Phenotypic value in *B. napus* population				
	Chiifu-401	Kenshin	Min	Max	Mean	Tapidor	Ningyou7	Min	Max	Mean
Glucosinolate (μmol/g)	101.1 ± 27.6	94.5 ± 15.6	35.6 ± 20.3	159.3 ± 24.2	104.3 ± 14.3	39.7 ± 16.4	110.9 ± 37.1	20.2 ± 10.4	127.8 ± 14.0	76.5 ± 17.0
Oil (% of seed dry weight)	39.3 ± 5.2	39.8 ± 2.7	33.5 ± 1.8	45.1 ± 2.2	40.3 ± 2.1	43.6 ± 1.2	42.7 ± 1.7	37.0 ± 2.3	48.6 ± 1.6	43.3 ± 1.2
Protein (% of seed dry weight)	23.6 ± 1.2	24.0 ± 2.6	19.7 ± 1.1	27.1 ± 1.9	23.6 ± 1.4	23.5 ± 0.3	21.7 ± 0.3	19.4 ± 1.2	25.2 ± 0.3	22.5 ± 0.6

The data are average values (±standard deviation) taken from multiple experiments

Table 5 Number of QTL detected for the seed quality traits on each linkage group of the A subgenomes of *B. rapa* (A^r) and *B. napus* (A^n)

Linkage group	Number of QTL for seed quality traits						Number and interval of conserved QTL
	Glucosinolate		Oil		Protein		
	A^r	A^n	A^r	A^n	A^r	A^n	
A1	3	1	1	2	1	3	2 AtT5J17-AtT19K4 (Glu) AtT4L20-KBrB066A08 (Oil)
A2	–	–	1	1	2	3	–
A3	–	–	1	3	–	1	1 KBrB056I08-KBrB049D17 (Oil)
A4	1	–	–	1	1	2	1 AtK15O15-AtF26B6 (Pro)
A5	3	–	2	–	3	1	1 AtF18O19-KBrB050K06 (Pro)
A6	–	–	4	3	3	1	1 KBrB086M23-KBrB011L09 (Oil/Pro)
A7	1	1	4	3	3	2	1 KBrB022C05-AtT7B11 (Oil)
A8	1	3	–	5	–	–	–
A9	3	2	1	–	–	1	1 KBrH010M17-KBrB092J12 (Glu)
A10	–	–	2	1	1	1	1 KBrB030F10-KBrH053G06 (Oil)
Sum	**12**	**7**	**16**	**19**	**14**	**15**	**9**

Genomic conservation and diversification at the structural level must have had an effect on the function of particular genomic regions (Laurie and Devos 2002), and resulted in either similarity or change of the final phenotypic expression, such as that observed for seed quality traits in the A subgenome of tetraploid oilseed crop *B. napus* in comparison with the diploid *B. rapa*, the parental lines of which in this study are used for vegetable production. We were quite surprised to identify 9 QTL for seed quality that were conserved between the two species, especially the conserved QTL at the bottom of linkage group A9, which controlled total glucosinolate content in seeds. In *B. napus*, many previous studies have identified this QTL as the most significant regulator of total glucosinolate content in seeds (Howell et al. 2003; Toroser et al. 1995; Uzunova et al. 1995). Given that the two parental lines of the *B. rapa* CK DH population, Chiifu-401 and Kenshin, both have a high level of glucosinolate in seeds, it is interesting that we also detected this QTL on A^r9 in *B. rapa*, and validated that the functional conservation was retained in spite of genomic rearrangement, which included an inversion associated with blocks Q and X that covered this QTL. Although this QTL has been accepted widely and used to improve seed quality in *B. napus*, the identification of its conserved counterpart in *B. rapa*, for which much sequence information is now available, could help us to identify the actual gene(s) associated with this QTL.

Seed oil content, which has been an important target for improvement of *B. napus* but not the vegetable *B. rapa*, did not differ widely between the parents of both the *B. napus* and *B. rapa* populations. Nevertheless, we detected a total of five QTL for seed oil content, one each on linkage groups A1, A3, A6, A7, and A10, that were conserved between the two species (Table 5). Although strong selection for seed oil content might not have happened in *B. rapa*, the *B. rapa* population that we used has a relatively high level of total oil content in seeds. The presence of QTL with large effects on seed oil content in *B. rapa* might be due to natural selection for high seed oil content in wild or landrace progenitors of vegetable *B. rapa*, or tight linkage of the seed oil and vegetable traits. Therefore, we assume that the elite alleles from *B. rapa* might represent an exotic source to improve seed oil content in *B. napus*, like the successful utilization of *B. rapa* as a source for breeding disease-resistant *B. napus* (Leflon et al. 2007; Yu et al. 2008).

As we described above, there were also many QTL in one species that had no counterparts controlling the same trait(s) in the corresponding locations of the contrasting species. Although such differences in QTL locations commonly exist even in different populations of the same species and result from diversified molecular polymorphisms, nevertheless the coincidence between locations of these non-conserved QTL and the presence of genomic rearrangements may imply that the genomic structural changes could also be the reason for their differences in functional expression between the two species. Previous studies have shown that structural changes in the genome could either result in loss of function or gain of novel function (Gaeta et al. 2007; Xiao et al. 2008). It should be possible to characterize the effect of such genomic rearrangements on functional properties more precisely in the near future when more sequences for both species are available.

Polyploidization has occurred widely in plant evolution, and many important crops and fruits are polyploid derivatives of old or wild ancestors with relatively simpler genomic constitution. Nowadays, several ancestral species with small genomes are undergoing genome sequencing, with the expectation that the sequence in the ancestors will assist dissection of the polyploid genomes. However, our study in *Brassica* reveals that significant genomic divergence has occurred between the common genomes of a diploid and its derived allopolypoid form, from which we conclude that caution must be applied to the use of the referential genomes for either comparative sequencing or agronomic trait improvement in derived polyploid species.

In this study we have provided a framework of genomic construction for the *Brassica* A subgenome which shows significant divergence between the model species (*B. rapa*) and the polyploid crop (*B. napus*), and this should help to avoid misleading results during comparative sequencing. Our genetic map of the tetraploid *B. napus* A subgenome, based on sequence-informative markers from diploid *B. rapa,* provides a basic foundation for further research on the complexity of *Brassica* genomes that have experienced various genomic rearrangements during polyploidization. The *B. napus* TN genetic map was updated with hundreds of sequence-informative markers which will improve its value as an advanced reference genetic map for *B. napus*. Markers derived from *B. rapa* BAC-sequences were mapped on the A subgenome of the *B. napus* TN genetic map and were separated by a moderate distance of 5–10 cM (Online Resource 1), thus providing a valuable framework for the sequencing project of the tetraploid *B. napus* genome. The released *B. rapa* sequences will provide reference for assembling pieces of *B. napus* sequence at segmental scales, which are being generated from some high-throughput sequencers (such as Roche-454 and Solexa sequencing); however, in building the complete A subgenome sequence in *B. napus* it will be important to take into account many rearrangements between the A^n and A^r subgenomes, some of which we have identified in this paper.

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