DNA Research **18**, 401–411, (2011)
Advance Access Publication on 4 August 2011
doi:10.1093/dnares/dsr027

Extensive Chromosome Homoeology among Brassiceae Species Were Revealed by Comparative Genetic Mapping with High-Density EST-Based SNP Markers in Radish (*Raphanus sativus* L.)‡

Feng Li[1], Yoichi Hasegawa[1,†], Masako Saito[1], Sachiko Shirasawa[1], Aki Fukushima[1], Toyoaki Ito[2], Hiroshi Fujii[3], Sachie Kishitani[1], Hiroyasu Kitashiba[1], and Takeshi Nishio[1,*]

Graduate School of Agricultural Science, Tohoku University, Aoba-ku, Sendai, Miyagi 981-8555, Japan[1]; Field Science Center, Graduate School of Agricultural Science, Tohoku University, Oosaki, Miyagi 989-6711, Japan[2] and National Institute of Fruit Tree Science, NARO, Shizuoka, Shizuoka 424-0292, Japan[3]

*To whom correspondence should be addressed. Tel. +81 22-717-8650. Fax. +81 2-717-8654.
Email: nishio@bios.tohoku.ac.jp

Edited by Katsumi Isono
(Received 24 March 2011; accepted 9 July 2011)

Abstract

A linkage map of expressed sequence tag (EST)-based markers in radish (*Raphanus sativus* L.) was constructed using a low-cost and high-efficiency single-nucleotide polymorphism (SNP) genotyping method named multiplex polymerase chain reaction–mixed probe dot-blot analysis developed in this study. Seven hundred and forty-six SNP markers derived from EST sequences of *R. sativus* were assigned to nine linkage groups with a total length of 806.7 cM. By BLASTN, 726 markers were found to have homologous genes in *Arabidopsis thaliana*, and 72 syntenic regions, which have great potential for utilizing genomic information of the model species *A. thaliana* in basic and applied genetics of *R. sativus*, were identified. By construction and analysis of the genome structures of *R. sativus* based on the 24 genomic blocks within the Brassicaceae ancestral karyotype, 23 of the 24 genomic blocks were detected in the genome of *R. sativus*, and half of them were found to be triplicated. Comparison of the genome structure of *R. sativus* with those of the A, B, and C genomes of *Brassica* species and that of *Sinapis alba* L. revealed extensive chromosome homoeology among Brassiceae species, which would facilitate transfer of the genomic information from one Brassiceae species to another.

Key words: comparative genomics; *Raphanus sativus*; SNP genotyping; synteny; chromosome homoeology

1. Introduction

Angiosperms evolved from a common ancestral genome that underwent duplications, rearrangements, and mutations in succeeding generations. Phylogenetic relatedness of different species correlates with a degree of synteny between their genomes.[1,2] Comparative genomics contributes greatly to understanding the basic processes of genome evolution from or to related organisms within a phylogenetic framework[3–5] and applying model species genome information to the study of related organisms.[6,7] Comparative mapping studies in the grass family (Poaceae) have pioneered the field of plant comparative genomics.[3,8] The collinear genomic regions between domesticated cereals and forage crops have been detected by such studies,

‡ After this manuscript was submitted, a paper by Shirasawa *et al.* (An EST-SSR linkage map of Raphanus sativus and comparative genomics of the Brassicaceae) (http://dnaresearch.oxfordjournals.org/content/current) was published. LG1 (R6), LG2 (R5), LG3 (R4), LG4 (R2), LG5 (R9), LG6 (R3), LG7 (R8), LG8 (R1), and LG9 (R7) in the present study are identified to correspond to LG2, LG3, LG4, LG5, LG6, LG8, LG7, LG9, and LG1 of Shirasawa *et al.*, respectively.

† Present address: National Institute of Crop Science, NARO, 2-1-18 Kannondai, Tsukuba, Ibaraki 305-8518, Japan.

which have opened the way to efficient map-based cloning and enabled inference of the basic organization of the ancestral grass genome.

The dicot family Brassicaceae consists of 338 genera with ca. 3700 species in 25 tribes,[9] and their chromosome number varies greatly from $n = 4$ to 128.[10] Comparative genomics in Brassicaceae has largely focused on direct comparisons between the model species *Arabidopsis thaliana* and the species of interest, especially, the agronomically important *Brassica* crops. Comparative linkage mapping among three diploid *Brassica* species, i.e. *Brassica nigra*, *Brassica oleracea*, and *Brassica rapa*,[6] and that between *A. thaliana* and three *Brassica* species[11] have suggested that genomes of the diploid *Brassica* species are composed of a triplicated and rearranged ancestral genome. Additional evidence for the genome triplication in *Brassica* has been shown by nucleotide sequencing[12,13] and cytogenetic methods.[14,15] However, some genomic regions are present in less or more than three copies in *Brassica* genomes.[7,16] Furthermore, ~30–40% of genes in the syntenic regions of *A. thaliana* have been lost in their counterparts of the *Brassica* genomes,[13,16] and genomic rearrangements and gene duplications have occurred in *A. thaliana* after the divergence of *Brassica* and *Arabidopsis*,[17,18] making the comparative maps between *A. thaliana* and *Brassica* species complicated.[7,11,19]

A Brassicaceae ancestral karyotype ($n = 8$) has been previously deduced from comparative genetic maps of two $n = 8$ species in the tribe Camelineae, i.e. *Capsella rubella* and *Arabidopsis lyrata*, with *A. thaliana*.[5,20–22] *Arabidopsis thaliana* and its closely related species with six or seven chromosome pairs have been suggested to be derived from the ancestral karyotype through a similar mechanism of chromosome reduction.[23] Based on a set of 21 conserved genomic regions within the *Arabidopsis* genome identified in *Brassica napus* by Parkin *et al.*,[19] Schranz *et al.*[5] have proposed a set of 24 genomic blocks within the Brassicaceae ancestral karyotype. These 24 genomic blocks represent the conserved segments among the Brassicaceae ancestral karyotype, *Arabidopsis*, and *Brassica*. Using these genomic blocks, the homoeologous relationship and evolution of the A, B, and C *Brassica* genomes have been studied,[24] and a conserved chromosome (AK1) of the Brassicaceae ancestral karyotype has been revealed in *Sinapis alba*.[25]

More recently, Mandáková and Lysak[4] have proposed a Proto-Calepineae karyotype ($n = 7$), which has been suggested to be descended from the Brassicaceae ancestral karyotype and to be the karyotype of the common progenitor of the tribes Calepineae, Conringieae, and Noccaeeae, and the Brassicaceae lineage II, including the tribes Brassiceae, Isatideae, Sisymbrieae, and Eutremeae. Two to three copies of genomic blocks associated closely in the Proto-Calepineae karyotype have also been found in the *Brassica* genome,[5,19] suggesting that the Proto-Calepineae karyotype has undergone the whole-genome triplication in the clade leading to the tribe Brassiceae. However, the genomic evolutionary process from the primary paleo-hexaploid ancestor to the present Brassiceae species is not clear. Reconstruction of karyotypes of different species using the common genomic blocks of the Brassicaceae ancestral karyotype is expected to solve this problem.

Radish (*Raphanus sativus* L., $2n = 2x = 18$) belonging to the tribe Brassiceae and closely related to *B. rapa* is an important commercial crop that is grown and consumed all over the world, especially in eastern Asia. *Raphanus sativus* has great variations in the root shape from round and thick with a diameter of more than 30 cm to thin and long with a length of more than 2 m. The thick roots are commonly harvested as vegetables, while there are some cultivars used as leafy vegetables, silique vegetables, or oil crops. As a genetic map with DNA markers can be utilized in applied genetics and breeding, several genetic linkage maps have been constructed in *R. sativus*[26–29] based on Amplified Fragment Length Polymorphism (AFLP), Random Amplified Polymorphism (RAPD), and Simple Sequence Repeats (SSR) markers, and quantitative trait loci (QTLs) for shape and colour of roots, club-root resistance, and cyst nematode resistance have been detected.[27,29,30] However, since the AFLP and RAPD markers are anonymous and SSR markers are usually located in non-coding sequences, the comparative genomics of *R. sativus* with other species has not been performed, and utilization of the genome information of *Arabidopsis* and *B. rapa* in the study of *R. sativus* is limited.

Recently, abundant information on expressed sequence tag (EST) sequences of radish has been published on the radish sequence database (Radish DB; http://radish.plantbiology.msu.edu). As single-nucleotide polymorphisms (SNPs) are the most abundant type of DNA polymorphism in genomes, in order to construct a linkage map of *R. sativus*, we explored these EST sequences to design primer pairs for specific amplification of genes, and identified SNPs for production of EST-based SNP markers by determining nucleotide sequences of two lines used for production of an F_2 population. Genotyping of EST-based SNP markers of F_2 plants was performed using dot-blot-SNP analysis combining multiplex polymerase chain reaction (PCR)[31] and mixed probe hybridization,[32] and a high-density linkage map was constructed to reveal synteny with the genome of *A. thaliana*. Furthermore, the genome structure of *R. sativus* was

reconstructed using the 24 genomic blocks of the Brassicaceae ancestral karyotype and was compared with those of other Brassiceae species.

2. Materials and methods

2.1. Plant materials

Two radish lines self-pollinated for three generations from 'Sayatori 26704' (National Institute of Vegetable and Tea Science, Japan) and 'Aokubi *S-h*' (Takii Seed Co., Japan), respectively, were used. 'Sayatori 26704' (hereafter 'Sayatori') is a seedpod vegetable with a very thin and small root like a rat tail cultivated in South Asia. 'Aokubi *S-h*' (hereafter 'Aokubi') is a Japanese radish with a long and thick root. F_1 hybrids were produced by crossing 'Aokubi' with 'Sayatori', and F_2 seeds were obtained by selfing with bud pollination of a single F_1 hybrid plant.

2.2. Analysis of DNA polymorphism between 'Aokubi' and 'Sayatori'

Genomic DNAs of the 'Aokubi' and 'Sayatori' inbred lines were extracted from leaves by the CTAB method.[33] For designing primer pairs, ~9000 unigenes were selected from the RS2 library of the Radish Database (http://radish.plantbiology.msu.edu). The exon/intron junction and the UTR region of the unigenes were predicted by the MAEZATO system, which aligns a target EST sequence with a possible homologous *Arabidopsis* cDNA.[34] Forward primers were designed within an exon region and reverse primers were designed within a region containing a predicted 3′-UTR using Primer3 plus (http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi). As flowering time is important for the production of vegetables in *R. sativus*, the coding sequence of an *FLC1* homologue in *B. rapa*[35] was also used for designing primers. PCR was conducted with these primer pairs using genomic DNA of the 'Aokubi' and 'Sayatori' inbred lines as templates. A 20 μl reaction mixture contained 20 ng of plant genomic DNA, 10 pmol of each primer, 1× *ExTaq* buffer, 2 nmol of each dNTP, and 0.5 U of *Taq* DNA polymerase (*ExTaq*, Takara Biomedicals, Japan). The thermal cycling condition was 35 cycles of 94°C for 30 s, 58°C for 30 s, and 72°C for 2 min. A 4 μl PCR product was electrophoresed on 1.2% agarose gel, and a single fragment amplified by PCR was sequenced by the Sanger method.[36] The sequences were aligned using SEQUENCHER version 4.7 (Gene Codes Corporation, MI, USA) to identify SNPs. Sequences having SNPs between 'Aokubi' and 'Sayatori' were used for designing bridge probes[37] for dot-blot-SNP analysis.

2.3. Dot-blot-SNP analysis

Genomic DNAs from 189 plants of the F_2 population were prepared using the modified CTAB method.[38] In order to achieve cost-effective and high-efficiency SNP genotyping, a dot-blot-SNP method combining multiplex PCR[31] and a mixed probe[37] was developed and named multiplex PCR–mixed probe (MPMP) dot-blot analysis. First, 36 primer pairs of SNP markers were grouped into six by MultiPLX 2.0[31] and were assigned to six lines in a table. Secondly, hybridization conditions of probes of the markers were predicted as described by Shiokai *et al.*[32] using the DINAMelt web server (http://mfold.rna.albany.edu/?q=DINAMelt/Hybrid2),[39] and the probes having similar hybridization conditions in different primer groups were placed into the same column to design an MPMP table (an example being shown in Supplementary Table S1). Multiplex PCR was conducted in a 10 μl reaction mixture containing 4 ng plant genomic DNA, 10 pmol of each primer mixed as designed in the MPMP table, 1× KAPATaq EXtra buffer (without Mg^{2+}), 1.75 mM $MgCl_2$, 0.3 mM of each dNTP, and 0.25 U of DNA polymerase (KAPATaq Extra, KAPABIOSYSTEMS, Boston, MA, USA). Thermal cycling conditions were as follows: 1 min denaturation at 94°C, 35 cycles of 94°C for 30 s, 56°C for 30 s, and 72°C for 1 min, and a final extension at 72°C for 2 min. Amplified DNA was denatured in a solution of 0.4 N NaOH and 10 mM EDTA, and dot-blotted onto a nylon membrane (Nytran, Pall, NY, USA) using Multi-pin Blotter (Atto, Tokyo, Japan). Probes in the same columns of the MPMP table were mixed and hybridized together with digoxigenin-labelled probes having sequences complementary to the bridge probe.[37] Allele-specific signals were detected according to Shiokai *et al.*[37] This MPMP dot-blot analysis using 36 EST-SNP markers was compared with dot-blot-SNP analysis using a single EST-SNP marker.

2.4. Linkage analysis

Linkage analysis was carried out using the JoinMap 4.0 software (Kyazma B.V., Wageningen, The Netherlands).[40] The SNP markers were grouped into nine linkage groups (LGs) at high LOD threshold (≥6). Marker order was subsequently determined by a regression mapping algorithm on the basis of a minimum LOD score of 1.0 and a recombination threshold of 0.4 in each LG. Recombination values were converted to genetic distance (cM) using the Kosambi mapping function.[41] Each map was graphically visualized with MapChart.[42] The EST-based SNP markers were named <Rs> <EST name or gene name> <s>.

2.5. Comparison with the A. thaliana genome sequences

Sequences in the *A. thaliana* genome homologous with the loci of EST-based SNP markers in the linkage map were surveyed using the BLASTN program of the National Centre for Biotechnology Information (http://www.ncbi.nlm.nih.gov/) with a threshold value of $E < 10^{-20}$. Syntenic regions were identified according to the conserved collinearity of the markers of *R. sativus* and corresponding homologous genes of *A. thaliana*. A single non-collinear homologue in a syntenic region was ignored.

3. Results and discussion

3.1. SNP identification and production of DNA markers

To identify SNPs between the parental lines, i.e. 'Sayatori' and 'Aokubi', 4709 primer pairs were designed and used for amplification of coding regions of genes containing 3′-untranslated regions, and single DNA fragments in both parental lines were amplified by PCR with 3576 primer pairs and sequenced. After trimming ambiguous sequences at both ends and excluding the sequences with a quality score <90% (Sequencher, http://www.genecodes.com/), 2290 sequences, covering 791.9 kb, were obtained (Supplementary Table S2). Among them, 1465 (64.0%) DNA fragments showed nucleotide polymorphism. The frequency of variable bases, i.e. SNPs and indels, was 1/72 bp, and the frequency of SNPs was 1/107 bp. As continuous SNPs or indels can be designed as one marker, such sites were regarded as one SNP site or one indel site. The frequencies of SNP sites and indel sites were 1/115 and 1/520 bp, respectively. These results suggest that the use of SNP markers enables construction of a high-density EST-based genetic map. Of the 1465 sequences having SNPs or indels between the parental lines, we randomly selected 1410 fragments for designing dot-blot-SNP markers.

3.2. Dot-blot-SNP analysis and mapping

Since dot-blot-SNP analysis allows hybridization of only a fully complementary sequence without hybridization of a mismatched sequence, this method ensures accuracy of genotyping,[37] and enables genotyping of multiple-copy genes in polyploid species, e.g. *B. napus*, and diploid species having ancestral genome replication, e.g. *B. rapa* and *R. sativus*. In MPMP dot-blot-SNP analysis, more than half of the makers showed allele-specific signals in the first round of probe hybridization under the conditions predicted by the method of Shiokai *et al.*[32] In the second round of probe hybridization, conditions were adjusted according to the signals shown in the first round. At most, three rounds of probe hybridization were conducted. The results of the genotyping data were consistent with the dot-blot-SNP method using a single SNP marker. In this way, the MPMP dot-blot-SNP method was found to speed up the genotyping and to reduce the cost of analysis. Of the 1410 dot-blot-SNP markers, 881 yielded clear dot-blot signals with distinct differences between SNP alleles.

Using 772 dot-blot-SNP markers among the 881 SNP markers, genotypes of 189 F_2 plants were analysed. By linkage analysis with the JoinMap 4.0 software, 746 were assigned to nine LGs. Primer sequences, probe sequences, and hybridization conditions of the 746 markers are listed in Supplementary Table S3. Nine LGs were designated as LG1–LG9 in decreasing order of map distances (cM) (Fig. 1). The longest group LG1 consisting of 88 markers and the shortest one LG9 consisting of 75 markers have lengths of 126.5 and 62.7 cM, respectively. The total length covered by the linkage map was 806.7 cM with an average distance of intervals between markers of 1.1 cM. Based on the physical length of 530 Mb in *R. sativus*,[43] 1 cM was estimated to be 657 kb.

Many ESTs, which were used for developing the markers in the present study, have high similarity to genes with known or hypothetical functions. For example, on the shortest LG9, *RsFLC1* amplified by the primers designed from *BrFLC1* (accession no. AY115678.1) was mapped. LG9 also contained RS2CL4436, a homologue of *AtPPa* in the *S* locus of *B. rapa* (AB257127.1), and Rs2CL2115, a homologue of the *B. rapa* disease resistance gene *BrTN3* (FJ842847.1). Furthermore, ESTs homologous to *A. thaliana* genes involved in cadmium ion transmembrane transportation (NM_104680.2), salt stress response (NM_106207.3), cold and drought response (NM_122163.2), sucrose synthesis (NM_122090.3), and amino acid biosynthesis (NM_001036839.2) were mapped on LG9. Other LGs also contain many interesting genes.

3.3. Synteny between R. sativus and A. thaliana

As protein-coding regions in the genomes are conserved in related species more than intergenic regions, EST-based markers are useful for comparative mapping between them. We searched for genes homologous with these markers in the *A. thaliana* genome by BLASTN. The results are shown in Supplementary Table S4. Under a significance threshold of $E < 10^{-20}$, *A. thaliana* loci homologous with 726 *R. sativus* markers were identified, these homologous loci covering nearly the whole genome of *A. thaliana*.

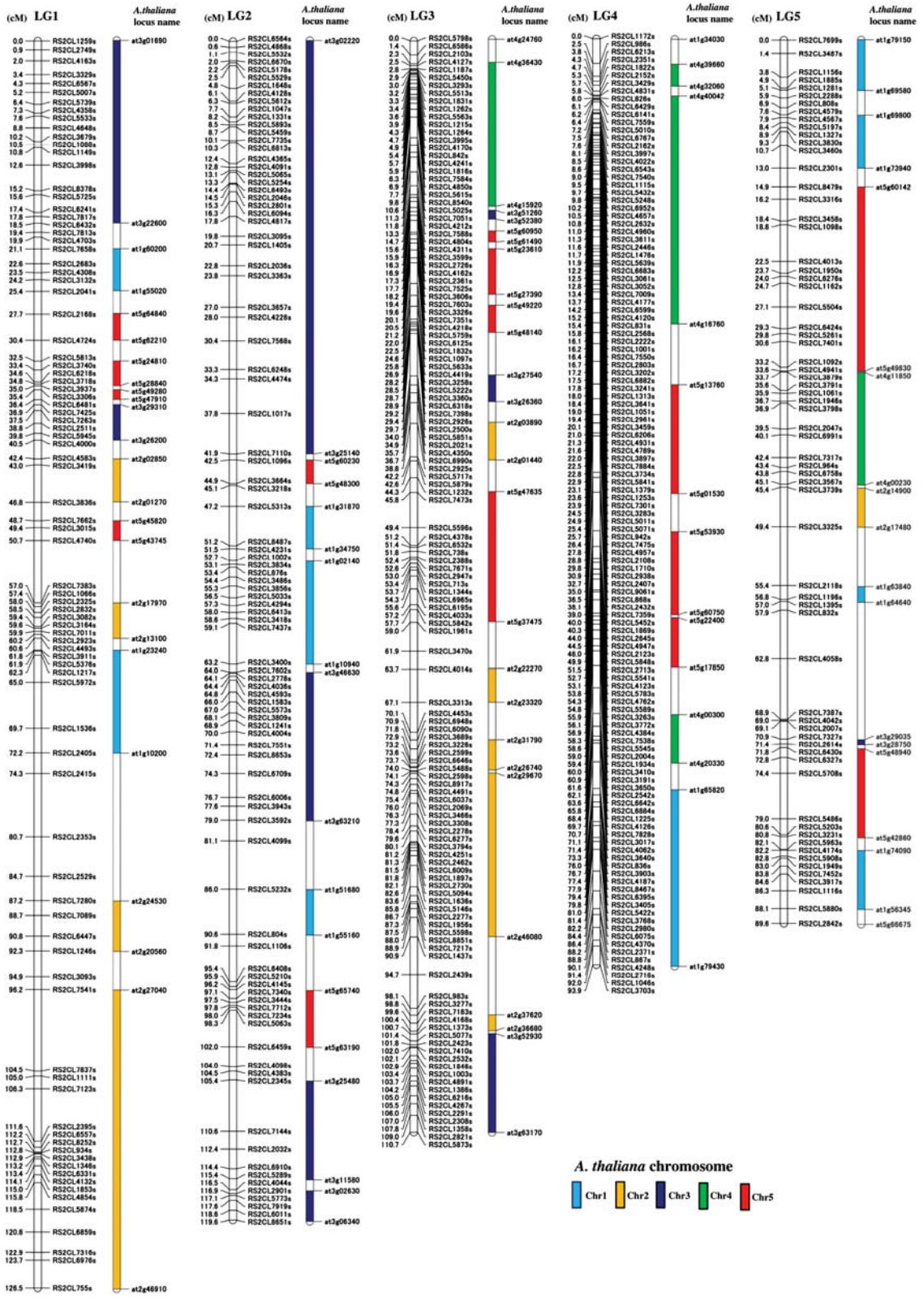

Figure 1. A linkage map of *R. sativus* with a comparative map of *A. thaliana*. Nine LGs are labelled as LG1−LG9 in the order of length. Marker positions (in cM) are shown on the left side and the corresponding marker names are shown on the right side of each LG. Each locus was tested for homology with *A. thaliana* using BLAST and segments of two or more markers showing homology with *A. thaliana* in collinearity are regarded as syntenic regions, which are shown to the right of the *R. sativus* LGs as coloured vertical bars. The *A. thaliana* chromosomes are coloured according to Parkin *et al*.,[19] and are shown at the bottom right of the figure. Except for both ends of each syntenic region, the names of identified homologous loci in *A. thaliana* are not shown, but are shown in Supplementary Table S4.

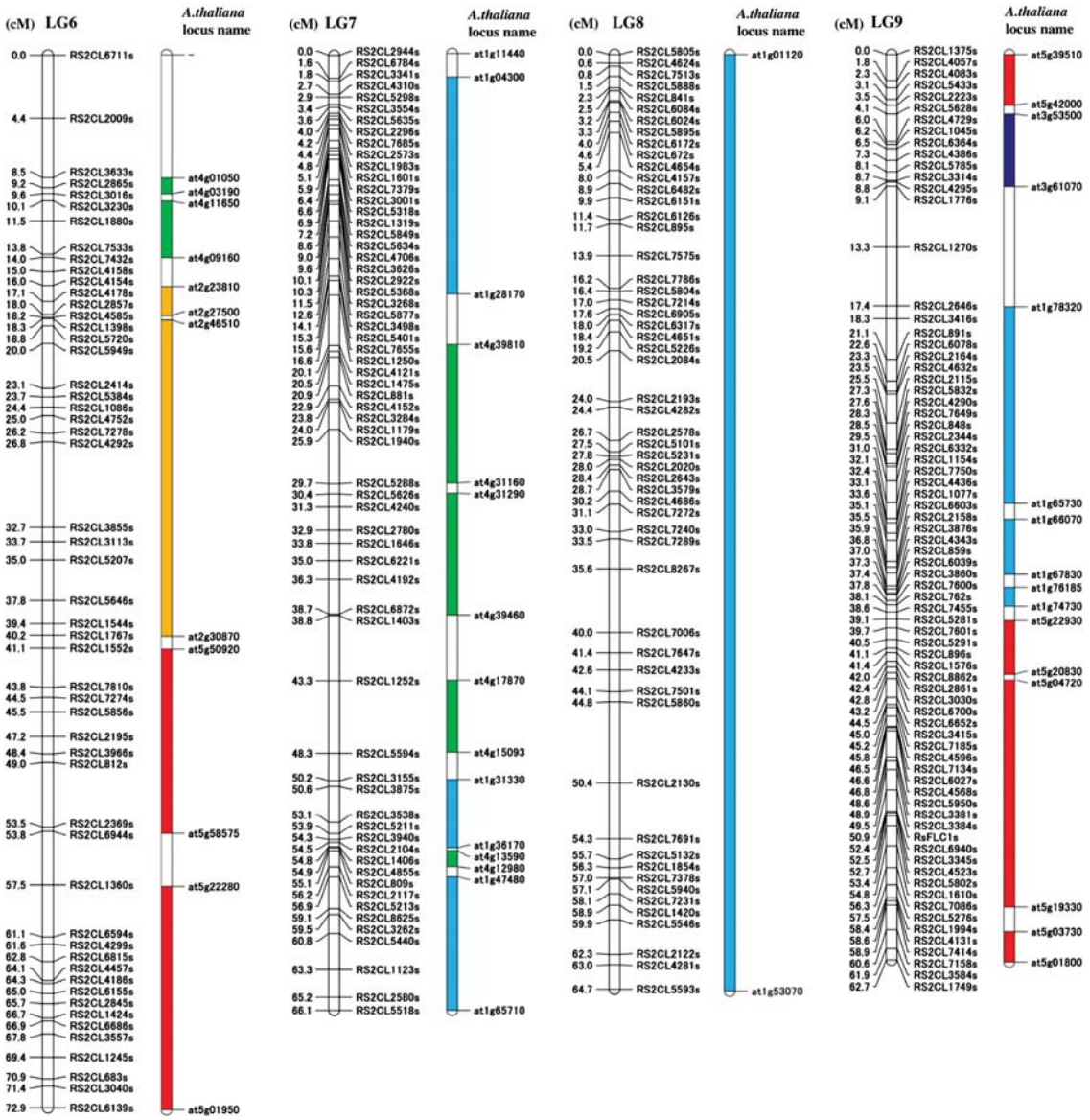

Figure 1. *Continued*

The five chromosomes of *A. thaliana* were divided into many segments and distributed to various regions of the *R. sativus* map (Fig. 1). There were 72 syntenic regions, mostly containing at least three markers and having conserved collinearity with the homologues in *A. thaliana*. The whole LG8 showed collinearity to more than half of chromosome 1 of *A. thaliana*. This synteny information is considered to be useful for utilizing the genome information of *A. thaliana* in the study of *R. sativus* and may speed up map-based cloning, as shown in *B. rapa*.[7,44]

The 24 genomic blocks within the ancestral karyotype proposed by Schranz *et al.*[5] have been identified in many species of the family Brassicaceae[4,16,23–25,45] and can contribute to build a unified comparative genomics system in the Brassicaceae. The genome structure of *R. sativus* was reconstructed by these genomic blocks (Fig. 2). Except for the G block, all the blocks were found in the genome of *R. sativus*. The absence of G block in the present map is possibly due to its shortness and closeness to the AK3 centromere of the Brassicaceae ancestral karyotype.[5] Half of the genomic blocks within the Brassicaceae ancestral karyotype, including A, B, C, E, F, I, J, O, P, R, U, and W, were triplicated. Some parts of A, U, and E blocks were revealed to have been further replicated, resulting in four, four, and six copies, respectively. It has also been reported that half of the genomic blocks are triplicated, while others occur only once or twice in *B. rapa* genome.[16] These results indicate recent replication and loss of blocks during genomic evolutionary processes from the paleohexaploid to the present

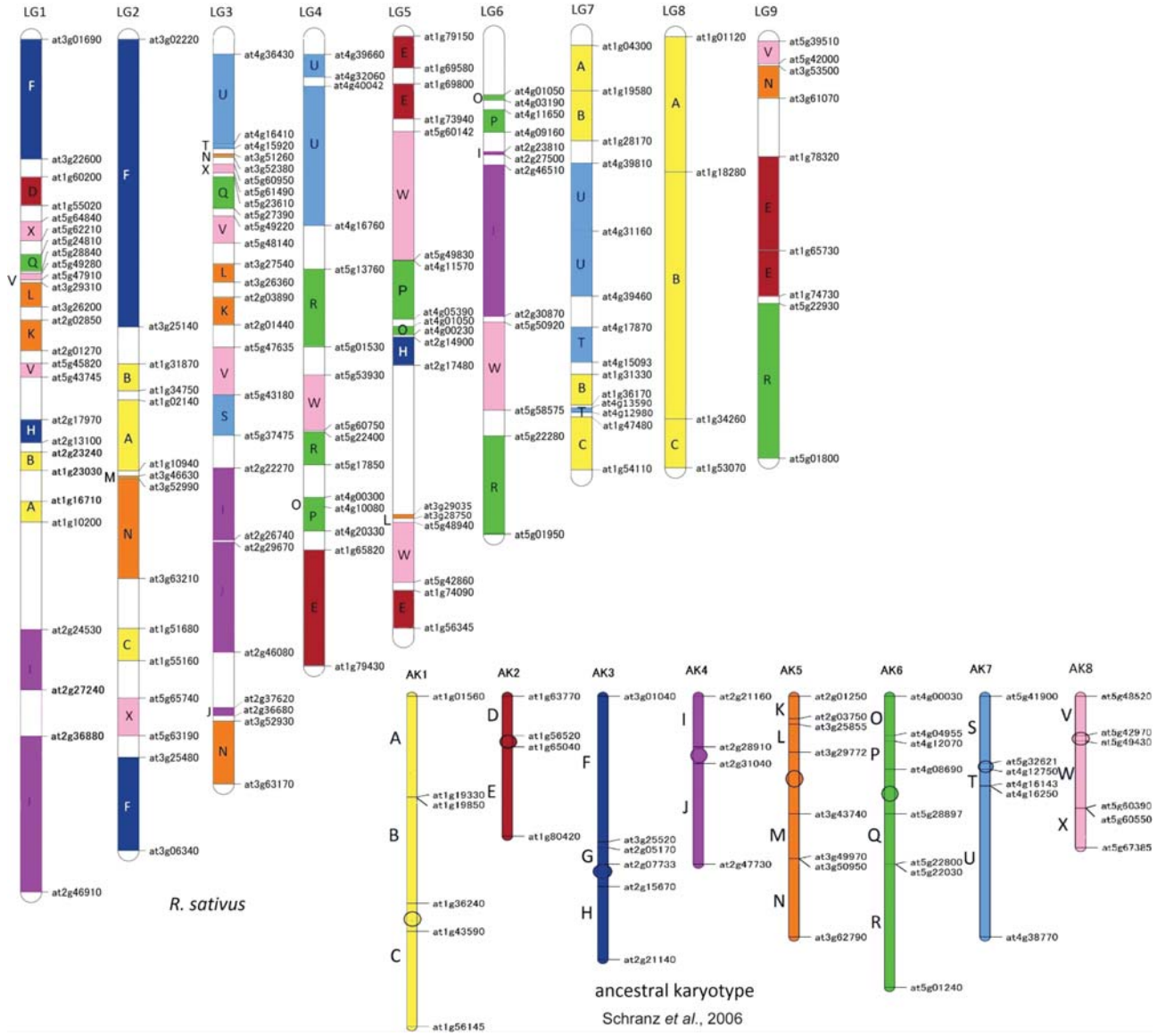

Figure 2. Genome structure of *R. sativus* based on 24 genomic blocks within the Brassicaceae ancestral karyotype. The genomic blocks of the Brassicaceae ancestral karyotype, shown on the bottom right, are labelled by the letters A to X. One of the eight colours corresponds to each chromosome. Boundaries of the blocks are defined by their flanking locus name of *A. thaliana*, according to Schranz *et al.*[5] The position of each genomic block in *R. sativus* was defined according to the comparative map of *R. sativus* and *A. thaliana* in Fig. 1.

Brassiceae species. Some neighbouring blocks, e.g. I/J, A/B, and O/P, in the Brassicaceae ancestral karyotype were not separated in the genome of *R. sativus*. One chromosome (AK1) of the Brassicaceae ancestral karyotype comprising A, B, and C blocks was found to be completely conserved, forming LG8 of *R. sativus*, as the case of S05 in *S. alba*.[25] The other copies of AK1 may have incurred genomic rearrangement and dispersed in LG1, LG2, and LG7. The blocks of V, K, L, Q, and X from different chromosomes of the Brassicaceae ancestral karyotype associated to form parts of LG1 and LG3. Similarly, the blocks of R, W, O, and P associated to form parts of LG4 and LG6, and possibly LG5. These V/K/L/Q/X, R/W, and O/P block associations have been detected in the Proto-Calepineae karyotype (Supplementary Fig. S1),[4] and found to be replicated in the A, B, and C genomes of *Brassica* species.[4,5,19,24] In *S. alba*, although V/K/L/Q/X block association has not been observed, probably due to the small number of markers used, R/W block association has been found to be triplicated.[25] These findings reinforce the hypothesis on the origin of the Brassicaceae lineage II from the common Proto-Calepineae karyotype.[4]

3.4. *Identification of homoeology between the genomes in Brassiceae species*

In terms of the 24 genomic blocks within the Brassicaceae ancestral karyotype, the structures of

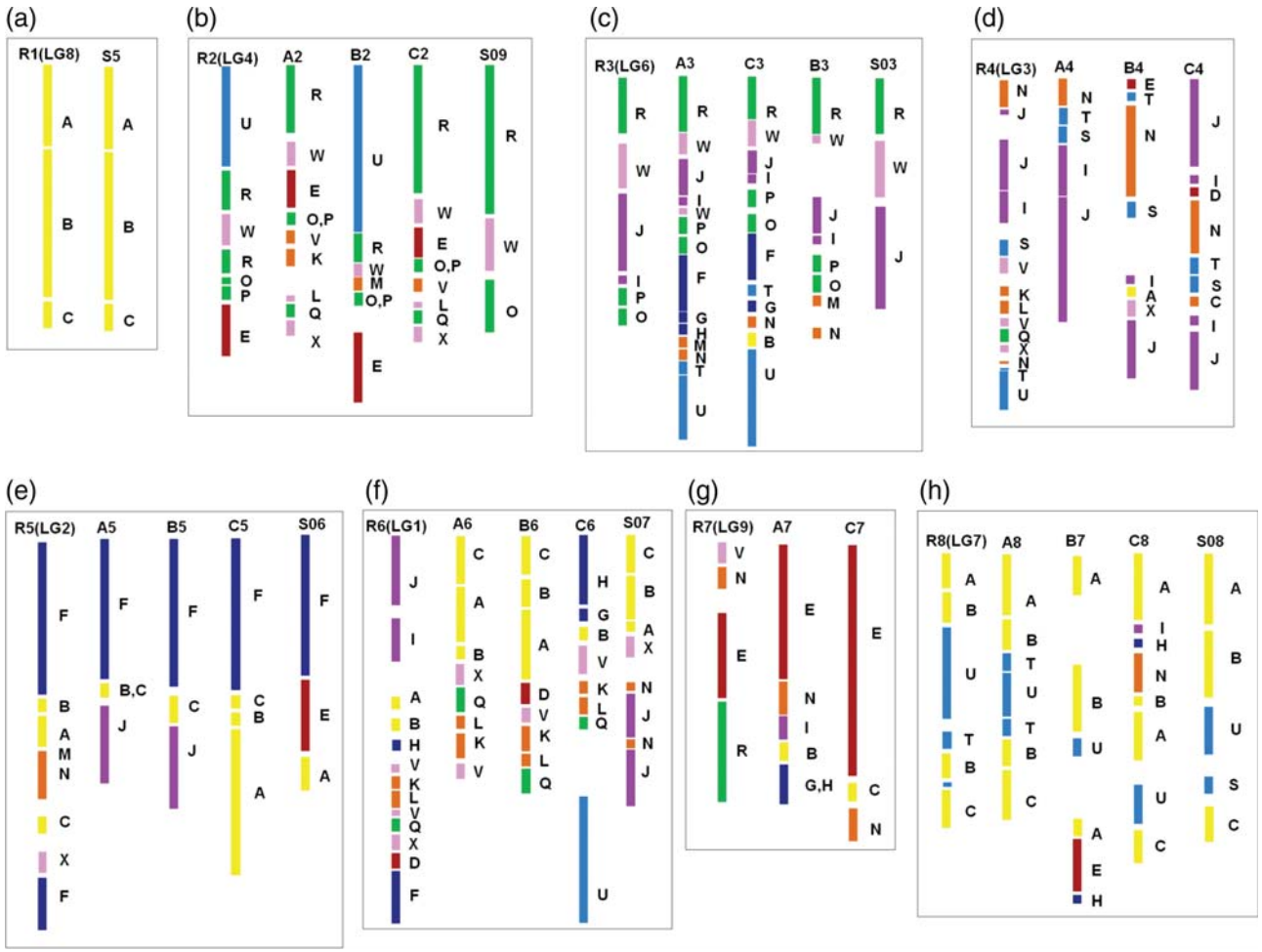

Figure 3. Comparative block arrangements in the genomes of *R. sativus*, *S. alba,* and the A, B, and C genomes of *Brassica* species. Eight homoeologous chromosome sets [from (a) to (h)] were grouped with the extent of homoeology between them. LG5 of *R. sativus* is not shown, because a corresponding homoeologous chromosome in other species was not identified. The block arrangements in the A, B, and C genomes of *Brassica* species are based on Panjabi *et al.*,[24] except that A3 and A8 are based on Mun *et al.*[24] and Trick *et al.*,[48] respectively. The block arrangements in *S. alba* are based on Nelson *et al.*[25]

the A, B, and C genomes of *Brassica* species and that of *S. alba* have been reconstructed.[5,16,24,25] The chromosome organization of *R. satiuvs* was compared with those of the four genomes using these genomic blocks. Eight groups were formed with the extent of homoeology between them, as shown in Fig. 3. Except for LG5 of *R. sativus*, in which only short regions such as W/E and O/P were shared by other chromosomes, all the chromosomes in *R. sativus* were inferred to have corresponding homoeologous chromosomes in other species. Large homoeologous regions could be observed in each set of homoeologous chromosomes. According to the homoeology shared between them, we assign a new nomenclature for the *R. satiuvs* LGs, i.e. R1, R2, R3, R4, R5, R6, R7, R8, and R9 to LG8, LG4, LG6, LG3, LG2, LG1, LG9, LG7, and LG5, respectively. Only R1 (LG8) and S05 are homoeologous to AK1, which was rearranged in the *Brassica* A, B, and C genomes (Fig. 3a). The structure of R2 (LG4) is nearly the same as that of B2, while the U block is not shared by A2, C2, and S09 (Fig. 3b). Since the *Rapa/Oleracea* lineage including *R. sativs* and the *Nigra* lineage are considered to have diverged ~7.9 Mya,[14] U/R/W/O/P/E shared by both *B. nigra* and *R. sativus* might be of a more ancestral Brassiceae chromosome. The genomic blocks in R3 (LG6) could be found in all the other homoeologous chromosomes except that I, P, and O blocks were not detected in S03 of *S. alba* (Fig. 3c). Block association N/S/T/I/J was conserved in all genomes except for *S. alba* (Fig. 3d), although rearrangements may have occurred in *R. sativus*, *B. nigra*, and *B. oleracea*. Block association F/C/J was shared by A5 and B5 (Fig. 3e), while F/C/B/A was conserved in R5 (LG2) and C5. Since the A, B, and C genomic blocks originally neighboured, formation of F/C/J needs more arrangement than that of A/B/C/F, indicating A/B/C/F to be more ancestral than F/C/J. Block association A/B/C was conserved in A6, B6, and S07 (Fig. 3f), and block association V/K/L/Q/X was shared by all

homoeologous chromosomes except for S07. N/E block association in R7 (LG9) was found to be shared by A7 and C7 (Fig. 3g), while it was not detected in *B. nigra* and *S. alba*, revealing that it was formed after the split time between the *Rapa/Oleracea* lineage and the *Nigra* lineage. The A, B, C, T, and U genomic blocks were present in both R8 (LG7) and A8 (Fig. 3h), and A, B, U, and C blocks were also detected in C8 and S08, indicating a high syntenic relationship between them.

Since the A, B, and C genomes of *Brassica* species are of major economic and calorific importance, contributing to a lot of world-wide edible vegetables and oil crops, great progress has been achieved in their genetics and genomics studies (www.brassica.info), especially in the A genome (http://www.brassica-rapa.org/BRGP/index.jsp). The chromosome A3 in *B. rapa* has been sequenced using the traditional Sanger technology.[46] Recently, next-generation sequencing technology has also been used to determine the genome sequence of *B. rapa*, and 183 scaffolds have been anchored onto the chromosomes, the total coverage of the genome being 88.9%.[47] The homoeology shared between *R. sativus* and the A, B, and C genomes of *Brassica* species indicates that genomic study of *R. sativus* would benefit from the rich genomic information available in *Brassica* species and inversely may also promote the study of *Brassica* species.

4. Conclusion

High frequency of SNPs and a high-efficiency SNP genotyping method developed in the present study facilitated the construction of a high-density *R. sativus* linkage map with EST-based SNP markers. Establishment of syntenic relationship between *R. sativus* and *A. thaliana* based on these EST markers would be greatly beneficial for identification and positional cloning of genes involved in important agronomic traits in *R. sativus*. Identification of the chromosome homoeology in Brassiceae species based on 24 genomic blocks of the ancestral karyotype would facilitate transfer of available genomic information between the homoeologous chromosomes to speeding up the exploration of genetic resources in Brassiceae species. Note: After this manuscript was submitted, a paper by Shirasawa *et al*. (An EST-SSR linkage map of *Raphanus sativus* and comparative genomics of the Brassicaceae) was published. LG1 (R6), LG2 (R5), LG3 (R4), LG4 (R2), LG5 (R9), LG6 (R3), LG7 (R8), LG8 (R1), and LG9 (R7) in the present study are identified to correspond to LG2, LG3, LG4, LG5, LG6, LG8, LG7, LG9, and LG1 of Shirasawa *et al*., respectively.

Acknowledgements: We are grateful to Mr Uno for management of plants and Dr Ashutosh for revising a manuscript.

Supplementary data: Supplementary data are available at www.dnaresearch.oxfordjournals.org.

Funding

This work was supported by the Program for Promotion of Basic and Applied Researches for Innovations in Bio-oriented Industry (BRAIN), Japan.

References

1. Gale, M.D. and Devos, K.M. 1998, Plant comparative genetics after 10 years, *Science*, **282**, 656–9.
2. Tang, H., Bowers, J.E., Wang, X., Ming, R., Alam, M. and Paterson, A.H. 2008, Synteny and collinearity in plant genomes, *Science*, **320**, 486–8.
3. Gale, M.D. and Devos, K.M. 1998, Comparative genetics in the grasses, *Proc. Natl Acad. Sci. USA*, **95**, 1971–4.
4. Mandáková, T. and Lysak, M.A. 2008, Chromosomal phylogeny and karyotype evolution in x = 7 crucifer species (Brassicaceae), *Plant Cell*, **20**, 2559–70.
5. Schranz, M., Lysak, M. and Mitchell-Olds, T. 2006, The ABC's of comparative genomics in the Brassicaceae: building blocks of crucifer genomes, Trends Plant Sci., **11**, 535–42.
6. Lagercrantz, U. and Lydiate, D.J. 1996, Comparative genome mapping in *Brassica*, *Genetics*, **144**, 1903–10.
7. Li, F., Kitashiba, H., Inaba, K. and Nishio, T. 2009, A *Brassica rapa* linkage map of EST-based SNP markers for identification of candidate genes controlling flowering time and leaf morphological traits, DNA Res., **16**, 311–23.
8. Moore, G., Devos, K.M., Wang, Z. and Gale, M.D. 1995, Cereal genome evolution. Grasses, line up and form a circle, Curr. Biol., **5**, 737–9.
9. Al-Shehbaz, I.A., Beilstein, M.A. and Kellogg, E.A. 2006, Systematics and phylogeny of the Brassicaceae (Cruciferae): an overview, Plant Syst. Evol., **259**, 89–120.
10. Warwick, S.I. and Al-Shehbaz, I.A. 2006, Brassicaceae: chromosome number index and database on CD-Rom, Plant Syst. Evol., **259**, 237–48.
11. Lagercrantz, U. 1998, Comparative mapping between *Arabidopsis thaliana* and *Brassica nigra* indicates that Brassica genomes have evolved through extensive genome replication accompanied by chromosome fusions and frequent rearrangements, *Genetics*, **150**, 1217–28.
12. Yang, T.J., Kim, J.S., Kwon, S.J., et al. 2006, Sequence-level analysis of the diploidization process in the triplicated *FLOWERING LOCUS C* region of *Brassica rapa*, *Plant Cell*, **18**, 1339–47.
13. Town, C.D., Cheung, F., Maiti, R., et al. 2006, Comparative genomics of *Brassica oleracea* and

Arabidopsis thaliana reveal gene loss, fragmentation, and dispersal after polyploidy, *Plant Cell*, **18**, 1348–59.
14. Lysak, M.A., Koch, M.A., Pecinka, A. and Schubert, I. 2005, Chromosome triplication found across the tribe Brassiceae, Genome Res., **15**, 516–25.
15. Lysak, M.A., Cheung, K., Kitschke, M. and Bures, P. 2007, Ancestral chromosomal blocks are triplicated in Brassiceae species with varying chromosome number and genome size, Plant Physiol., **145**, 402–10.
16. Mun, J.H., Kwon, S.J., Yang, T.J., et al. 2009, Genome-wide comparative analysis of the *Brassica rapa* gene space reveals genome shrinkage and differential loss of duplicated genes after whole genome triplication, Genome Biol., **10**, R111.
17. Cho, K., O'Neill, C.M., Kwon, S.J., et al. 2010, Sequence-level comparative analysis of the *Brassica napus* genome around two stearoyl-ACP desaturase loci, Plant J., **61**, 591–9.
18. Lysak, M.A. and Lexer, C. 2006, Towards the era of comparative evolutionary genomics in Brassicaceae, *Plant Syst. Evol.*, **259**, 175–98.
19. Parkin, I.A., Gulden, S.M., Sharpe, A.G., et al. 2005, Segmental structure of the *Brassica napus* genome based on comparative analysis with *Arabidopsis thaliana*, *Genetics*, **171**, 765–81.
20. Yogeeswaran, K., Frary, A., York, T.L., et al. 2005, Comparative genome analyses of *Arabidopsis* spp.: inferring chromosomal rearrangement events in the evolutionary history of *A. thaliana*, Genome Res., **15**, 505–15.
21. Kuittinen, H., de Haan, A.A., Vogl, C., et al. 2004, Comparing the linkage maps of the close relatives *Arabidopsis lyrata* and *A. thaliana*, *Genetics*, **168**, 1575–84.
22. Koch, M.A. and Kiefer, M. 2005, Genome evolution among cruciferous plants: a lecture from the comparison of the genetic maps of three diploid species—*Capsella rubella*, *Arabidopsis lyrata* subsp. *petraea*, and A. *thaliana*, Am. J. Bot., **92**, 761–7.
23. Lysak, M.A., Berr, A., Pecinka, A., Schmidt, R., McBreen, K. and Schubert, I. 2006, Mechanisms of chromosome number reduction in *Arabidopsis thaliana* and related Brassicaceae species, *Proc. Natl Acad. Sci. USA*, **103**, 5224–9.
24. Panjabi, P., Jagannath, A., Bisht, N.C., et al. 2008, Comparative mapping of *Brassica juncea* and *Arabidopsis thaliana* using Intron Polymorphism (IP) markers: homoeologous relationships, diversification and evolution of the A, B and C *Brassica* genomes, *BMC Genomics*, **9**, 113.
25. Nelson, M.N., Parkin, I.A. and Lydiate, D.J. 2011, The mosaic of ancestral karyotype blocks in the *Sinapis alba* L. genome, *Genome*, **54**, 33–41.
26. Bett, K.E. and Lydiate, D.J. 2003, Genetic analysis and genome mapping in *Raphanus*, *Genome*, **46**, 423–30.
27. Kamei, A., Tsuro, M., Kubo, N., et al. 2010, QTL mapping of clubroot resistance in radish (*Raphanus sativus L.*), Theor. Appl. Genet., **120**, 1021–7.
28. Tsuro, M., Suwabe, K., Kubo, N., Matsumoto, S. and Hirai, M. 2005, Construction of a molecular linkage map of radish (*Raphanus sativus L.*), based on AFLP and *Brassica*-SSR markers, Breed. Sci., **55**, 107–11.
29. Budahn, H., Peterka, H., Mousa, M.A., Ding, Y., Zhang, S. and Li, J. 2009, Molecular mapping in oil radish (*Raphanus sativus L.*) and QTL analysis of resistance against beet cyst nematode (*Heterodera schachtii*), Theor. Appl. Genet., **118**, 775–82.
30. Tsuro, M., Suwabe, K., Kubo, N., Matsumoto, S. and Hirai, M. 2008, Mapping of QTLs controlling root shape and red pigmentation in radish, *Raphanus sativus L.*, Breed. Sci., **58**, 55–61.
31. Kaplinski, L., Andreson, R., Puurand, T. and Remm, M. 2005, MultiPLX: automatic grouping and evaluation of PCR primers, *Bioinformatics*, **21**, 1701–2.
32. Shiokai, S., Kitashiba, H. and Nishio, T. 2010, Prediction of the optimum hybridization conditions of dot-blot-SNP analysis using estimated melting temperature of oligonucleotide probes, Plant Cell Rep., **29**, 829–34.
33. Murray, M.G. and Thompson, W.F. 1980, Rapid isolation of high molecular weight plant DNA, Nucleic Acids Res., **8**, 4321–5.
34. Fujii, H., Ogata, T., Sugiyama, A., et al. 2010, Development of software to improve the design efficiency of theintron-spanning PCR primer from EST utilizing genomic information of *Arabidopsis thaliana*. In: Abstracts of the International Conference 'Plant & Animal Genomes XVIII Conference', Sherago International, Inc., San Diego, CA, USA.
35. Schranz, M.E., Quijada, P., Sung, S.B., Lukens, L., Amasino, R. and Osborn, T. C. 2002, Characterization and effects of the replicated flowering time gene *FLC* in *Brassica rapa*, *Genetics*, **162**, 1457–68.
36. Sanger, F., Donelson, J.E., Coulson, A.R., Kossel, H. and Fischer, D. 1973, Use of DNA polymerase I primed by a synthetic oligonucleotide to determine a nucleotide sequence in phage fl DNA, *Proc. Natl Acad. Sci. USA*, **70**, 1209–13.
37. Shiokai, S., Shirasawa, K., Sato, Y. and Nishio, T. 2010, Improvement of the dot-blot-SNP technique for efficient and cost-effective genotyping, Mol. Breed., **25**, 179–85.
38. Doyle, J.J. and Doyle, J.L. 1990, Isolation of plant DNA from fresh tissue, *Focus*, **12**, 13–15.
39. Markham, N.R. and Zuker, M. 2005, DINAMelt web server for nucleic acid melting prediction, Nucleic Acids Res., **33**, W577–81.
40. Van Ooijen, J.W. 2006, *JoinMap 4.0, Software for the Calculation of Genetic Linkage Maps in Experimental Populations*. Kyazma, B.V.: Wageningen, The Netherlands.
41. Kosambi, D.D. 1994, The estimation of map distance from recombination values, Ann. Eugen., **12**, 172–75.
42. Voorrips, R.E. 2002, MapChart: software for the graphical presentation of linkage maps and QTLs, J. Hered., **93**, 77–8.
43. Marie, D. and Brown, S.C. 1993, A cytometric exercise in plant DNA histograms, with 2C values for 70 species, *Biol. Cell*, **78**, 41–51.
44. Li, F., Kitashiba, H. and Nishio, T. 2010, Association of sequence variation in *Brassica GLABRA1* orthologs with

leaf hairiness, Mol. Breed., doi:10.1007/s11032-010-9508-z (Online).
45. Schranz, M.E., Windsor, A.J., Song, B.H., Lawton-Rauh, A. and Mitchell-Olds, T. 2007, Comparative genetic mapping in *Boechera stricta*, a close relative of Arabidopsis, *Plant Physiol*, **144**, 286–98.
46. Mun, J.H., Kwon, S.J., Seol, Y.J., et al. 2010, Sequence and structure of *Brassica rapa* chromosome A3, Genome Biol., **11**, R94.
47. Wang, Y., Sun, S., Liu, B., et al. 2011, A sequence-based genetic linkage map as a reference for *Brassica rapa* pseudochromosome assembly, *BMC Genomics*, doi:10.1186/1471-2164-12-239.
48. Trick, M., Kwon, S.J., Choi, S.R., et al. 2009, Complexity of genome evolution by segmental rearrangement in *Brassica rapa* revealed by sequence-level analysis, *BMC Genomics*, **10**, 539.