Assessing the level of collinearity between *Arabidopsis thaliana* and *Brassica napus* for *A. thaliana* chromosome 5

I.A.P. Parkin, D.J. Lydiate, and M. Trick

Abstract: This study describes a comprehensive comparison of chromosome 5 of the model crucifer *Arabidopsis* with the genome of its amphidiploid crop relative *Brassica napus* and introduces the use of in silico sequence homology to identify conserved loci between the two species. A region of chromosome 5, spanning 8 Mb, was found in six highly conserved copies in the *B. napus* genome. A single inversion appeared to be the predominant rearrangement that had separated the two lineages leading to the formation of *Arabidopsis* chromosome 5 and its homologues in *B. napus*. The observed results could be explained by the fusion of three ancestral genomes with strong similarities to modern-day *Arabidopsis* to generate the constituent diploid genomes of *B. napus*. This supports the hypothesis that the diploid *Brassica* genomes evolved from a common hexaploid ancestor. Alignment of the genetic linkage map of *B. napus* with the genomic sequence of *Arabidopsis* indicated that for specific regions a genetic distance of 1 cM in *B. napus* was equivalent to 285 Kb of *Arabidopsis* DNA sequence. This analysis strongly supports the application of *Arabidopsis* as a tool in marker development, map-based gene cloning, and candidate gene identification for the larger genomes of *Brassica* crop species.

Key Words: comparative mapping, *Brassica* species, model crucifer, genome evolution, genome duplication.

Résumé : Une comparaison détaillée entre le chromosome 5 d'*Arabidopsis*, une crucifère-modèle, et le génome de l'amphiploïde cultivé apparenté, *Brassica napus*, a été réalisée en faisant appel à la recherche d'homologie in silico pour identifier les locus conservés entre les deux espèces. Une région du chromosome 5 mesurant 8 Mb était présente en six copies hautement conservées au sein du génome du *B. napus*. Une seule inversion constitue apparemment le réarrangement prédominant qui distingue les deux branches évolutives ayant mené au chromosome 5 d'*Arabidopsis* et à son homologue chez le *B. napus*. Les résultats observés pourraient s'expliquer par la fusion de trois génomes ancestraux, montrant de grandes similitudes avec l'*Arabidopsis* contemporain, afin de produire les génomes diploïdes qui composent le *B. napus*. Cela supporte l'hypothèse selon laquelle les génomes diploïdes du genre *Brassica* ont évolué à partir d'un génome ancestral commun et hexaploïde. L'alignement de la carte génétique du *B. napus* avec la séquence génomique d'*Arabidopsis* montre que, pour des régions spécifiques, une distance génétique de 1 cM chez le *B. napus* équivaut à un intervalle de 285 kb au sein de la séquence d'*Arabidopsis*. Cette analyse confirme l'intérêt d'exploiter *Arabidopsis* comme outil dans le développement de marqueurs, dans la cartographie positionnelle et l'identification de gènes candidats au sein des génomes plus grands chez les espèces cultivées du genre *Brassica*.

Mots clés : cartographie comparée, espèces du genre *Brassica*, crucifère-modèle, évolution des génomes, duplication des génomes.

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Introduction

The application of molecular markers, particularly restriction fragment length polymorphisms (RFLPs), in genetic mapping has been a powerful tool in the study of plant genome organization (Schmidt 2000). There are now a number of dense genetic linkage maps published for a wide variety of plant species. The value of these maps has been enhanced by the use of common marker sets that, through the identification of homologous fragments of DNA, allow cross-map comparisons. Such comparative mapping analysis began with the Solanaceae, where the genetic linkage maps of tomato, potato, and pepper revealed virtually identical gene content and large regions with conserved gene order across the species (Tanksley et al. 1992; Prince et al. 1993). The extent of the genome collinearity varied across the species with only five inversions separating tomato and potato, but 22 chromosomal rearrangements differentiating the genomes of tomato and pepper. In the Poaceae, extensive mapping in 11 different grass species revealed that by dividing the genome of rice, the simplest genome studied, into 19

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linkage blocks, these blocks can be rearranged to explain the chromosomal linkage karyotypes observed for each of the 11 species (Moore et al. 1995).

Comparative mapping between the genomes of model species, such as rice and related crop species, allows the transfer of information and resources from the more widely studied models to research efforts in the more challenging genomes of their crop relatives. Model species are characterized by having smaller, simpler genomes that latterly have become the focus of international research programs to deduce their entire DNA nucleotide sequences. For dicots, the most widely accepted model species is *Arabidopsis thaliana*, for which the whole genome sequence has recently been published (*Arabidopsis* Genome Initiative 2000). With caution, it should be possible to identify and characterize candidate genes in *Arabidopsis* that control important agronomic traits in related crop species.

Arabidopsis is a member of the family Cruciferae and is one of a diverse range of species that includes the widely grown crop species *Brassica napus* (canola or oilseed rape).

B. napus is an amphidiploid formed as the result of a fusion between the diploids *Brassica rapa* (the A genome donor) and *Brassica oleracea* (the C genome donor) (U. 1935; Parkin et al. 1995). A number of publications have addressed the level of conserved synteny between regions of the *Arabidopsis* genome and different *Brassica* species. Considering only *B. napus*, two published comparisons between it and *Arabidopsis* each examined a region covering approximately 30 cM of the *Arabidopsis* genome and both revealed six homologous regions within the *B. napus* genome with highly conserved gene content and gene order (Scheffler et al. 1997; Cavell et al. 1998). A third comparison between these two species described a smaller region (approximately 20 cM) in *B. napus* from which selected markers mapped to four separate chromosomes of *Arabidopsis*, suggesting numerous discontinuities can exist in the conserved synteny (Osborn et al. 1997).

This paper describes a detailed comparison of the whole of *Arabidopsis* chromosome 5 with the genome of *B. napus* by genetic mapping and correlation of this data with the in silico mapping of *Brassica* markers to the genomic sequence of *Arabidopsis*. The data presented support the suggested evolution of the *Brassica* lineage from a hexaploid ancestor formed from the fusion of three possibly distinct genomes of similar size to present day *Arabidopsis*. This study also highlights some of the difficulties that are faced when attempting to elucidate chromosome evolution in lineages that have involved numerous polyploidization events.

Materials and methods

Populations and DNA probes

A population of 100 recombinant inbred lines, derived from a cross between *Arabidopsis* ecotypes Columbia and Landsberg described in Lister and Dean (1993), was used for genetic linkage analysis in *Arabidopsis*. A population of 50 doubled-haploid lines, derived from a cross between an oilseed rape cultivar ('N-o-9') and a newly resynthesised *B. napus* line (SYN1) described in Parkin et al. (1995), was used for genetic linkage analysis in *B. napus*.

The DNA clones used for Southern hybridization analysis consisted of 25 *Pst*I genomic clones from *Arabidopsis* (prefix mi), described in Liu et al. (1996), and 38 genomic clones from *Brassica* species (prefix pO, pR, pN, or pW), described in Sharpe et al. (1995); three *Arabidopsis* cDNA clones purchased from the *Arabidopsis* Biological Resource Centre (Ohio State University, Columbus, Ohio) (atts2094, atts2506, and atts2524) (http://www.arabidopsis.org); one *B. napus* cDNA clone (cA69), kindly donated by Dr. Andrew Sharpe (Saskatoon Research Centre, Saskatoon, Sask.); and the homologue of the *Arabidopsis* flowering-time gene CONSTANS (CO) from *B. napus* linkage group N10 (Robert et al. 1998).

DNA preparation, Southern analysis, filter design, and sequencing of *Brassica* DNA probes

DNA was prepared and restriction digests and Southern analyses were carried out as described in Sharpe et al. (1995), except that all washes were carried out at low stringency (2× SSC (0.30 M NaCl and 0.03 M trisodium citrate) and 0.1% SDS).

For *Arabidopsis*, screening filters were composed of the two parental lines restricted with 15 different enzymes (*Bcl*I, *Bgl*II, *Bst*UI, *Cfo*I, *Cla*I, *Eco*RI, *Eco*RV, *Hae*III, *Hpa*II, *Hin*dIII, *Hpa*I, *Msp*I, *Sca*I, *Spe*I, and *Xba*I), and mapping filters were composed of the two parental lines and either 30 or 90 of the 100 recombinant inbred lines digested with one of the 15 enzymes. For *B. napus*, screening filters were composed of the two parental lines and a panel of 10 doubled haploid lines cut with five different enzymes (*Bam*HI, *Eco*RI, *Eco*RV, *Hin*dIII, and *Xba*I), and mapping filters were composed of the two parental lines and 30 doubled haploid lines digested with one of the five enzymes.

Sequencing reactions were carried out for 48 *Brassica* DNA probes using primers from each end of the plasmid and the BigDye™ Terminator cycle sequencing kit (Applied Biosystems, Foster City, Calif.) according to the instructions of the manufacturer. They were then electrophoresed on an automated 377 DNA Sequencer (Applied Biosystems). Sequences were analysed for homology to the *Arabidopsis* genomic sequence using the BLAST programs of the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/). Default parameters were used for all homology searches.

Linkage analysis

RFLP loci detected by each of the probes were assigned to the 19 linkage groups of *B. napus* (Parkin et al. 1995) and the five chromosomes of *A. thaliana* (Lister and Dean 1993) using Mapmaker version 3.0, with an LOD score of at least 3.0 (Lander et al. 1987). The nomenclature for the 19 linkage groups of *B. napus* is as described in Parkin et al. (1995); N1–N10 refer to the 10 linkage groups derived from the A-genome progenitor of *B. napus* and N11–N19 refer to the nine linkage groups derived from the C-genome progenitor of *B. napus*.

Sequence homology between the *Brassica* markers and the available *Arabidopsis* genomic sequence was used to validate the map position of the *Brassica* probes in *Arabidopsis* using the combined genetic, physical, and sequence map of *Arabidopsis* (available from http://www.arabidopsis.org).

Table 1. Comparative map position in *Arabidopis* and *B. napus* for the *Arabidopsis* markers from chromosome 5.

Arabidopsis markers from C5[a]	No. of loci in *Arabidopsis*[b]	Map position in *Arabidopsis* (cM)[c]	No. of loci in *B. napus*[b]	Map position in *B. napus*[d]
mi121	2s	C5 (3.7)	2s	N3
mi97	1	C5 (14.3)	6–8	N2, N10, N13 (s), N19
atts2506	NA	C5 (16)	repetitive	N2, N5, N10, N11, N13, N14, N19
mi174	1s, 1f	C5 (21.1)	6	N2, N3, N10, N12, N13, N19
atts2524	NA	C5 (26.4)	4	N2 (s), N10, N12 (s), N19
mi322	1s, 2–3f	C5 (31.8)	2s	N10, N19
mi438	1s, 1f	C5 (31.8)	2s, 1f	N10 (s), N13 (f)
mi90	1	C5 (42.4)	2s	N10 (s), N12
mi138	2	C5 (42.4)	≥8	N1, N2, N10 (s), N11, N13, N19 (s)
mi433	1	C5 (42.4)	2s	Mono
mi219	1	C5 (53.8)	2s, 1–2f	N2 (f)
mi125	1–2	C5 (53.8)	6–8	N2 (s), N6, N12 (s), N17
mi291	2s	b: C5 (64.7) a: C1	4	N8, N10, N18
mi137	1s, 1f	C5 (66.4)	4	N5, N11
mi323	2s, 1f	as: C5 (77.6) bf: C3	6–8	N3, N4, N13, N14, N19
mi194	1	C5 (93.8)	2	N6, N17
mi83	1s, 1f	C5 (99.4)	4	N9, N19
mi423[e]	3–4s (L), 1f (C)	b: C5 (100.2) a: C1	repetitive	N1, N5, N8, N17, N19
mi61	1s, 1f	C5 (101)	4	N6, N9, N19
atts2094	1	C5 (102.7)	6	N1, N2, N6, N9, N17 (s), N19
mi271	1s, 1f	C5 (104.4)	4–6	N2, N6 (s), N17
mi2	2s, 2f	a: C5 (106.1) b: C2	4–6	N4, N7, N14
mi69	1s, 1–2f	C5 (123.2)	4	N3, N10, N13
mi70	1	C5 (123.2)	4	N10, N12, N19
mi74	1s, 1f	af: C5 (123.2) bs: C3	4	N1, N5
mi184	1s, 2f	C5 (123.2)	4–6	N2, N10, N12, N13, N19
mi418	1s, 2f	C5 (123.2)	2s, 2f	N2, N12
mi335	1s, 1–2f	C5 (142.9)	3–4s, 3–4f	N9 (s), N17, N19 (s)

Note: C5, chromosome 5.

[a]*Arabidopsis* markers from chromosome 5 listed according to their genetic position on the map as described in Liu et al. (1996).

[b]The number of loci identified in both *Arabidopsis* and *B. napus* is estimated based on RFLP patterns from the screening filters described in Materials and methods. The strength of the hybridizing bands is indicated: s, strongly hybridizing; f, faintly hybridizing.

[c]The genetic distance on *Arabidopsis* chromosome 5 is shown in parentheses. When a duplicate locus was identified, the suffix on the locus name is indicated along with its map location (a or b) and the strength of hybridization (s, strong; f, faint).

[d]The linkage groups to which loci were mapped in *B. napus* are shown and, where possible to distinguish, the most strongly hybridizing loci are indicated.

[e]Upon hybridisation to *Arabidopsis*, mi423 gave an unusual pattern. Differential hybridisation to the two parental ecotypes was observed for 'Columbia' (C) and 'Landsberg' (L), as shown.

Results

Comparative copy number of the RFLP probes in the genomes of the two species

The genomic copy number for 28 *Arabidopsis* markers, 25 of which had been previously mapped to *A. thaliana* chromosome 5 (Liu et al. 1996), was estimated in both *A. thaliana* and *B. napus* using polymorphism screening filters (Table 1). The 28 probes identified 91 polymorphic loci (average of 3.4 loci per probe) that could be positioned on 16 of the 19 linkage groups of the genetic linkage map of *B. napus* (Table 1). *Brassica* markers were selected from these 16 linkage groups and their copy number estimated in *Arabidopsis* and *B. napus* using the same polymorphism screening filters (Table 2). The 40 *Brassica* probes identified 60 polymorphic loci (average of 1.5 loci per probe) that could be positioned on the genetic linkage map of *Arabidopsis*. Tables 1 and 2 present estimates for the total number of loci detected by each probe, including both monomorphic and polymorphic loci. The multiple loci detected by each probe in *B. napus* were expected based on both the amphidiploid origin of *B. napus* and the extensive intra-chromosomal duplication previously observed within the A and C genomes (Parkin et al. 1995). However, based on previous mapping studies (Liu et al. 1996), the level of duplication in *Arabidopsis* was higher than expected, with

only 7 of the 28 *Arabidopsis* markers and only 6 of the 40 *Brassica* markers being obviously single copy in the *Arabidopsis* genome. Four *Brassica* probes detected duplicate loci and two of the homologues mapped to *Arabidopsis* chromosome 5. None of these four intrachromosomal duplications were observed in the homologous regions of the *B. napus* genome. More recent sequence analysis has revealed a high level of redundancy within the *Arabidopsis* genome, with only 35% of the predicted proteins estimated as unique (*Arabidopsis* Genome Initiative 2000).

In silico positioning of *Brassica* markers on the *Arabidopsis* genomic sequence

48 *Brassica* clones, 40 of which were used as RFLP probes in this study, were sequenced from each end and the resultant sequence was then aligned with the *Arabidopsis* genomic sequence (Table 2). Sequence analysis (apart from the second hit of pN91) was based upon BLASTn results, which measures identity at the nucleotide level. In two instances, this allowed markers that were monomorphic in *Arabidopsis* (based on the 16 enzymes tested) to be mapped to chromosome 5 (i.e., pN105 and pO112). The observed differences in hybridization intensity for genetic loci mapped in *Arabidopsis* could be directly correlated with the level of sequence identity (the expect value (*E*)) found between those probes and the *Arabidopsis* genomic sequence (Table 2). For example, pW122 identified three genetic loci that mapped to chromosomes 1, 3, and 5; the most strongly hybridizing locus was mapped to chromosome 1. The corresponding sequence analysis identified homology to chromosome 1 BAC clone T15M6 with an *E* value of 1×10^{-26} and homology to chromosome 5 BAC clone MLF18 with an *E* value of 1×10^{-5} (Table 2). Based on a comparison between the genetic map position and the physical position on the *Arabidopsis* genome for the *Brassica* markers, an *E* value of $\leq 10^{-5}$ can be considered significant.

Conservation of a 30-cM (8-Mb) region from *Arabidopsis* in six copies in the *B. napus* genome

Figure 1 (1*a*–1*c*) depicts the collinear relationships between *Arabidopsis* chromosome 5 and six regions of the *B. napus* genome, arranged in three pairs of homoeologous A- and C-genome linkage groups. The top of *Arabidopsis* chromosome 5, from pW109 to pW152 (equivalent to approximately 8 Mb), displayed strong conservation of gene content and gene order in all six regions of the *B. napus* genome. The few disruptions in synteny demonstrate the importance of identifying duplicate loci in the *Arabidopsis* genome. For example, pN180 and pW240 each detect two loci on *Arabidopsis* chromosome 5, one that disrupts the collinear region and one that does not.

This region of chromosome 5 represents approximately 40 cM of the genetic map of *Arabidopsis* and covers approximately 8 Mb of the *Arabidopsis* genomic sequence. The region is equivalent to genetic map distances in *B. napus* of 30 and 33 cM on N2 and N12, 32 and 25 cM on N10 and N19, and 31 and 21 cM on N3 and N13, respectively. This means that for this region every 1 cM of genetic map distance in *B. napus* is equivalent to between 285 and 380 kb (average 285 kb) of *Arabidopsis* genomic sequence.

Inversion of the lower half of *Arabidopsis* chromosome 5 relative to six homologous regions of the *B. napus* genome

The mapping data presented in Fig. 1 (1*a*–1*c*) indicate that the lower half of *Arabidopsis* chromosome 5 was inverted with respect to each of the homologous regions detected in the *B. napus* genome.

A comparison of *Arabidopsis* chromosome 5 with the *B. napus* linkage groups N2 and N12 (Fig. 1*a*) revealed a chromosomal rearrangement that had inverted the lower half of chromosome 5 with respect to N2 and N12. The loci on N2 and N12 detected by markers pO3, pR4, pN181, and pR72 at the centre of the inverted region showed no homology to *Arabidopsis* chromosome 5. However, the adjacent loci detected by markers pW161–mi271, appeared to be in the correct order with respect to chromosome 5, suggesting that these segments were inserted subsequent to the inversion event.

What appeared to be the same inversion event was evident from the comparison of chromosome 5 with N10/N9 and N19 (Fig. 1*b*). There is a breakpoint in the homoeology between the A (N10) and C (N19) genomes of *B. napus* that occurs below locus *mi70* on each group (Fig. 1*b*). Extending down from this breakpoint, linkage group N10 shows homology to the top of *Arabidopsis* chromosome 1 and conversely, a number of markers (pW233, pW180, pN173, pW203, and pW122; mapping below the breakpoint on N19 and on the homologous region of N9) map to the bottom of chromosome 1. The centre of *Arabidopsis* chromosome 5 was found to be part of an inter-chromosomal duplication within *Arabidopsis*, with markers pW167, mi291, pW161, pW122, and pW180 detecting strongly hybridizing loci on the bottom of *Arabidopsis* chromosome 1 (Fig. 1*a*, inset). Because some of the markers found within this inter-chromosomal duplication detect loci on N2–N12 and N9–N19, it seems likely that the ancestral chromosome from which these two homoeologous pairs of chromosomes evolved carried a copy of the duplicated region.

Comparison between *Arabidopsis* chromosome 5 and N3 and N13 of *B. napus* (Fig. 1*c*) revealed that the markers from mi184 to pW102 mapped to the bottom of *Arabidopsis* chromosome 5. In contrast to the previously identified segments, it appeared that the inversion event was followed by additional rearrangements that removed further homology between N3–N13 and chromosome 5. The central regions of N3 and N13 have previously been shown to have homology to *Arabidopsis* chromosome 3 (Scheffler et al. 1997). However, two markers from chromosome 5, mi138 and mi438, detected loci on N13. mi138 was highly duplicated in *B. napus* (Table 1) and might belong to a gene family that would not necessarily follow the underlying pattern of conserved synteny, whereas the locus detected by mi438 on N13 was very faint and might not represent a true orthologue or paralogue of the chromosome 5 copy of mi438.

Discontinuities in the collinearity between *Arabidopsis* chromosome 5 and the identified homologous regions of *B. napus*

It is apparent that some regions from *Arabidopsis* chromosome 5 do not show a simple relationship with their homolo-

Table 2. Comparative map position in *Arabidopis* and *B. napus* for selected *Brassica* markers.

Brassica marker[a]	No. of loci in *B. napus*[b]	Map position in *B. napus*[c]	No. of loci in *A. thaliana*[b]	Genetic map position in *A. thaliana*[d]	Physical map position in *A. thaliana*[e]
***Brassica* markers mapping to *Arabidopsis* chromosome 5**					
pW109a[f]	repetitive	N2 (s), N12 (s)	4–6	C4c (s), C5a (s), C4d, C5b	C5(F12E4) 10^{-35}, C5(T14C9) 10^{-33}, C4(F9H3) 10^{-33}
pW200	4	N10 (s), N19 (s)	2	C3, C5	1.9 Mb (MBL20) 10^{-46}, C3(F24K9) 10^{-14}
pN3	4	N3, N10, N13, N19	1	C5	1.9 Mb (MBL20) 10^{-44}
pW240a	2	N10, N19	2–3	C5b (s), C5a	C5(MQJ2) 10^{-37}
pO118	2–4	N10, N19	NA	C5[g]	3.5 Mb (F2I11) 10^{-24}
pO7	4	N10, N19 (s)	NA	C5[g]	3.6 Mb (F15N18) 10^{-14}
pN180	6–8	N2, N3, N10, N12, N13, N14, N19	3–5	C5 (×2) (s), C1, C2	3.9 Mb (MXC9) 10^{-17}, C5(T20D1) 10^{-34}, C2(T5E7,T12J2) 10^{-11}
pN102	6–8	N2, N10 (s), N12, N19 (s)	4	C5 (s)	5 Mb (T20K14) 10^{-94}, C3(T4P13) 10^{-54}
CO	6	N10 (s), N12, N19 (s)	2	C5 (s)	5.1 Mb (F14F8) 10^{-101}, C3(F11A12) 10^{-27}
pW189	4–6	N3 (s), N10, N13 (s), N19	2	C5 (s)	5.3 Mb (MQK4) 10^{-104}
pO160	4	N3, N10, N13, N19 (s)	2–3	C5 (s)	5.4 Mb (MTG13) 10^{-30}
pN105	6–8	N3 (s), N10, N13 (s), N19	2–3	Mono	5.7 Mb (T10B6) 10^{-71}
pN91	4–6	N10 (s), N14, N19 (s)	3–4	C5 (s), C4	5.7 Mb (T10B6) 10^{-107} [i],C4(F20O9) 10^{-20}
pW212	2	N19	1	C5	6.4 Mb (T24G5) 10^{-12}
pN180	6–8	N2, N3, N10, N12, N13, N14, N19	3–5	C5 (×2) (s), C1, C2	6.6 Mb (T20D1) 10^{-34}, C5(MXC9) 10^{-17}, C2(T5E7,T12J2) 10^{-11}
pW152	≥6	N3, N10, N13	2–3	C5 (s)	7.3 Mb (T6G12) 10^{-72}, C2 (T20B5) 10^{-3}
cA69	8	N2, N9, N17, N19	2	C5 (s)	8.1 Mb (MQM1) 10^{-22}
pN64	4–5	N14	2–3	C5 (s)	9.6 Mb (F2P16) 10^{-80}
pW167	2–4	N6 (s), N17 (s)	NA	NA	9.9 Mb (F15A18) 10^{-39}, C1(F15C21) 10^{-8}
pO120	4	N2 (s), N6, N12 (s), N17	2–3	C5 (s)	10 Mb (T1G16) 10^{-33}
pN101	6	N6 (s), N15, N17 (s), N19	2	C5 (s)	10.1 Mb (F14I23) 10^{-59}
pW161	3–4	N2 (s), N12 (s)	2	C1 (s), C5	NA
pW122	3–4	N4, N9 (s), N14, N19 (s)	3	C1 (s), C3, C5	15.4 Mb (MLF18) 10^{-5}, C1(T15M6) 10^{-26}
pW180	4–6	N2 (s), N7, N9, N12 (s), N19	2	C1 (s), C5	C1(F516) 10^{-37}
pW148	3–4	N3 (s), N12	2	C4 (s), C2, C5	C4(T15B16) 10^{-57}, C2(section 12) 10^{-11}
pW218	4	N6, N12 (s), N14	2	C5 (s), C3	18 Mb (MBD2) 10^{-17}
pW233	2	N9, N19	NA	NA	18.5 Mb (MRH10) 10^{-41}, C1(F20D22) 10^{-25}
pO112	4	N6	2	Mono	19.3 Mb (MRA19) 10^{-9}
pN86	2	N6, N17	1	C5	19.9 Mb (MQD22) 10^{-74}
pR3	6–8	N6 (s), N17 (s)	NA	NA	20 Mb (MNJ7) 10^{-46}, C4(FCA8) 10^{-29}, C3(MTC11) 10^{-18}
pW176	2	N2, N12 (s)	NA	NA	20.8 Mb (K7J8) 10^{-28}, C3(F3E22) 10^{-9}
pO119a	6–8	N2, N6, N12, N13, N19	3	C5b (s), C5a (s), C1 (f)	21.1 Mb (K9P8) 10^{-29}, C5(MQB2) 10^{-37}
pW154	3–4	N2 (s), N12 (s)	NA	C5[g]	21.3 Mb (MXI22) 10^{-63}
pR34	2–4	N19 (s)	NA	NA	21.6 Mb (MWD22) 10^{-43}
pW109b[f]	repetitive	N2 (s), N12 (s)	4–6	C4c (s), C5a (s), C4d, C5b	C5(F12E4) 10^{-34}, C5(T14C9) 10^{-33}, C4(F9H3) 10^{-33}
pR115a	3–4	N2 (s), N10, N12 (s), N19	2–3	C5(s)	24.3 Mb (K21L19) 10^{-80}
pO155	4	N3, N10 (s), N13, N19 (s)	NA	NA	24.35 Mb (MCK7) 10^{-162}
pW240a	2	N10, N19	2–3	C5a (s), C5b	24.4 Mb (MQJ2) 10^{-37}
pO119b	6–8	N2, N6, N12, N13, N19	3	C5b (s), C5a (s), C1 (f)	26 Mb (MQB2) 10^{-37}, C5(K9P8) 10^{-29}
pW102a	8	N2, N3, N6 (s), N10	2	C5 (s)	26.7 Mb (MXK3) 10^{-73}
pW191	4–6	N2, N12, N7	2	C5 (s)	27.7 Mb (K8K14) 10^{-43}
***Brassica* markers not mapping to *Arabidopsis* chromosome 5**					
pN23	6	N9, N10, N18	2	C1 (s)	C1(F10K1) 10^{-28}
pN173	5–6	N1 (s), N9, N11 (s), N14	2	C1	C1(T8L23) 10^{-55}
pN181	4	N2 (s), N9, N12 (s)	2–3	C4 (×2)	C4(T26M18) 10^{-59}, C4(F7H19) 10^{-31}

pN199	5–6	N6 (s), N10	2–3	C3 (f)	C1(F14N23) 10^{-27}, C3(F27H5) 10^{-25}
pN216	≥8	N6, N8, N9 (s), N15, N18, N19 (s)	2	C1 (s)	C1(F7H2) 10^{-53}
pO3	4–6	N2 (s), N7, N12 (s)	NA	NA	C1(F23N20) 10^{-108}, C4(F4I10) 10^{-11}, C3(MLN21) 10^{-7}
pO29	6	N1, N3, N8, N11, N17, N18	NA	C4[h]	C4(ATFCA5) 10^{-32}, C3(F3E22) 10^{-8}
pR4	2	N2, N12	1–2	C4 (s)	NA
pR6	2	N6 (s), N13	1–2	C3 (s)	C3(T21J18) 10^{-48}
pR43	4–5	N6 (s), N17	3–4	C3 (s), C4	C3(MLJ15) 10^{-87}, C4(T24H24) 10^{-20}
pR72	2	N2, N12	2	C3 (s), C1	C3(MPE11) 10^{-16}, C1(F8L10) 10^{-9}
pR116	5–6	N13, N19 (s)	2–3	C4 (s)	C4(F6N15) 10^{-36}, C2(CIC5B11) 10^{-14}
pW130	4	N4, N7 (s), N9, N14, N18	2–3	C3 (s), C4	C3(F5K20) 10^{-26}
pW203	5–6	N1, N9 (s), N19	1	C1	C1(F16M22) 10^{-16}

[a]The *Brassica* markers are listed according to their map position on *Arabidopsis* chromosome 5. Markers are also listed that did not map to *Arabidopsis* chromosome 5, but that were tested because their linkage group of origin was identified by an *Arabidopsis* marker from chromosome 5 (Table 1).
[b]The *Brassica* and *Arabidopsis* screening filters, as described in Materials and methods, were used to estimate the total number of loci that could be detected by each marker.
[c]The map position in *B. napus* (linkage groups N1–N19) of all polymorphic loci is indicated; where mapped and where possible to differentiate, the most strongly hybridizing loci are indicated.
[d]The genetic map position in *Arabidopsis* of each marker is shown and the relative hybridization intensity where known is indicated.
[e]The physical map position of each marker is shown in megabase pairs on *Arabidopsis* chromosome 5 as determined from sequence similarity searches. For each hit on the *Arabidopsis* genome, when not on chromosome 5, the *Arabidopsis* chromosome is indicated followed by the BAC and the *E* value from the BLASTN result.
[f]pW109 was found to have homology to a tRNA, giving numerous hits across the *Arabidopsis* genome.
[g]The genetic map position of these markers in *A. thaliana* was reported in Osborn et al. (1997).
[h]The genetic map position of this marker in *A. thaliana* was reported in Cavell et al. (1998).
[i]The result shown is from a TBLASTX analysis.

gous *Brassica* chromosomes. Breakpoints in synteny may simply correspond to chromosomal rearrangements that have occurred during the divergence of the A and C genomes from a common ancestor, such as that between chromosome 5 and N10 (Fig. 1*b*) as described above.

A number of markers from chromosome 5 were found to identify loci in the homoeologous regions of linkage groups N6 and N17 (Fig. 1*d*). The markers at the bottom of chromosome 5 would need to be inverted to give some semblance of the locus order observed for N6–N17. However, there is a large section from mi291 to pW148 that shares no homology with N6–N17. Because this region is duplicated on *Arabidopsis* chromosome 1 (Fig. 1*a*, inset), it is possible that either the chromosome that gave rise to N6–N17 did not have the duplicated segment or the region was translocated from its original position on N6–N17. The presence on N6–N17 of a locus detected by pW167 mapping to both chromosomes 1 and 5 could be evidence for the latter. A number of rearrangements involving further *Arabidopsis* chromosomes would need to be invoked to derive linkage groups N6 and N17; further comparative mapping with markers from N6–N17 would be necessary to uncover the extent of those rearrangements.

Finally, no homology was detected between chromosome 5 and N2–N12 for markers pO3, pR4, pN181, and pR72, that detected homologous loci on three separate *Arabidopsis* chromosomes, 1, 3, and 4. Additionally, closely linked markers from this region of N2–N12 detected duplicate loci on these same three *Arabidopsis* chromosomes (Fig. 1*a*) where they appeared to map in small conserved blocks. Using a combination of cytological identification of telocentric chromosomes and RFLP mapping the centromere for N12 has been positioned approximately in the region pR4–pW176 (Kelly 1996). In the Gramineae and the Solanaceae, numerous discontinuities in collinearity have been observed to accumulate at centromeric regions (Moore et al. 1997; Livingstone et al. 1999). It is possible that the breakdown in collinearity observed between *Arabidopsis* chromosome 5 and N2–N12 is the result of the forces of karyotypic evolution that specifically affect centromeric regions.

Discussion

Allopolyploidy has played a significant role in the evolution of the *Brassica* crops, because three amphidiploid crop species (*B. napus*, *B. carinata*, and *B. juncea*) were formed from the pairwise fusions of three different diploid *Brassica* species (U 1935). This study focused on amphidiploid *B. napus* (canola or oilseed rape) that evolved from a hybridization event between *B. rapa* (A-genome donor) and *B. oleracea* (C-genome donor) (Parkin et al. 1995). There is a growing body of evidence to suggest that polyploidy has been an important evolutionary process in the generation of the diploid *Brassica* genomes themselves (Lagercrantz and Lydiate 1996; Lan et al. 2000). Similarly, other diploid plant species, such as soybean (Shoemaker et al. 1996) and maize (Gaut et al. 2000), have been shown to have genomic structures indicative of degenerate tetraploids. In *Brassica* species, the published data (Lagercrantz and Lydiate 1996; Scheffler et al. 1997; Cavell et al. 1998) and the data presented in this paper strongly suggest that triplication has

Fig. 1. (*a–d*) Comparative genetic map positions of RFLP probes from *Arabidopsis* chromosome 5 on regions of the *B. napus* genome. Each figure depicts the relationship between chromosome 5 and a different pair of homoeologous regions from the *B. napus* genome. Lines drawn between the linkage groups indicate loci detected by common makers. Intrachromosomal duplications within *Arabidopsis* chromosome 5 are indicated to the left of that linkage group. Where *Brassica* markers identify duplicate loci on alternate *Arabidopsis* chromosomes, the other *Arabidopsis* chromosomes are indicated (in italics) to the right of the locus name. Markers that were positioned on *Arabidopsis* chromosome 5 based solely on sequence similarity are indicated in parentheses. Fig. 1*b*, the curved lines on N10 and N19 indicates the breakpoint in the homology between these two linkage groups.

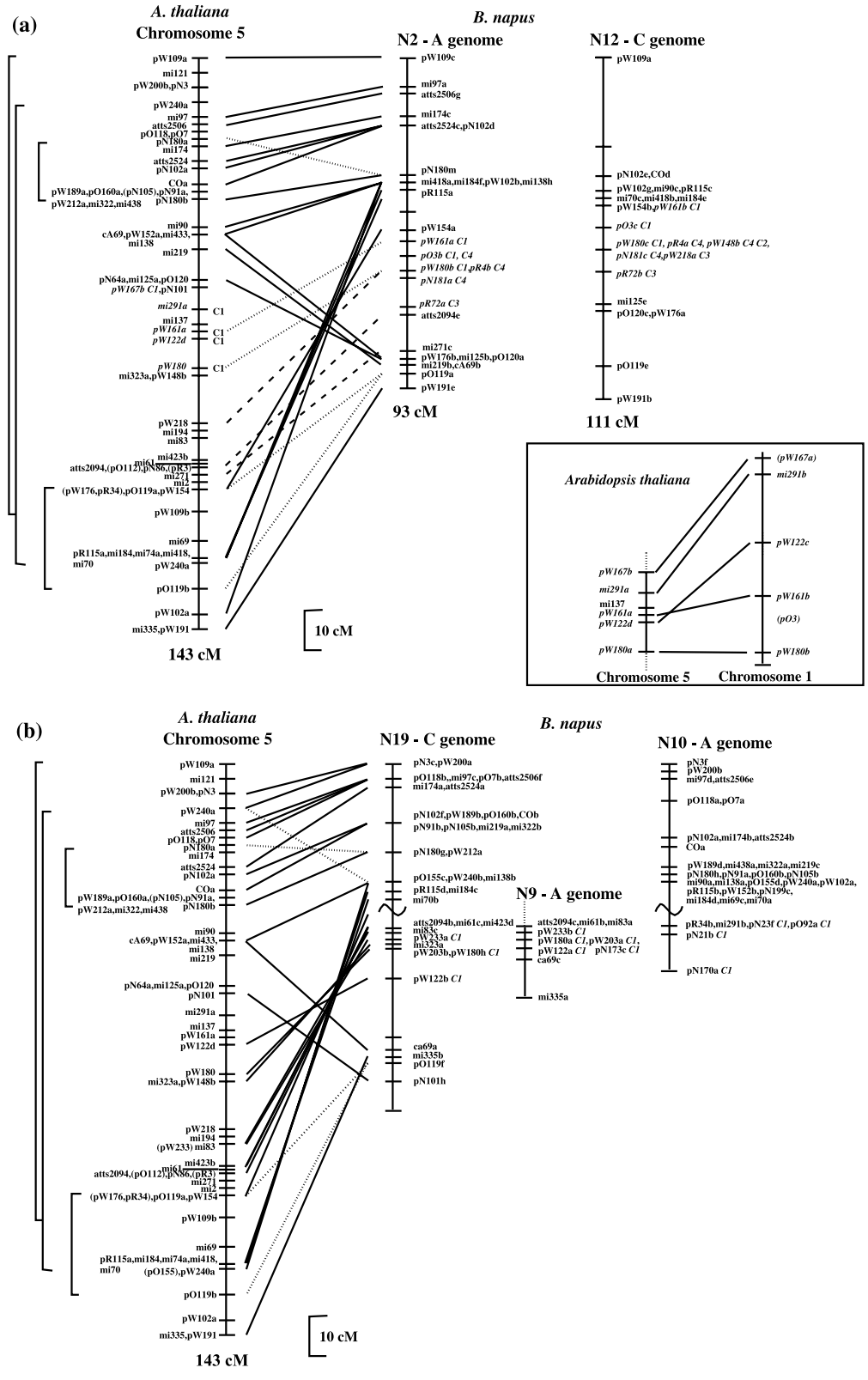
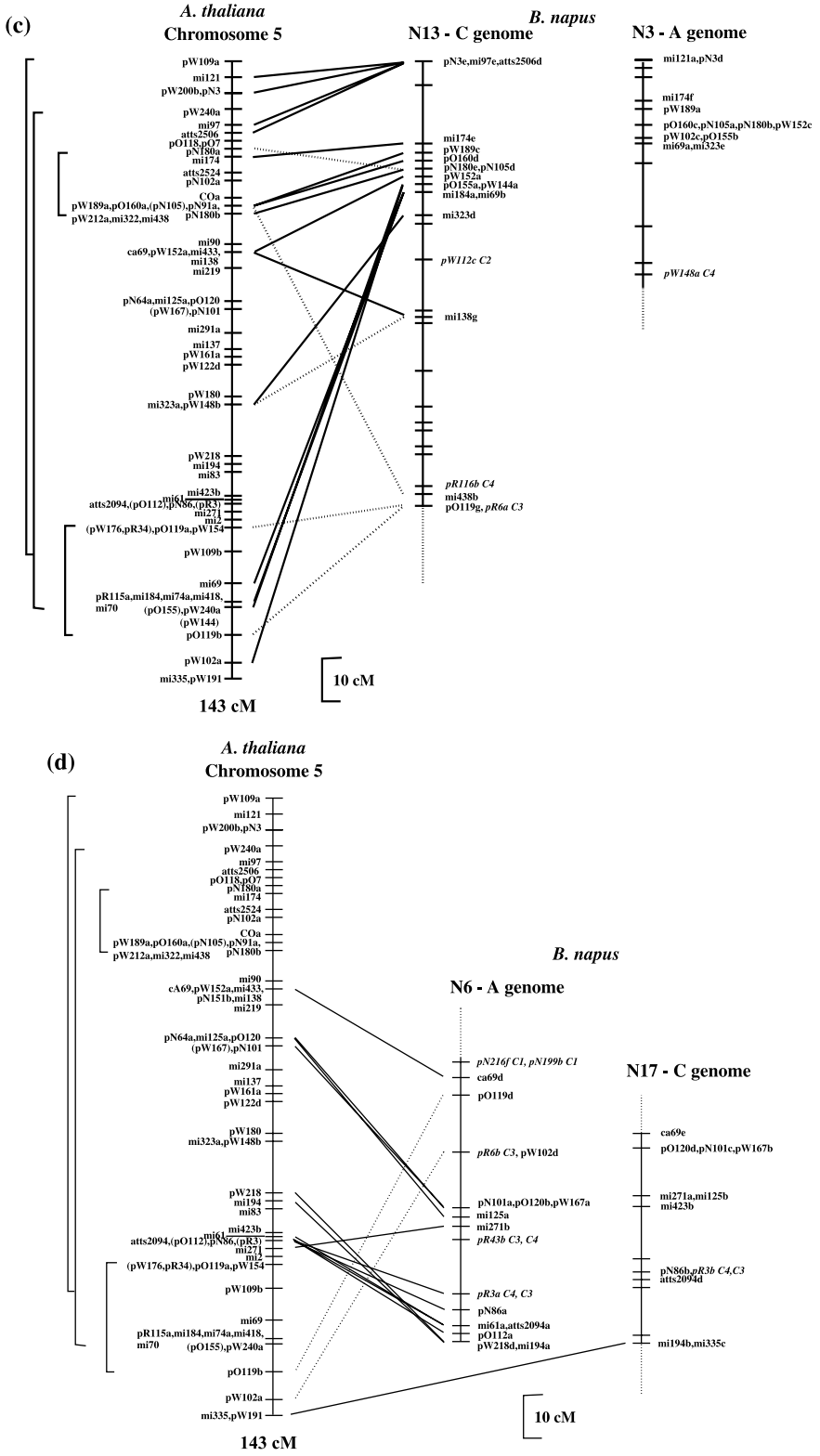


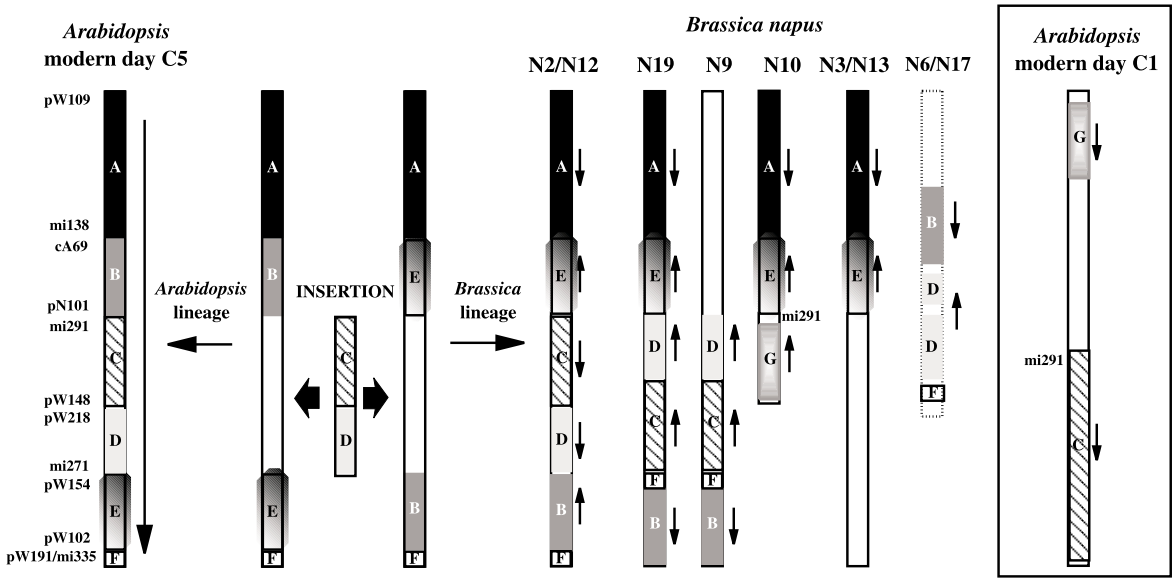
Fig. 1. (*concluded*).



played a role in the evolution of the diploid genomes and that the extant genomic structures are the remains of an ancestral hexaploid genome.

Because *Arabidopsis* is now thought to have evolved through a tetraploid ancestor (Blanc et al. 2000), the timing of the duplication events in the *Brassica* and *Arabidopsis* lineages should be reflected in comparative mapping studies. For example, recent physical mapping results for *B. oleracea* suggested that the *Brassica* hexaploid ancestral genome was formed after at least one duplication event in

Fig. 2. Schematic representation of the evolution of *B. napus* chromosomes from *Arabidopsis* chromosome 5. Conserved blocks of markers are identified as six segments of chromosome 5 (blocks A–F) and one segment of chromosome 1 (block G). The most extreme markers of those blocks are indicated. Arrows indicate the relative order of the markers on each linkage group. For linkage groups N6 and N17, the origin of only a portion of these linkage groups has been uncovered and is indicated by the dotted lines.



Arabidopsis (O'Neill and Bancroft 2000). Probes from a duplicated segment of the *Arabidopsis* genome identified six collinear regions in the *B. oleracea* genome, three equivalent to chromosome 4 and three equivalent to the duplicated region of chromosome 5. In this study, the largest duplicated segment was found between chromosomes 1 and 5 of *Arabidopsis*. The fact that markers from this region identified no more than six regions within the *B. napus* genome suggests that the triplication of this region occurred before the duplication event in *Arabidopsis*.

A possible scheme for the evolution of the observed relationships between the chromosomes of *B. napus* and *Arabidopsis* chromosome 5 is shown in Fig. 2. From the data presented here it is possible to identify six significantly conserved blocks of markers on *Arabidopsis* chromosome 5 that are representative of ancestral blocks found in the progenitor Crucifer genome. The presence of block A and an inverted block E in six regions of the *B. napus* genome (N2 and N12, N3 and N13, and N10 and N19), suggests that the chromosomal rearrangement that inverted blocks B and E with respect to *Arabidopsis* chromosome 5 must have occurred before the triplication event in the *Brassica* lineage. This chromosomal rearrangement has also been observed for the B genome of the diploid *Brassica nigra*, where the use of a common set of markers from blocks A and E by Lagercrantz (1998) has uncovered the same organization on three *B. nigra* linkage groups, G2 (pW200–mi69), G5 (pW200–pW189), and G8 (pW200–mi69). The fact that blocks C and D are in the same orientation in both *Brassica* N2 and N12 and *Arabidopsis* chromosome 5 suggests that these segments were inserted after the inversion event. To generate the observed structures for N9, N10, and N19, N3 and N13, and N6 and N17, either a number of inversions and translocations must have occurred subsequent to the triplication event or the original triplication event must have involved the fusion of three related, but divergent, chromosomes. The bottom of linkage group N10 has homology to a segment from chromosome 1, designated block G (Fig. 2). Interestingly, half of B-genome linkage group G2 of Lagercrantz (1998) has the same A – E – G block structure, indicating that the observed organization of N10 has remained fixed since the divergence of the A and B *Brassica* diploid genomes.

Block C is duplicated in *Arabidopsis* and is found on both chromosomes 1 and 5. The genetic distance covered by the markers is much greater on chromosome 1 compared with both chromosome 5 and the N2–N12 region. However, the *Brassica* markers found at the centre of N2–N12 are most closely related, based on sequence homology and hybridization strength, to chromosome 1. Each of the postulated blocks will have undergone significant divergence in the approximately 14–20 million years of evolution since the separation of the *Brassica* genomes from *Arabidopsis* (Yang et al. 1999). It seems likely that the ancestral chromosome that gave rise to at least the *B. napus* chromosomes N2–N12 and N9 – N10 – N19 would have comprised regions from both chromosomes 1 and 5. The centromere for *Arabidopsis* chromosome 5 has been positioned between markers mi125 and mi137 (Copenhaver et al. 1999), which places it approximately at the junction between blocks B and C. The positioning of centromeres and telomeric-associated sequences at breakpoints of chromosomal rearrangements is now a common feature of comparative maps (Moore et al. 1997; Lagercrantz 1998; Livingstone et al. 1999). Interestingly, the centromeric region of *Arabidopsis* chromosome 1 is reported to start approximately at marker mi291 (Copenhaver et al. 1999). The duplication of block C, which appears to include the centromere, suggests that the centromeric regions of

chromosomes 1 and 5 are thus evolutionarily related. This further suggests a common ancestral chromosome from which *Arabidopsis* chromosomes 1 and 5 have evolved.

As with any such scheme of evolutionary reconstruction, the organization of progenitor chromosomes and the mode of evolution have to be inferred from the ghostly impressions found within the modern-day karyotypes. It is difficult to ally the data presented here with other modes of evolution, such as multiple, independent, segmental duplications or the generation of a tetraploid *Brassica* ancestor, followed by independent duplications of certain regions that has previously been suggested from analysis of limited data sets (Sadowski and Quiros 1998). However, a whole-genome comparison between *Arabidopsis* and *B. napus* and the integration of that data with maps from a wide range of diploid crucifers should assist in answering the question as to how the diverse species of the Cruciferae have evolved.

The ability to compare the sequence of mapped *Brassica* markers with the available *Arabidopsis* genomic sequence has allowed us to assess the level of physical collinearity between the two species. In one highly conserved region, approximately 8 Mb of *Arabidopsis* genomic sequence was equivalent to a genetic distance of approximately 30 cM in the *B. napus* genome. A similar comparison identified a 7.5-Mb region of *Arabidopsis* chromosome 4 that was again equivalent to a genetic distance of approximately 30 cM in *B. napus* (Cavell et al. 1998). It will be interesting to determine both the physical length of the corresponding triplicate regions in the *Brassica* genome and the level of DNA nucleotide sequence identity across the three copies. Comparable analyses have not been carried out in other degenerate polyploids. However, the pattern of microcollinearity between maize, sorghum, and rice has shown numerous minor rearrangements and it appears that the relative expansion of the maize genome is largely because of the insertion of multiple retrotransposable elements (Bennetzen 2000). It is likely that the pattern of microcollinearity between *Brassica* and *Arabidopsis* will also uncover similar minor rearrangements. However, assuming the *Brassica* diploids have evolved through a hexaploid ancestor, triplication of a genome of similar size to *Arabidopsis* (150 Mb) would leave little room for major genome expansion (as the estimated size of diploid *Brassica* genomes is 500–600 Mb) (Arumuganathan and Earle 1991). Comparative mapping by in situ hybridisation of *Arabidopsis* BAC clones onto extended DNA fibers of *B. rapa* provides additional evidence that regional expansion of the genome has not been a driving force in crucifer genome evolution (Jackson et al. 2000).

The ability to exploit the relationship between the linkage groups of *Brassica* species and the genomic sequence of the *Arabidopsis* model will be entirely dependent upon the region of the *Brassica* genome that is being studied and the accuracy of the genetic mapping in that region. The *B. napus* genome can be broken down into segments of various sizes that correspond to conserved regions of the *Arabidopsis* genome. In some cases these regions can show startling levels of conservation, such as the top 8 Mb of *Arabidopsis* chromosome 5, suggesting some evolutionary advantage in maintaining particular gene complements or even particular gene orders. In other cases, major chromosomal rearrangements have obscured the observed synteny, but as with the identified inversion that separated chromosome 5 from three homoeologous pairs of *B. napus* linkage groups, this still allows the identification of defined blocks of homology. In the final instance there appears to be a complete breakdown in synteny, which could be due to the vestige of ancient duplications within the *Arabidopsis* genome or the reported instability of centromeric regions (Moore et al. 1997; Livingstone et al. 1999). However, even in these highly disparate regions order is observed, even if somewhat limited, with adjacent markers in the *B. napus* genome being physically linked in *Arabidopsis*, as was observed at the centre of N2–N12.

A chart identifying all the conserved regions between the *Arabidopsis* and *Brassica* genomes will soon be available and the genomic sequence for all of the chromosomes of *Arabidopsis* is now available (*Arabidopsis* Genome Initiative 2000). The combination of these two factors will allow *Brassica* researchers the exciting prospect of access to a plethora of molecular markers, a definitive physical map of the model crucifer genome, and, through data mining of the nucleotide sequence, the possibility of identifying novel candidate genes for traits of interest.

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