Theor Appl Genet (1995) 90: 157–165

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Centromeric repetitive DNA sequences in the genus *Brassica*

Received: 17 May 1994 / Accepted: 30 May 1994

Abstract Representatives of two major repetitive DNA sequence families from the diploid *Brassica* species *B. campestris* and *B. oleracea* were isolated, sequenced and localized to chromosomes by *in situ* hybridization. Both sequences were located near the centromeres of many chromosome pairs in both diploid species, but major sites of the two probes were all on different chromosome pairs. Such chromosome specificity is unusual for plant paracentromeric repetitive DNA. Reduction of stringency of hybridization gave centromeric hybridization sites on more chromosomes, indicating that there are divergent sequences present on other chromosomes. In tetraploid species derived from the diploids, the number of hybridization sites was different from the sum of the diploid ancestors, and some chromosomes had both sequences, indicating relatively rapid homogenization and copy number evolution since the origin of the tetraploid species.

Key words Maps (genetic) · Nuclear architecture Repetitive DNA · Ribosomal DNA · Satellite DNA *Brassica* · Crucifereae

Introduction

Knowledge about the distribution of repetitive DNA sequences is necessary to gain insight into the organization, evolution and behaviour of plant genomes. In higher plant genomes, the highly repetitive DNA sequences represent at least 20% and sometimes more than 90% of the DNA. Individual sequence classes may represent some 10% of the total genome, but they vary widely in characteristics. A particular sequence may be present in many species within a taxonomic family, or be essentially species specific; such results indicate that some repetitive sequences evolve rapidly, while others may be conserved (Anamthawat-Jónsson and Heslop-Harrison 1992). The genomic distribution of a repetitive sequence is often characteristic for a particular sequence: some are located in subtelomeric or paracentromeric domains on chromosomes, while others are widely dispersed (see Heslop-Harrison 1991); the distribution can indicate mechanisms and constraints on both genome and species evolution. Microsatellites may have a two base pair (bp) repeat motif, while other repeat units, such as the rDNA, may be 10 kbp long. However, a repeat unit of 160–180 bp, the length of sequence wrapped around a single nucleosome, is a frequent length in both plants and animals.

In situ hybridization is valuable for studying the chromosomal distribution of sequences and the copy number at different sites, and to follow evolutionary changes in their physical organization in the genome. In the *Brassicas*, where allopolyploidy occurs regularly, evolutionary changes are of particular interest because of the complex, and sometimes controversial, relationships within the genus (Song et al. 1988, 1990) and its economic importance. In the investigation presented in this paper, we aimed to identify repetitive DNA sequences that make up the centromeric heterochromatin of some *Brassica* species and to characterize their chromosomal distribution in tetraploid species and their diploid progenitors.

Materials and methods

The plants used in this study were *B. campestris* L. (Chinese cabbage cv 'Chinese Courage', NVRS-HRI Accession 6202), *B. nigra* (L.) Koch (black mustard cv 'Vince'), *B. oleracea* L. (kale cv 'Dwarf green curled'), *B. juncea* (L.) Czrm. (accession 81794, CRM 87109), *B. napus* L. (winter oil seed rape cv 'Falcon') and *B. carinata* A. Braun. (Abbesynian mustard). Figure 5 gives the genome designations, ploidies and chromosome numbers of these species. Plant DNA was extracted from young leaves taken from greenhouse grown material using standard techniques.

Nuclear DNA from the diploid species *B. campestris*, *B. nigra* and *B. oleracea* was digested with *Hin*dIII. After agarose gel electrophoresis and ethidium bromide staining, prominent bands of about 360 bp were seen only in *B. campestris* and *B. oleracea*. These bands

Communicated by F. Mechelke

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were cut out and the DNA cloned in pUC18 using standard techniques (Sambrook et al. 1989); the two clones chosen for extensive analysis were named pBcKB4 and pBoKB1, from *B. campestris* and *B. oleracea,* respectively, and KB for Karyobiology to identify the library. Clones were sequenced in both directions on a Pharmacia ALF DNA sequencer (Fig. 2).

Genomic DNA from the diploid and tetraploid species was digested with restriction enzymes, separated by electrophoresis and transferred to nylon membranes (Hybond N+, Amersham) using standard techniques. Probes were non-radioactively labelled (ECL, Amersham) and hybridization sites detected following the methods of Anamthawat-Jónsson et al. (1990) at a stringency of 85%.

Seedling root tips from the six species above were used to make chromosome preparations. Seeds were germinated on moist filter paper in petri dishes at 25°C. When the roots were 2–3 cm long, seedlings were transferred to 4°C for 24 h and then returned to 25°C for a further 24 h to synchronize cell divisions. Root tips were removed and treated with 8-hydroxyquinoline (0.002 *M*) for 30 min at room temperature followed by 2 h at +4°C, then fixed (3:1 100% ethanol:glacial acetic acid) and stored at –20°C until use. Fixed root tips were washed in enzyme buffer (6 m*M* sodium citrate, 4 m*M* citric acid, pH 4.6) followed by digestion with cellulase and pectinase (1.6% cellulase, Calbiochem, 0.4% cellulase Onazaka, 20% pectinase, Sigma, in enzyme buffer) at 37°C for 35 min. Squashes were made in 45% acetic acid. Coverslips were removed after freezing on dry ice, and slides were allowed to air dry before storing at +4°C until use.

In situ hybridization followed the techniques of Schwarzacher et al. (1989) and Maluszynska and Heslop-Harrison (1993b). Detailed protocols are given in Schwarzacher et al. (1994). Briefly, chromosome preparations were pretreated with RNase (100 mg/ml, 1 h, 37°C) and then pepsin (20 U/ml) in 0.01 *M* HCL, fixed in 4% paraformaldehyde, dehydrated through an alcohol series and air dried. The DNA sequences pBcKB4 and pBoKB1 were labelled with digoxigenin-11-dUTP (Boehringer Mannheim) or biotin-11-dUTP (Sigma) by oligolabelling. The hybridization mixture consisted of 50% formamide, 10% dextran sulphate, 0.1–0.5% SDS with autoclaved salmon sperm DNA (25–100 ng/l) and SSC (20× stock: 0.3 *M* sodium citrate and 3 *M* sodium chloride) at 4× (high stringency of hybridization; used where not otherwise stated) or 0.66× (low stringency) with 50 ng/slide of labelled pBcKB4 probe or 80 ng/slide of pBoKB1. The probe mix was denatured at 70°C for 10 min and held on ice for 5 min before being added to the slides. Probe and root tip preparations were denatured in a modified thermal cycling machine at 80°C for 5 min (Heslop-Harrison et al. 1991; improved versions available from Hybaid, London) before hybridization overnight at 37°C. Several washes were carried out, with the most stringent being in 50% formamide in 2×SSC at 42°C (high stringency) or 37°C (low stringency). Sites of hybridization were detected using either antidigoxigenin:fluorescein (Boehringer Mannheim) or Avidin:Texas red (Vector). Where probes labelled with biotin and digoxigenin were simultaneously hybridized, both haptens were detected together (Leitch et al. 1991). In other cases, slides were reprobed with a second label following methods described previously (Heslop-Harrison et al. 1992). The chromosomes were counterstained with DAPI (4′,6 diamidino-2-phenylindole, 2 µg/ml in McIlvaines citric buffer, pH 7; blue emission) and, sometimes, propidium iodide (PI, 5 µg/ml in buffer; red emission) before examination by epifluorescent light microscopy. Photographs were taken on Fujicolor 400 print film and digitized with the Kodak PhotoCD system. Final prints were prepared with Adobe Photoshop using only the Image and Channels menus, except in Fig. 3a where some background was removed. The listed functions apply the same adjustments to all parts of the image but enable contrast and brightness adjustment and overlaying of different exposures.

Results

Size fractionated *Hin*dIII digests of *B. campestris* and *B. oleracea* DNA showed prominent bands corresponding to fragments of 180 bp and 360 bp. No equivalent bands were present in *B. nigra*. The 360 bp fragments from *B. campestris* and *B. oleracea* were cloned, and two of the resulting plasmids, pBcKB4 and pBoKB1 (from *B. campestris* and *B. oleracea* respectively) were sequenced (Fig. 2) and used as probes for Southern (Fig. 1) and *in situ* hybridization (Figs. 3, 4).

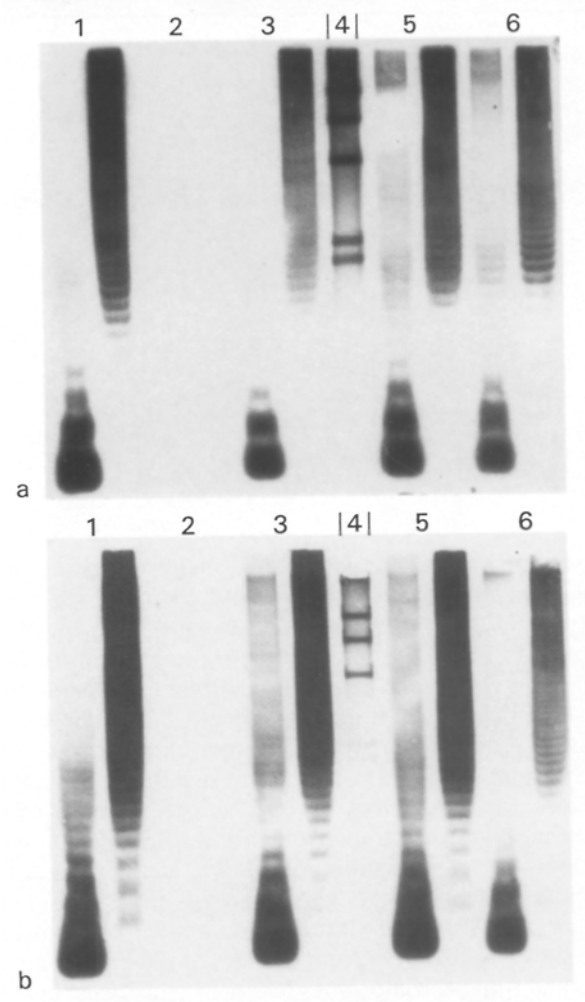

Fig. 1 Luminographs showing Southern hybridization of tandemly repeated DNA sequences isolated from *Brassica oleracea* (pBoKB1, **a**) and *B. campestris* (pBcKB4, **b**) to restriction enzyme digests (*Hin*dIII, *left*, and *Dra*I, *right* of each pair) of genomic DNA from: *track* 1 *B. campestris*, 2 *B. nigra*, 3 *B. juncea*, 4 Lambda *Hin*dIII marker, bands (from top to bottom 23.1, 9.4, 6.6, 4.4, 2.3, 2.1 kb), 5 *B. napus*, 6 *B. oleracea*

Figure 1 shows *Hin*dIII- and *Dra*I-digested total genomic DNA from *B. campestris, B. nigra, B. juncea, B. napus* and *B. oleracea* probed with the clones. Both probes showed similar hybridization to all species containing the A and C genomes, with prominent ladders characteristic of a satellite pattern in the *Hin*dIII digest. No probe hybridization was seen to *B. nigra* digests, and there was some variation in hybridization strength between the other tracks.

Fig. 2 The DNA sequences of **a** pBoKB1 and **b** pBcKB4. See Results and Discussion

(a) clone pBoKB1

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        10          20          30          40          50          60
AAGCTTGCTA TAAGATTTTC GTTTGTACAC AGCTTGAAAA TGAAAGCAAT ATTAATGCTG
TTCGAACGAT ATTCTAAAAG CAAACATGTG TCGAACTTTT ACTTTCGTTA TAATTACGAC

        70          80          90          100         110         120
GTCGTGAATC CCTCTTTAGA CCACAACTTC ATTGCAAAAA CAGATAATTA TTTTGCCATG
CAGCACTTAG GGAGAAATCT GGTGTTGAAG TAACGTTTTT GTCTATTAAT AAAACGGTAC

       130         140         150         160         170         180
TTTAGAAAAT ACAATGATGT TTGTTTTGCC ATTTGTTATC ATTCTCTTCA TATTATTATT
AAATCTTTTA TGTTACTACA AACAAAACGG TAAACAATAG TAAGAGAAGT ATAATAATAA

       190         200         210         220         230         240
TTTCTTTAAA ATTTTCTGTT GGTTCTGACG TTGTGAGACT TGTTGTACAC ACTTACAGGT
AAAGAAATTT TAAAAGACAA CCAAGACTGC AACACTCTGA ACAACATGTG TGAATGTCCA

       250         260         270         280         290         300
ATGTCTGCTG TTGTTCATAC TCCACAACGT GGGCCCCCCA AGGGAAAGTT CATCATCGCA
TACAGACGAC AACAAGTATG AGGTGTTGCA CCCGGGGGGT TCCCTTTCAA GTAGTAGCGT

       310         320         330         340
TTTGTTTCTA ACAGATGCAG AGACTGATAA CCCTCAACCC TG
AAACAAAGAT TGTCTACGTC TCTGACTATT GGGAGTTGGG AC
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(b) clone pBcKB4

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        10          20          30          40          50          60
AAGCTTGATT TGGATACATA AAGTGTTGGA GAATTTACCA GGAAGTTGAA TAAACCGCAT
TTCGAACTAA ACCTATGTAT TTCACAACCT CTTAAATGGT CCTTCAACTT ATTTGGCGTA

        70          80          90          100         110         120
AGGAGTTGGG ATGAAGAAGT TACCCCACTT TCAAATCAGG TGATTCCAGG TTTGCAGTTT
TCCTCAACCC TACTTCTTCA ATGGGGTGAA AGTTTAGTCC ACTAAGGTCC AAACGTCAAA

       130         140         150         160         170         180
GAGAATAGAA CAACTTCTTC ATCATTCCAA TCAAACCAGG ATGAATAACT TTGTAAGAAT
CTCTTATCTT GTTGAAGAAG TAGTAAGGTT AGTTTGGTCC TACTTATTGA AACATTCTTA

       190         200         210         220         230         240
CTTGATTTGG ATACATAAAT TGGTGAAGAA TCACCAGGAA TTTGAATAAA TCTCATAGGA
GAACTAAACC TATGTATTTA ACCACTTCTT AGTGGTCCTT AAACTTATTT AGAGTATCCT

       250         260         270         280         290         300
CTTAGGATAA AGAAGTTATC CCACTTTCAA ATCAAGTGAT TCCACTTTGC TAGTTTGGGA
GAATCCTATT TCTTCAATAG GGTGAAAGTT TAGTTCACTA AGGTGAAACG ATCAAACCCT

       310         320         330         340         350
ATAGGACATC TTCGTCGTTC CAATCAAACC AGGATGAATC ACTTTGTAAG
TATCCTGTAG AAGCAGCAAG GTTAGTTTGG TCCTACTTAG TGAAACATTC
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Sites of *in situ* hybridization of both probes were detected on chromosomes from metaphases and interphase nuclei (Figs. 3 and 4), and always co-located with brightly DAPI stained heterochromatic blocks at or very close to the centromeres of metaphase or prophase chromosomes. Hybridization sites were distributed over most the area of interphase nuclei, again at DAPI staining chromocentres; nucleoli were visible as areas of less strong DAPI fluorescence, but sometimes had hybridization sites near or overlaying (above or below) the nucleoli in the flattened preparation. Since hybridization sites were not normally clustered or joined at interphase, the numbers of sites which could be counted corresponded with the number of sites visible at metaphase. Although only one or two examples of each probe and species combination are shown, the results quoted arise from counting hybridization sites in many metaphases and interphase nuclei (summary: Fig. 5).

The clone pBcKB4 hybridized to 8 (low stringency; Fig. 3a, b–d) or else 7 (high stringency; Fig. 3e, f) of the 10 pairs of chromosomes (Fig. 3a) in *B. campestris* at metaphase and interphase. The probe hybridized to 7 pairs of sites in *B. oleracea* (Fig. 3 g). As expected from the Southern hybridization result, pBcKB4 showed no defined hybridization to *B. nigra* (not shown).

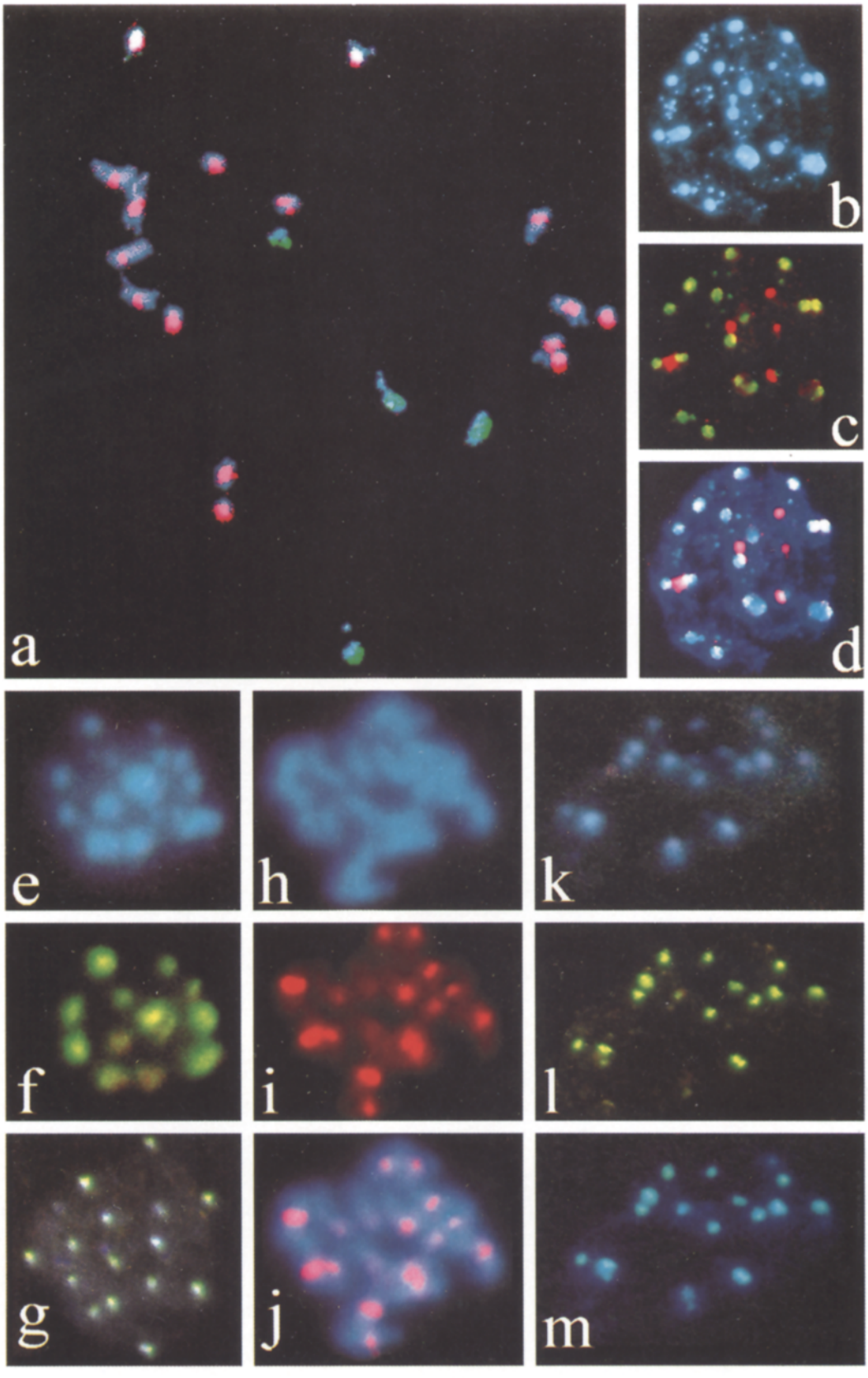

Fig. 3a–m Metaphase, prophase and interphase nuclei of diploid *Brassica* species probed with the repetitive DNA sequences from *B. oleracea* (BoKB1) and *B. campestris* (pBcKB4). All nuclei or chromosomes are stained with the fluorochrome DAPI (blue). The chromosome number in each species and number of sites of hybridization found with each probe/target combination is summarized in Fig. 5 and not repeated here. **a** *B. campestris* metaphase probed with pBcKB4 (pink/white) and pBoKB1 (blue-green), **b–f** *B. campestris* interphase nuclei probed with pBcKB4 (green-yellow in *c*, *f*) and pBoKB1 (red in *c*), **g** *B. oleracea* interphase probed with pBcKB4 (green-white), **h–j** *B. oleracea* metaphase probed with pBoKB1 (red or pink), **k–m** *B. oleracea* prophase probed with pBoKB1 (green or blue-green)

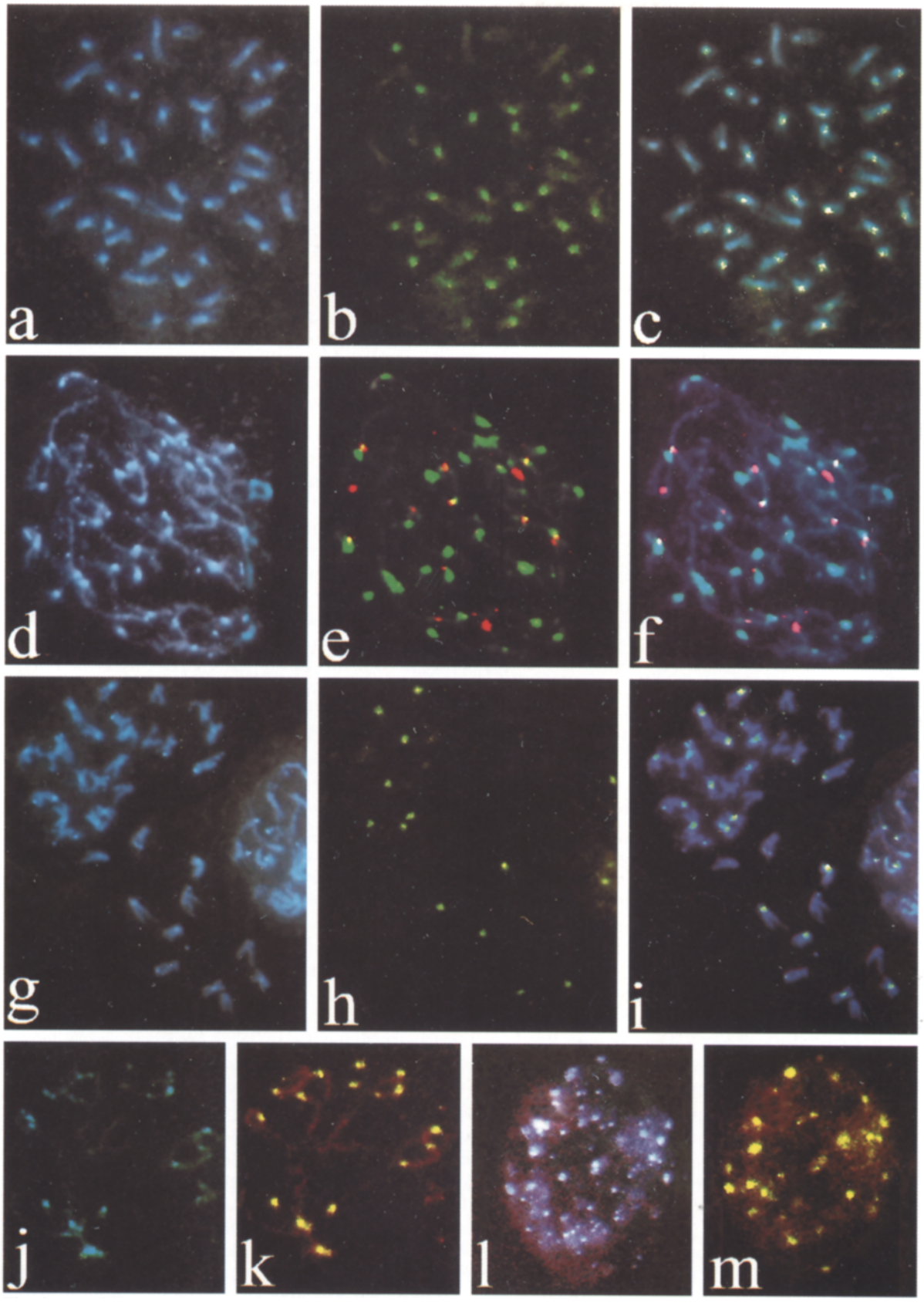

Fig. 4a–m Metaphase, prophase and interphase nuclei of tetraploid *Brassica* species (see Fig. 3). **a–f** *B. napus* metaphase and prophase probed with pBcKB4 (green) and (**d–f** only) pBoKB1 (red). **g–i** *B. carinata* metaphase probed with pBcKB4 (green or yellow where it overlaps red probe), **j, k** *B. juncea* pro-metaphase probed with pBcKB4 (yellow), **l, m** *B. juncea* interphase probed with pBoKB1 (yellow). **k** and **m** show propidium iodide counterstaining (red-brown)

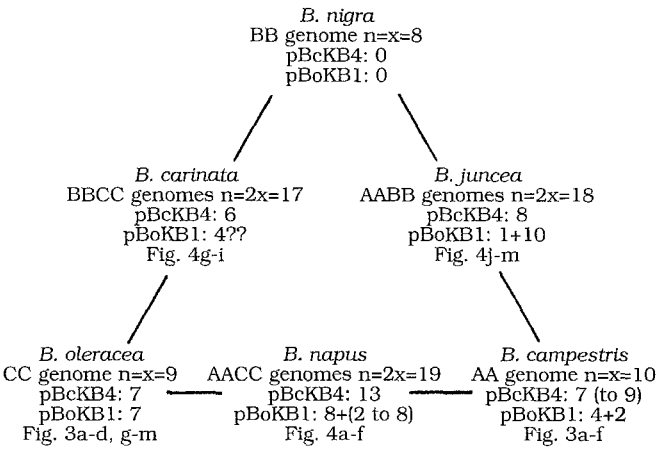

Fig. 5 A summary of the relationships (after U 1935), chromosome numbers, genome designations and number of hybridization sites of pBoKB1 and pBoKB4 on diploid and amphidiploid *Brassica* species

pBoKB1 hybridized to the centromeres of 7 of the 9 pairs of chromosomes in *B. oleracea* at high stringency in both metaphase (Fig. 3h–j) and interphase (Fig. 3k–m). One pair of centromeres showed a stronger signal than th others. When hybridized to *B. campestris*, the prob showed 4 major and 2 minor sites of hybridization (Fig 3a, b–d). None of the major sites co-located with sites o pBcKB4.

The tetraploid species *B. napus* has genomes from *B campestris* and *B. oleracea*. pBcKB4, expected to hav 14–16 pairs of sites in *B. napus*, hybridized to the centro meres of 13 pairs of chromosomes (Fig. 4a–f). pBoKB1 expected to show 11– 13 pairs of sites, showed hybridiza tion of varying strength to considerably fewer centromeres (Fig. 4d–f). This was found in both reprobed (see Heslop-Harrison et al. 1992) and in simultaneously hybridized preparations (Fig. 4d–f). About 8 pairs of chromosomes showed strong hybridization in all cases, and 2–8 pairs showed weaker hybridization. All centromeres were strongly labelled by one or the other probe.

The tetraploid species *B. carinata* (BC) and *B. juncea* (AB) were also investigated. In *B. carinata*, pBcKB4 showed 6 pairs of major hybridization sites (Fig. 4g–i) and approximately 4 pairs of sites of pBoKB1 (although the data, not shown, were inconclusive). *B. juncea* showed 8 pairs of sites of pBcKB4 (Fig. 4j, k) and 1 strong and 10 weaker pairs of sites of pBoKB1 (Fig. 4l, m).

Discussion and conclusions

Repetitive sequences and homologies

Highly repetitive DNA sequences were isolated from *B. oleracea* (pBoKB1) and *B. campestris* (pBcKB4; Fig. 1). Both species, their amphidiploid hybrid and other hybrids including the A and C genomes have DNA homologous to both sequences, and both sequences show a similar tandem-repeat organization giving a ladder hybridization pattern (Fig. 1). The sequences of the two clones (Fig. 2) showed no significant homology.

pBcKB4 is a dimer with a change of a G to T in the *Hin*dIII recognition site (AAGCTT, underlined in Fig. 2) at position 178. There are three TTTNAA sites, one or more of which presumably alters occasionally to give a DraI site and hence the ladder pattern seen with this enzyme (Fig. 1). The clone is a representative of a family that has been sequenced many times and is represented in the GenBank/EMBL database (Release 80) by no less than 18 accessions from *B. campestris, B. napus, B. juncea, B. oleracea* (Lakshmikumaran and Ranade 1990), and the related genera *Diplotaxis erucoides* (L.) DC., *Raphanus sativus* L. (Grellet et al. 1986) and *Sinapis alba* L., which contain from 90% to 70% similarity over 140–180 base pairs, overlaps with pBcKB4. A homologous sequence has been reported from *B. nigra* (EMBL accession number X16589) but is almost certainly due to misidentification of the plant involved (M. Lakshmikurmaran, personal communication). Figure 1 shows that the sequence is undetectable in *B. nigra*. The satellite has a remarkable structure with many features, some of which have been described before (Grellet et al. 1986). The sequence shows a significantly reduced frequency of CpG ($P < 0.05$) and excess of TpT ($P < 0.01$) dinucleotides compared to the random expectation from its nucleotide composition. No deviations in trinucleotide frequencies are evident. As suggested previously, the monomeric unit itself has three extensively diverged internal repeats of about 60 bp, so may have arisen from a shorter sequence related to a tRNA gene, and may hence have similarities to SINEs (short interspersed elements) elsewhere in the genome (Grellet et al. 1986). The two monomeric units of pBcKB4 show less than 90% homology (lengths 173 and 177 bp, differing by 19 substitutions, 5 insertions and 1 deletion). The degree of variation between the monomeric units is much higher than that in human alphoid repeats, where sequence variations are generally chromosome-specific (see Laurent et al. 1994). Choo et al. (1991) have surveyed 293 human alpha-satellite sequences. Unlike plants, it seems that there is a very high level of conservation within each sequence, with considerable chromosome specificity of each sub-repeat.

Since its introduction in 1990 (Williams et al. 1990), the use of RAPD genetic linkage mapping [arbitrary random oligomer primers for amplifying polymorphic *DNA* segments] has proved valuable for genome analysis. When cloned, RAPDs have proved to include parts of single-copy, middle and highly repetitive DNA sequences (Williams et al. 1993). Dos Santos et al. (1994) anticipate that a linkage map in *B. oleracea* based upon RAPD markers will soon be produced and show that RAPDs and restriction fragment length polymorphisms (RFLPs) give equivalent levels of resolution for determination of genetic relationships between *B. oleracea* genotypes, indicating that the sources of the polymorphisms are similar. Other results indicate that the use of RAPDs as markers may give more uniform coverage of the genome than randomly chosen

RFLP probe-enzyme combinations, since RAPD polymorphisms result from both single-copy and repetitive sequences (Williams et al. 1993). It is notable that pBcKB4 contains a 12-mer GATGAAGAAGTT (bold in Fig. 2) on the forward and reverse strands at positions 70 and 132 in the first monomeric repeat; hence the 12-mer could be used as a primer in a polymerase chain reaction. Whether priming sites for this sequence are too frequent in the genome, or the product sizes too polymorphic, to allow mapping is unknown, but it would be interesting to use the sequence as a primer to examine polymorphisms between different lines and *Brassica* species.

pBoKB1, in contrast to the *B. campestris* clone, has an unusual sequence with no significant homology to previously described sequences. (The highest homologies in the Genbank/EMBL database represent homologies in AT-rich regions.) The sequence has a single *Dra*I site (position 185, the centre of a near-palindromic 21-mer, bold in Fig. 2), so could also be isolated with that enzyme, but there are few other noteworthy features. No significant deviations in dinucleotide or trinucleotide frequencies were detected. There are numerous AT-rich stretches that could be internal repeat motifs. The sequence is not a dimer, although it may have arisen from a duplication. Given its high abundance in the genome, it is surprising that no repeat similar to pBoKB1 is included in the Genbank/EMBL database. In the present work, a targeted cloning strategy was adopted, and it is possible that the sequence is under-represented in genomic libraries because of selective cloning, perhaps due to methylation or secondary structure.

Chromosomal Localization of sequences

While details of the evolution and relationships between *Brassica* species remain controversial, the basic framework of relationships of the species studied here was established by U (U 1935). Figure 5 summarizes the number of sites of hybridization of each probe to the chromosomes of the diploid and tetraploid species investigated. Both sequences were homologous to regions at or near the centromere of the *Brassica* chromosomes, and pairs or groups of sites did not regularly cluster at interphase. There was no strong gradient across the genome in hybridization or strength of DNA staining, contrasting with the situation in some cereals where centromeres are clustered at one end of the nucleus and there is an opposite pole with a lower proportion of the nucleus filled with DNA (Anamthawat-Jónsson and Heslop-Harrison 1990). Unlike other repetitive sequences occurring at the centromeric regions of plant chromosomes (such as the pAL1 sequence in *Arabidopsis thaliana* L.; Maluszynska and Heslop-Harrison 1991), the *Brassica* sequences analysed here are not located on all chromosomes and hence show chromosome specificity. Alteration of the stringency of hybridization changed the number of sites of hybridization of the probes as with human paracentromeric satellites (Schwarzacher et al. 1988), indicating that the sequences present on different chromosomes include some diverged members of the same sequence family (Warburton et al. 1991; Willard 1990), although, as discussed above, the structure of the centromeric satellites in *Brassica* seems to be more variable than that of human. In all cases, the pBcKB4 probe gave strong and discrete hybridization signals, while pBoKB1 often showed a number of weak hybridization sites. Iwabuchi et al. (1991) reported the localization of a sequence homologous to pBcKB4, pBT11, and stated that it was located near the centromere of all chromosomes in *B. campestris*, and also in distal locations on some smaller chromosomes. Their results probably differ from ours because a lower stringency of hybridization and washing was used; at our low stringency (Fig. 3d–f), we found that 9 of the 10 chromosome pairs showed hybridization.

Both sequences were present in the diploid species *B. oleracea* and *B. campestris*, but not *B. nigra*, so it is probable that the sequences arose after the divergence of *B. nigra* (or its true diploid ancestors; see below) from the common evolutionary lineage of the *Brassica*s, but before the separation of *B. oleracea* and *B. campestris*. This phylogeny is consistent with the evolutionary tree reconstructed from RFLP data (Song et al. 1988; 1990) and from analysis of chromosome pairing (Attia and Robbelen 1986). Song et al. (1988) and Hosaka et al. (1990) showed that some C genome (*B. oleracea*) markers had apparently introgressed into *B. campestris* (A genome), indicating the existence of a horizontal route for transfer of the sequences between the genomes. Thus, the possibility of a recent, horizontal, transfer of the satellite sequences, perhaps followed by amplification and dispersion in the genome, cannot be ruled out. It is probable that the 'diploid' species investigated are actually secondary polyploids (Quiros et al. 1987), which, along with chiasmatic exchange, indicates a mechanism for the dispersion of the sequences between chromosomes. It will be interesting to know if the differential hybridization of the two probes (e.g. Fig. 3a) is identifying chromosomes originating from the two ancestral genomes. As yet, there are no clear cytogenetic markers that enable easy identification of all the *Brassica* chromosomes (Olin-Fatih and Heneen 1992), but when physical chromosomes can be correlated with the genetic linkage groups, the structure of the ancestral genomes should become clear.

Two of the three tetraploid species examined showed a reduced number of signals compared to the number expected from the diploid ancestors. This result is most likely to occur because of a rapid change in the copy number of sequences located at or near the centromeres since the tetraploid species arose, a conclusion supported by the high number of minor hybridization sites (Fig. 5). All of the species used are cultivars or selections from plant breeding programmes and so have been subjected to some pressure for agronomic performance.

Based on RFLP analysis Song and Osborn (1992) reported that multiple origins could be common among the *Brassica* amphidiploids. They proposed that a number of different interspecific hybridizations may have taken place between different diploid parents and therefore that different morphotypes of amphiploids may have different num-

bers of sites of the two sequences. Investigations of such variability would show the utility and effectiveness of studying taxonomic relationships between species using molecular or molecular cytogenetic methods with repetitive DNA sequences (Orgaard and Heslop-Harrison 1993; Molnar et al. 1989; Gupta et al. 1989).

As with other repetitive DNA sequences, the structural role and relationships to genetic behaviour of these *Brassica* sequences is not clear. Interestingly, in *Pennisetum glaucum* (L.) R. Br., the predominant sequence at the centromeres of all chromosomes is undermethylated (Kamm et al. 1994), and we know that alteration in methylation levels affects the segregation of B chromosomes (Neves et al. 1992). The results presented here indicate that analysis of the distribution and sequences of repetitive DNA is a valuable part of genome analysis. Significant differences in chromosome specificity, genome specificity and changes in the hybrids are reported, and the *Brassica* species differ from other groups, particularly the cereals and human, where most work has been carried out.

Acknowledgements and note We thank Prof. J. Maluszynska, Dr. T. Schmidt and Dr. T. Schwarzacher for valuable discussions and help with the experiments described. pBoKB1 and pBcKB4 appear in the EMBL and Genbank databases as Z23004 (Bosatdnaa) and Z22947 (Bcsatdnaa), respectively.

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