

## Comparative Analysis of rDNA Distribution in Chromosomes of Various Species of Brassicaceae

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• **Background and Aims** The Brassicaceae family encompasses numerous species of great agronomic importance, belonging to such genera, as *Brassica*, *Raphanus*, *Sinapis* and *Armoracia*. Many of them are characterized by extensive intraspecific diversity of phenotypes. The present study focuses on the polymorphism of number, appearance and chromosomal localization of ribosomal DNA (rDNA) sites and, when possible, in relation to polyploidy, in 42 accessions of *Brassica* species and ten accessions of *Diplotaxis*, *Eruca*, *Raphanus* and *Sinapis* species.

• **Methods** Chromosomal localization of ribosomal DNA was carried out using dual colour fluorescence *in situ* hybridization (FISH) with 5S rDNA and 25S rDNA sequences as probes on enzymatically digested root-tip meristematic cells.

• **Key Results** Loci for 5S and 18S–5.8S–25S rDNA were determined for the first time in six taxa, and previously unreported rDNA constellations were described in an additional 12 accessions. FISH revealed frequent polymorphism in number, appearance and chromosomal localization of both 5S and 25S rDNA sites. This phenomenon was most commonly observed in the A genome of *Brassica*, where it involves exclusively pericentromeric sites of 5S and 25S rRNA genes. The intraspecific polymorphism was between subspecies/varieties or within a variety or cultivar (i.e. interindividual).

• **Conclusions** The number of rDNA sites can differ up to 5-fold in species with the same chromosome number. In addition to the eight previously reported chromosomal types with ribosomal genes, three new variant types are described. The extent of polymorphism is genome dependent. Comparing the A, B and C genomes revealed the highest rDNA polymorphism in the A genome. The loci carrying presumably inactive ribosomal RNA genes are particularly prone to polymorphism. It can also be concluded that there is no obvious polyploidization-related tendency to reduce the number of ribosomal DNA loci in the allotetraploid species, when compared with their putative diploid progenitors. The observed differences are rather caused by the prevailing polymorphism within the diploids and allotetraploids. This would make it difficult to predict expected numbers of rDNA loci in natural polyploids.

**Key words:** 5S rDNA, 25S rDNA, 18S–5.8S–25S ribosomal RNA genes, Brassicaceae, chromosomes, FISH.

### INTRODUCTION

The Brassicaceae family comprises about 350 genera and more than 3000 species and is one of the most important crop plant families. To date, detailed chromosome analyses in Brassicaceae have been almost exclusively limited to the leading crop species of *Brassica*, *Sinapis* and *Raphanus*, while knowledge of the genome organization in the wild species is still fragmentary (Ali *et al.*, 2005) or non-existent. Investigated species of *Brassica* and other related genera possess small and morphologically uniform chromosomes. The number of chromosomes is often relatively high due to widespread allopolyploidy. These features make karyotype analysis difficult when simple chromatin staining and morphometric studies of chromosome organization are carried out (Olin-Fatih and Heneen, 1992).

The two types of ribosomal RNA genes, 5S rDNA and 45S rDNA encoding for 18S–5.8S–25S ribosomal RNAs, are widely used as probes for fluorescence *in situ* hybridization (FISH), providing chromosome markers in

experiments performed on various plant species (Maluszynska, 2002). In species of *Brassica*, *Raphanus* and *Sinapis*, the simultaneous use of these probes revealed a high number of ribosomal RNA loci at different chromosomal locations, which in some genomes offers simple but reliable chromosome landmarks (Kamisugi *et al.*, 1998; Schrader *et al.*, 2000; Snowdon *et al.*, 2000, 2002; Hasterok *et al.*, 2001; Kulak *et al.*, 2002).

Analysis of the chromosomal localization of 45S ribosomal DNA sites (Maluszynska and Heslop-Harrison, 1993; Cheng *et al.*, 1995; Snowdon *et al.*, 1997; Fukui *et al.*, 1998; Hasterok and Maluszynska, 2000) followed later by 5S rDNA sites (Snowdon *et al.*, 2000, 2002; Hasterok *et al.*, 2001; Kulak *et al.*, 2002) was performed independently in several different institutions, both for the key crop species of *Brassica* and also, but to a lesser extent, for other Brassicaceae species (Fransz *et al.*, 1998; Schrader *et al.*, 2000; Ali *et al.*, 2005). Since the first FISH-based studies, discrepancies regarding the number and distribution of rDNA loci have been reported both for diploid and allotetraploid species, confirming similar observations made in earlier molecular analyses (Delseny *et al.*, 1990; McGrath *et al.*,

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1990; Kianian and Quiros, 1992). Importantly, in the studies above, determination of rDNA chromosomal distribution was usually based on a single variety or cultivar. Such variation was also reported in some other plant species, e.g. rice (Fukui *et al.*, 1994) and common bean (Pedrosa *et al.*, 2003), and in the case of *Brassica* species was usually briefly attributed to different origins of the material studied, and differences in technical approaches and sensitivity of FISH carried out by different groups (Maluszynska and Heslop-Harrison, 1993; Snowdon *et al.*, 1997, 2000; Fukui *et al.*, 1998; Hasterok *et al.*, 2001).

The aim of the present study was to analyse the chromosomal distribution of 5S and 45S ribosomal DNA loci in the key crop species of *Brassica*, their wild relatives and in species closely related to the *Brassica* genus. Special emphasis is placed on the analysis of polymorphism of rDNA loci, a variation that seems to be particularly widespread in this group of plants.

## MATERIALS AND METHODS

### *Plant material*

Fifteen species of the Brassicaceae family belonging to five genera and comprising 52 accessions were analysed in this study. Seeds were obtained from botanical gardens, plant breeding stations, research centres and commercial sources. Information on the origins of this plant material is provided in Table 1.

### *Somatic chromosome preparations*

All seeds were germinated on filter paper moistened with tap water at 20–22 °C in the dark for 3–5 d. Further treatment was carried out as described earlier (Hasterok *et al.*, 2001). Briefly, to ensure optimal chromatin condensation at metaphase, whole seedlings with roots 1.0–2.0 cm long were immersed for 2 h at room temperature in 2 mM 8-hydroxyquinoline, then fixed in 3 : 1 (v/v) methanol : glacial acetic acid and stored at –20 °C. Excised roots were washed in 0.01 M citric acid–sodium citrate buffer (pH 4.8) for 20 min prior to digestion in an enzyme mixture of 20 % (v/v) pectinase (Sigma), 1 % (w/v) cellulase (Calbiochem) and 1 % (w/v) cellulase ‘Onozuka R-10’ (Serva) for 1.5–2 h at 37 °C. Meristems were dissected out from root tips, squashed in drops of 45 % acetic acid and frozen. After removal of coverslips, the preparations were post-fixed in 3 : 1 ethanol : glacial acetic acid, followed by dehydration in absolute ethanol and air-dried.

Successful chromosome preparations made from at least five individual plants from each accession were subjected to FISH analysis. In the case of the two accessions of *B. carinata*, 20 individuals from each were studied for a detailed comparison of interindividual variation. One root tip per seedling (individual) was used to make one preparation.

### *DNA probes and FISH*

The following ribosomal DNA sequences were used as probes: 5S rDNA (pTa794) (Gerlach and Dyer, 1980)

labelled using PCR with tetramethyl-rhodamine-5-dUTP (Roche), and a 2.3-kb *Cla*I subclone of the 25S rDNA coding region of *Arabidopsis thaliana* (Unfried and Gruendler, 1990) labelled by nick translation using digoxigenin-11-dUTP (Roche). The latter probe was used to determine the chromosomal localization of 18S–5.8S–25S rRNA genes (45S rDNA). The labelling procedure followed the methods described in detail by Hasterok *et al.* (2002).

The hybridization mixture consisted of 50 % deionized formamide, 20 % dextran sulphate, 2× SSC and salmon sperm blocking DNA in 50–100× excess of labelled probes. The ribosomal DNA probes were mixed to a final concentration of about 2.5 ng  $\mu\text{l}^{-1}$  and pre-denatured (80 °C for 10 min). The slides with chromosome material were then denatured along with the hybridization mixture (70 °C for 4.5 min) and allowed to hybridize for 12–18 h in a humid chamber at 37 °C. The post-hybridization washes were carried out in two changes of 20 % deionized formamide in 0.1× SSC, 5 min each, at 42 °C, which is equivalent to 85 % stringency. Immunodetection of digoxigenated probes was done according to standard protocol using FITC-conjugated anti-digoxigenin antibodies (Roche). The preparations were mounted and counterstained in Vectashield (Vector Laboratories) containing 2.5  $\mu\text{g ml}^{-1}$  of DAPI (4',6-diamidino-2-phenylindole; Serva).

### *Image capturing and processing*

All images were acquired using either an Olympus Camedia C-4040Z digital camera attached to a Leica DMRB epifluorescence microscope or a Hamamatsu C5810 CCD camera attached to an Olympus Provis AX epifluorescence microscope and processed uniformly using Micrografix (Corel) Picture Publisher software.

## RESULTS AND DISCUSSION

Total numbers of 5S rDNA and 25S rDNA sites in all 52 accessions of the 15 species studied are summarized in Table 1. The fractions of sites at which the two classes of ribosomal genes are co-localized are also indicated. Figures 1–5 illustrate selected chromosomal distributions of rDNA sites in 18 accessions representing 13 species of Brassicaceae. Table 2 provides detailed comparative information about types and distributions of rDNA-bearing chromosomes found in these selected examples, without listing other distributions recorded in these accessions (Table 1). Emphasis in Table 2 has been placed on taxa or rDNA distributions not reported before. The idiograms of the base types of chromosomes bearing 5S rDNA and 25S rDNA loci and their variants are presented in Table 3. The base chromosomal types, numbered I–VIII, have been introduced and described in detail by Hasterok *et al.* (2001), with the exception that the 5S rDNA site in chromosome type V is now assigned to the short arm. These types have originally been used to describe the chromosomal distribution of rDNA sites in the three genomes (A, B and C) that constitute the six most studied crop species of *Brassica*. Most of these types have also been found in related species and genera.

TABLE 1. Origins, chromosome numbers, total numbers of 5S and 25S rDNA sites, and the fraction of these sites at which the two types of rDNA are co-localized, of the 52 accessions of the 15 Brassicaceae species studied\*

Taxon (common name; genome)	2n	Number of rDNA sites			Origin <sup>†</sup>
		5S	25S	(5S + 25S)	
<i>Brassica</i>					
<i>B. fruticulosa</i> (Mediterranean cabbage; F) subsp. <i>fruticulosa</i>	16	4	4	(2)	a
<i>B. nigra</i> (L.) Koch (black mustard group; B)					
var. <i>occidentalis</i> '128'	16	2	6	–	b
var. <i>occidentalis</i> '1858'	16	2	6	–	–
var. <i>occidentalis</i>	16	2	6	–	c
<i>B. cretica</i> Lam. (C <sub>7</sub> )	18	2	4–5	–	d
<i>B. oleracea</i> group (cabbage group; C)					
subsp. <i>oleracea</i> convar. <i>botrytis</i> var. <i>alboglabra</i> (Bail.)	18	2	4	–	–
subsp. <i>oleracea</i> convar. <i>botrytis</i> var. <i>alboglabra</i> (Bail.) 'No. 4003'	18	2	4 <sup>‡</sup>	–	e
subsp. <i>oleracea</i> convar. <i>botrytis</i> var. <i>italica</i> Plenck (broccoli)	18	2	4	–	f
subsp. <i>oleracea</i> convar. <i>botrytis</i> var. <i>botrytis</i> L. (cauliflower)	18	2	4	–	g
subsp. <i>oleracea</i> convar. <i>acephala</i> var. <i>gongylodes</i> L. 'Delikates Biala' (kohlrabi)	18	2	4–5	–	h
subsp. <i>oleracea</i> convar. <i>acephala</i> var. <i>gongylodes</i> L. 'Niebieska Maslowa' (kohlrabi)	18	2	4	–	g
subsp. <i>oleracea</i> convar. <i>viridis</i> L. 'Jarmuz Sredniowysoki' (kale)	18	2	4	–	i
subsp. <i>oleracea</i> convar. <i>capitata</i> var. <i>capitata</i> L. 'Kissendrup Swe' (common cabbage)	18	2	4	–	g
subsp. <i>oleracea</i> convar. <i>capitata</i> var. <i>capitata</i> L. 'Koda' (common cabbage)	18	2	4	–	g
subsp. <i>oleracea</i> convar. <i>gemmifera</i> var. <i>gemmifera</i> DC. 'Maczuga' (Brussels sprouts)	18	2	4	–	g
subsp. <i>oleracea</i> convar. <i>capitata</i> var. <i>sabauda</i> L. 'Langedijker Dauer' (Savoy cabbage)	18	2	4	–	g
subsp. <i>oleracea</i> convar. <i>capitata</i> var. <i>capitata</i> L. 'Kamienna Glowa' (common cabbage)	18	2	4	–	j, k
subsp. <i>oleracea</i> convar. <i>capitata</i> var. <i>capitata</i> L. 'Amager' (common cabbage)	18	2	4	–	g
subsp. <i>oleracea</i> (ornamental form)	18	2	4	–	g
'Bpl 3-1' (rapid-cycling form)	18	2	4	–	l
<i>B. oxyrrhina</i> (O)	18	4	4	(2)	d
<i>B. rapa</i> group (turnip/bird rape group; A)					
subsp. <i>oleifera</i> (DC.) 'Schneeball' (oilseed turnip)	20	6	6	(4)	g
subsp. <i>pekinensis</i> (Lour.) (Chinese cabbage)	20	6	10	(4)	g
subsp. <i>rapa</i> 'Goldball' (turnip)	20	6	10	(4)	g
subsp. <i>rapa</i> 'Goldball' (turnip)	20	8	10	(6)	m
'Bpl 1-1' (rapid-cycling form)	20	9	10	(7)	l
subsp. <i>trilocularis</i> (Roxb.) 'K-151' (Indian colza)	20	10	10	(8)	e
<i>B. tournefortii</i> Gouan '1412' (Asian mustard; T)	20	4	2	(2)	a
<i>B. carinata</i> group (mustard collar group; BC)					
's-67'	34	4	8–10	–	n
'Yellow Dodolla'	34	4	8–10	–	n
<i>B. juncea</i> group (India mustard group; AB)					
var. <i>crispa</i> '685'	36	8–10	12	(4–6)	o
'684'	36	10	14	(6)	o
'Muasiao'	36	10	16	(6)	p
'9565'	36	10	16	(6)	o
'Malopolska'	36	10	16	(6)	q
<i>B. napus</i> group (rape; AC)					
'429'	38	9–11	10–11	(5–7)	o
subsp. <i>napus</i> 'Kana' (rapeseed)	38	10	12	(6)	r
subsp. <i>napobrassica</i> 'Brukiew Wilhelmburska' (swede)	38	10	14	(6)	g
'Bpl 5-1' (rapid-cycling form)	38	10	14	(8)	l
'K-151' × 'No. 4003' (resynthesized)	38	12	14	(8)	e
subsp. <i>napus</i> 'Licosmos 00' (rapeseed)	38	12	14	(8)	s
subsp. <i>napus</i> 'Marita' (rapeseed)	38	12	14	(8)	r
<i>Diplotaxis</i>					
<i>D. muralis</i> '690' (wall rocket; D <sub>m</sub> ) (putative allotetraploid)	42	6	6	(2)	t
<i>Eruca</i>					
<i>E. vesicaria</i> (garden rocket; E) subsp. <i>sativa</i>	22	2	7	(2)	o
<i>Raphanus</i>					
<i>R. sativus</i> (radish; R)					
convar. <i>sativus</i> '186'	18	4	6	(2)	b
convar. <i>sativus</i> 'Opolanka'	18	5	5	(3)	g
convar. <i>sativus</i> 'Agata' (autotetraploid)	36	8	8	(4)	g
<i>Sinapis</i> (mustard; S)					
<i>S. arvensis</i> L. '1524' (charlock; S <sub>ar</sub> )	18	4	6	(2)	a
<i>S. alba</i> (white mustard; S <sub>al</sub> )					
subsp. <i>alba</i> '755'	24	4	8	(2)	t
subsp. <i>alba</i> 'Kamis'	24	4	8	(2)	u
subsp. <i>dissecta</i> (Lag.)	24	4	8	(2)	a
subsp. <i>alba</i> var. <i>oleifera</i> '774' (autotetraploid)	48	8	16	(4)	o

Botanical nomenclature used in this study follows Mansfeld's Taxonomic Database of Crop Plants (<http://mansfeld.ipk-gatersleben.de/mansfeld/Query.htm>).

\* Five individuals from each accession, except for the two accessions of *B. carinata* from which 20 individuals, were studied.

<sup>†</sup> a, Botanical Garden (BG) Berlin, Dahlem, Germany; b, Conservatoire et jardins Botaniques de Nancy, France; c, BG Powsin, Poland; d, Coimbra, Portugal; e, Swedish University of Agricultural Sciences, Alnarp, Sweden; f, CNOS Wrocław, Poland; g, PNOS Ozarów Mazowiecki, Poland; h, Coleus Mosina, Poland; i, CNOS Poznań, Poland; j, PNOS Toruń, Poland; k, Flora-Samen sp. z o.o., Jazgarzew k/Piaseczna, Poland; l, CrGC Madison, USA; m, PNOS Kraków, Poland; n, Holetta R. Center, Ethiopia; o, UMCS BG Lublin, Poland; p, Glasgow, UK; q, PHN Lublin, Poland; r, ZDHiAR Malyszyn, Poland; s, PPHU Kruszwica, Poland; t, BG Gottingen, Germany; u, Kamis, Przyprawy SA, Wolka Kosowska, Poland.

<sup>‡</sup> Polymorphism regarding the localization of two sites.

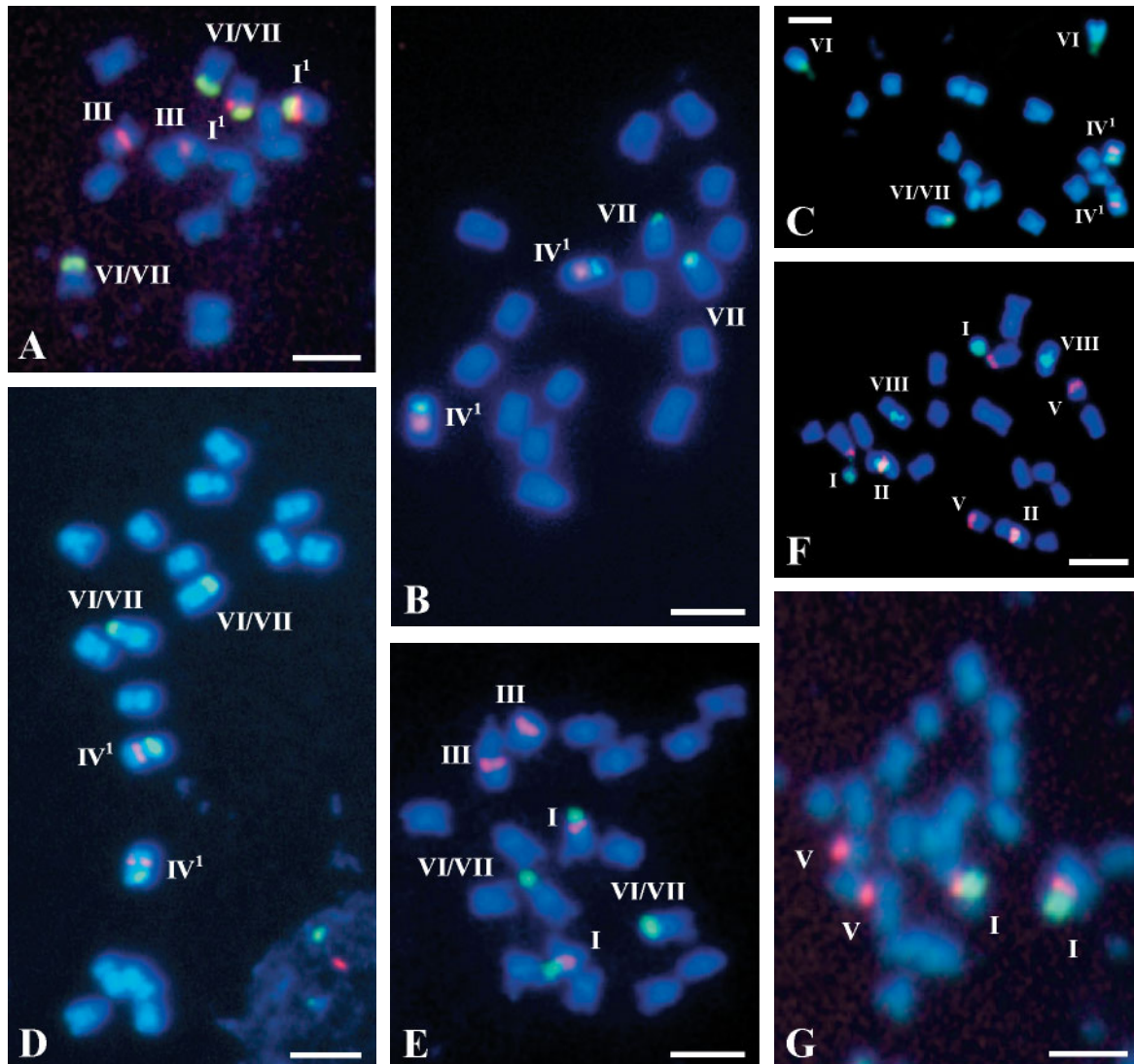


FIG. 1. Fluorescence *in situ* hybridization of 5S (red) and 25S (green) ribosomal DNA probes to somatic metaphase chromosomes of (A) *Brassica fruticulosa*, (B and C) *B. cretica*, (D) *B. oleracea* 'No. 4003', (E) *B. oxryrhina*, (F) *B. rapa* 'Schneeball', (G) *B. tournefortii* '1412'. The nomenclature of rDNA-bearing chromosomal types (Roman numerals) in Fig. 1–5 is according to Hasterok *et al.* (2001), supplemented by the new and variant chromosomal types identified in the present study. The chromosomes are counter-stained with DAPI (blue). White arrows visible on some photographs help to link the Roman numerals with their rDNA-bearing chromosomes. All scale bars = 5  $\mu$ m.

To be emphasized here is the fact that a certain type may refer to a specific chromosome in a defined complement (Hasterok *et al.*, 2005). It may also refer to undefined chromosomes in the same complement and/or homoeologues or non-homoeologues in different complements.

As far as is known, localization of 5S and 25S rDNA has been determined for the first time in six taxa: *Brassica fruticulosa*, *B. cretica*, *B. oxryrhina*, *B. tournefortii*, *Diplotaxis muralis* and *Eruca vesicaria* (Figs 1A–C, E and G and 4A and B and Tables 1 and 2). In addition to the occurrence of the eight base types and possible loss of rDNA loci in types II, VI, VII and VIII, the presence of three new types of chromosomes with rDNA sites is documented from the Brassicaceae material studied (Table 3). The new types are treated as variants of types I (I<sup>1</sup> and I<sup>2</sup>) and IV (IV<sup>1</sup>). Thus, type I might appear as type I<sup>1</sup> by having a

non-distended secondary constriction and/or a minor satellite, or by lacking the satellite. Alternatively, type I<sup>1</sup> may represent a different intact or structurally altered chromosome, especially in those cases that have the expected number of type I chromosomes (*B. napus* 'Bpl 5-1' and *Raphanus sativus* 'Opolanka'; Figs 3D and 4D, respectively). The variant type I<sup>2</sup> was identified in the chromosome complement of *Sinapis arvensis* (data not shown). This type is similar to the variant type I<sup>1</sup> except that the 5S rDNA site is localized proximally in the long arm and not distally on the short arm. The distension/non-distension of the secondary constriction, the presence/absence of the satellite or difference between satellite size resulting in variants I<sup>1</sup> and I<sup>2</sup> could well explain the difficulty in distinguishing types VI and VII in certain cases (Table 2), exemplified by *B. carinata* (Fig. 2A and B).

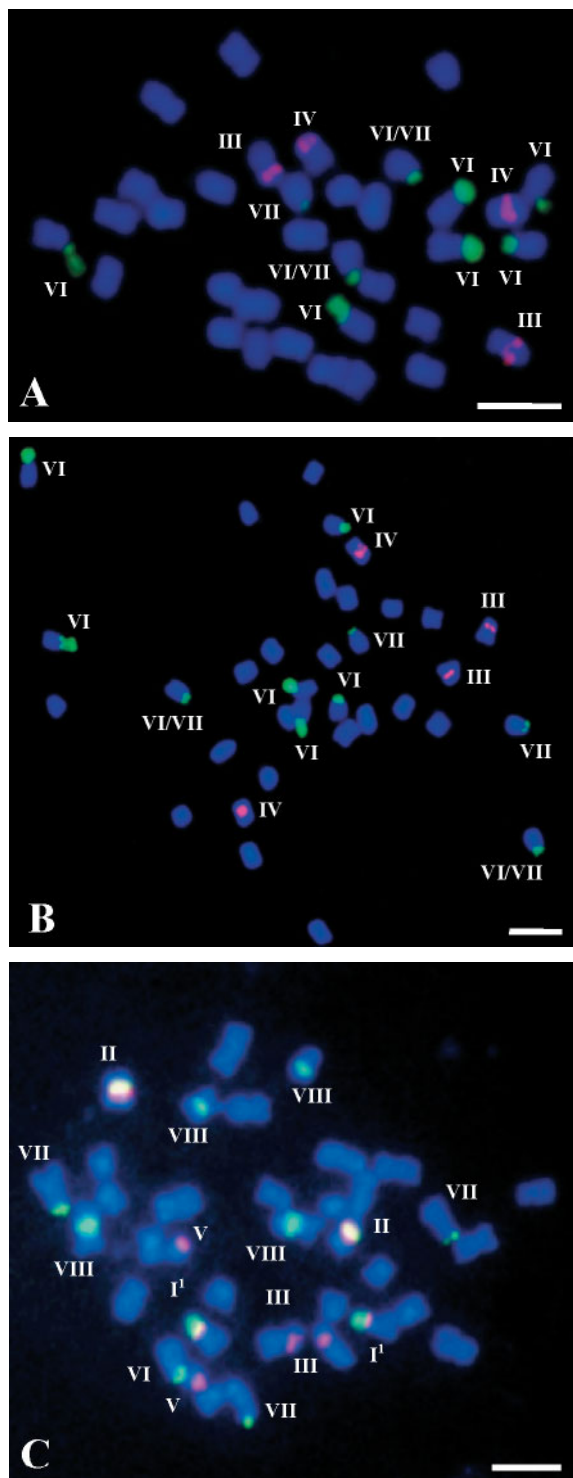


FIG. 2. FISH of 5S and 25S rDNA probes to somatic metaphase chromosomes of (A and B) *B. carinata* 'Yellow Dodolla' and (C) *B. juncea* '685'.

Type IV<sup>1</sup>, a variant of type IV specific for the C genome, is characterized by the presence of an intercalary site of 25S rDNA on the short arm and one proximal site of 5S rDNA, instead of two, on the long arm. This chromosomal type, present in the genome of the wild species *B. cretica*

(Fig. 1B and C), was occasionally observed in some forms of *B. oleracea* (e.g. in some individuals of 'No. 4003'; Fig. 1D).

Possibly interrelated base and variant chromosome types, occasionally indistinguishable types and loss of rDNA from certain types are indicated by arrows in Table 3. The prevalence of these features in many Brassicaceae species is the basis of the polymorphism in number, appearance and chromosomal distribution of both 5S and 25S ribosomal DNA loci.

#### Intergenomic variation in number of ribosomal DNA loci

The genomes of the materials studied are indicated in Table 1. For simplification the genome of *Diplotaxis muralis* ( $2n = 42$ ) has been marked as D<sub>m</sub>, although this species is considered to be an allotetraploid, most likely from *D. viminea* ( $2n = 2x = 20$ ) and *D. tenuifolia* ( $2n = 2x = 22$ ) (Martinez-Laborde, 1997). The number of 5S and 25S rDNA loci differs significantly between different genomes. The lowest somatic number of 25S rDNA sites (2) among the diploids was observed in the T genome of the wild species *B. tournefortii* (Fig. 1G), while the highest (10) was found in most accessions of *B. rapa* with the same chromosome number but containing the A genome (Table 1). Also, all three accessions of *Sinapis alba* invariably had eight sites of 25S rDNA (Fig. 5A). However, the most commonly observed number of 25S rDNA sites in the diploids was four (found in *B. fruticulosa*, *B. oxyrrhina*, the majority of individuals in *B. cretica* and almost all accessions of *B. oleracea* studied) and six (*B. nigra*, one accession of *B. rapa*, *D. muralis*, one accession of *R. sativus* and in *S. arvensis*).

Similarly, in the case of 5S rDNA there were several diploids, i.e. three accessions of *B. nigra*, one accession of *B. cretica* (Fig. 1B and C), all accessions of *B. oleracea* (Table 1 and Fig. 1D) and one accession of *E. vesicaria* (Fig. 4B) that possessed the minimum number of two sites per somatic chromosome complement. A large group of diploid species comprising *B. fruticulosa* (Fig. 1A), *B. oxyrrhina* (Fig. 1E), one accession of *R. sativus* (Fig. 4C), *S. arvensis* and three accessions of *S. alba* (Fig. 5A) has four sites. The highest number of 5S rDNA sites was again observed in *B. rapa*, from six (Hasterok et al., 2001) up to ten (Hasterok et al., 2005).

The two kinds of rDNA sites appeared on the same chromosome in 12 out of the 15 species studied. In these cases the sites were either co-localized on the same arm or were on different arms. It can be concluded from the above that despite having the same chromosome number, different genomes of Brassicaceae may differ up to 5-fold in the number of rDNA loci. A correlation between the increase in the number of rDNA sites and the increase of chromosome number in the genome, if existing at all, is very weak.

#### Variation in chromosomal distribution of rDNA loci

Comparative analyses revealed that polymorphism in the number and chromosomal distribution of 5S and 25S rDNA occurs both among different subspecies and varieties of a

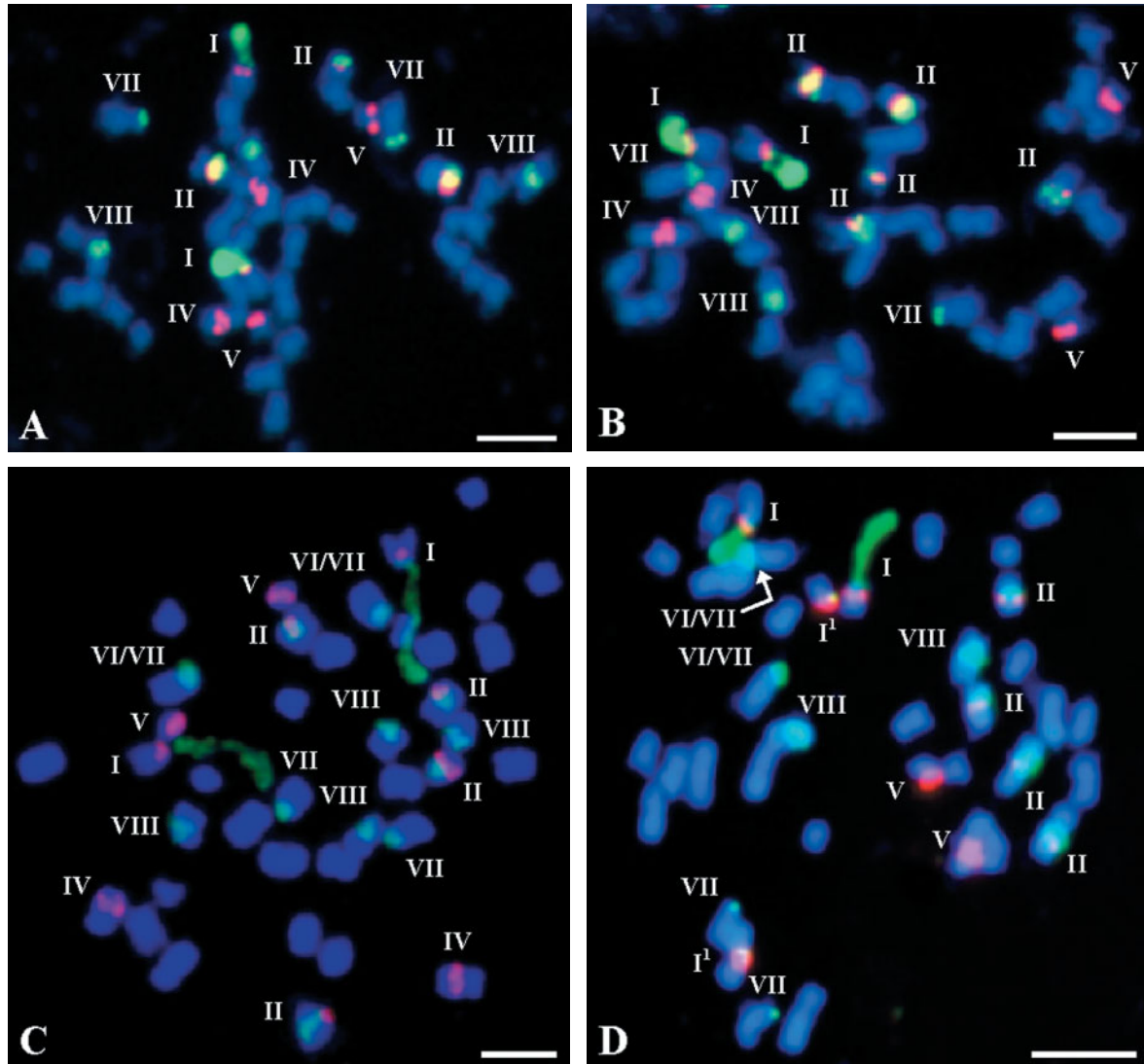


FIG. 3. FISH of 5S and 25S rDNA probes to somatic metaphase chromosomes of (A and B) *Brassica napus* '429', (C) *B. napus* 'Brukiew Wilhelmburska' and (D) *B. napus* 'Bpl 5-1' (rapid-cycling form).

given species and within a population of individuals that belong to one variety or cultivar. However, no polymorphism was encountered within an individual. Inter- and/or intravarietal polymorphism was observed in seven out of 15 species, i.e. in *B. cretica*, *B. oleracea*, *B. rapa*, *B. carinata*, *B. juncea*, *B. napus* and *R. sativus* (Table 1). Noticeably, all species that contain the A genome in their chromosome complement, i.e. *B. rapa*, *B. juncea* and *B. napus*, displayed high polymorphism in number and chromosomal localization of rDNA sites. The chromosomes of this genome carry extraordinary high numbers of both 5S rDNA and 25S rDNA sites (Hasterok *et al.*, 2001), a feature which is possibly the cause of their polymorphism in number and distribution.

Detailed analysis of rDNA distribution in the six accessions of *B. rapa* revealed that only the chromosomes with proximally and/or pericentromerically distributed ribosomal rRNA genes were polymorphic. These chromosomes

represented types II and VIII. It seems that the same chromosome may occur in different materials as type II, type VIII or as an unidentified chromosome without any rDNA landmark, depending upon the presence or absence of proximal and pericentromeric 5S and 25S rDNA sites (Table 3). It seems that the proximal 5S rDNA site in the A genome is more liable to change than its pericentromeric 25S rDNA counterpart, as absence of the 5S rDNA site was more common than the lack of the 25S rDNA site. This was observed in three out of six accessions of *B. rapa* (Table 1) leading to the more frequent occurrence of type VIII compared with type II chromosomes.

In *B. rapa* 'Schneeball', two chromosomes of type I and only two chromosomes of each of types II and VIII constitute a total of six sites of 25S rDNA (Fig. 1F), which is the lowest so far observed for *B. rapa* (Table 1). All potential sites lacking rDNA were invariably proximal and pericentromeric in the five individuals studied. Absence of

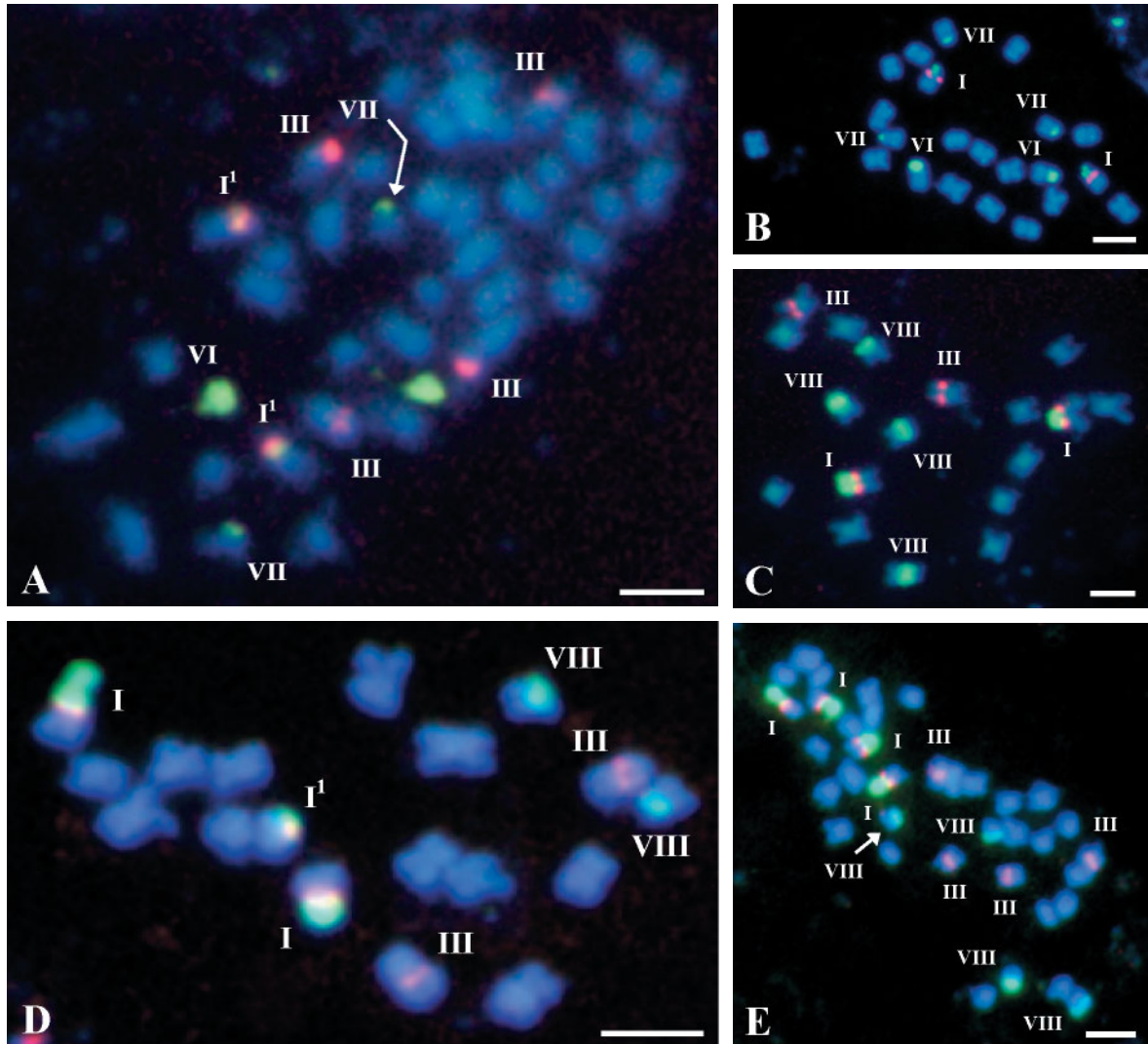


FIG. 4. FISH of 5S and 25S rDNA probes to somatic metaphase chromosomes of (A) *Diplotaxis muralis* '690', (B) *Eruca vesicaria*, (C) *Raphanus sativus* '186', (D) *R. sativus* 'Opolanka' and (E) *R. sativus* 'Agata' (autotetraploid).

such loci from type II and VIII chromosomes makes these chromosomes unidentifiable. At the other extreme, the presence of both 5S and 25S rDNA sites in all potential sites leads to over-representation of type II chromosomes, raising the total number of each of the 5S and 25S rDNA sites to ten (subsp. *trilocularis* 'K-151') (Hasterok *et al.*, 2005). Interestingly, no accession of A genome-containing *Brassica* species had only the 5S rDNA site retained at the proximal site of a putative type II chromosome (Table 3). Variation in numbers of chromosome types II and VIII also partially contributed to the polymorphism of rDNA sites in the two allopolyploid *Brassica* species that contain the A genome, i.e. *B. juncea* (Fig. 2C) and *B. napus* (Fig. 3A–D). On the other hand chromosome types V and I were not prone to such variation. Chromosome type V with a solitary 5S rDNA locus in the distal part of the short arm was never involved. Also chromosome type I, carrying 5S rRNA genes in the short arm and 25S rDNA in the secondary constriction and its adjacent regions, was almost constant in this

genome. Cytochemical analysis of 18S–5.8S–25S rRNA gene activity by silver staining revealed that chromosome type I is the only type in the A genome that carries transcriptionally active clusters of these genes, while those in loci near centromeres are not expressed (Hasterok and Maluszynska, 2000). Possibly, the sites that contain such inactive 45S rDNA are more prone to loss from the chromosome. It is more difficult, however, to speculate if the same applies to 5S rDNA sites, as there is no cytochemical method available that could distinguish active and inactive loci of these genes.

In contrast to the A genome, genome B in *B. nigra* and genome C in *B. oleracea* contain more than one pair of chromosomes with distal sites of 25S rDNA (types VI and VII) which are either actually (Hasterok and Maluszynska, 2000) or potentially (Hasterok and Maluszynska, 1999) transcriptionally active. That the type I pair of chromosomes is the only one in the A genome that carries active 25S rDNA genes, possibly relates to the

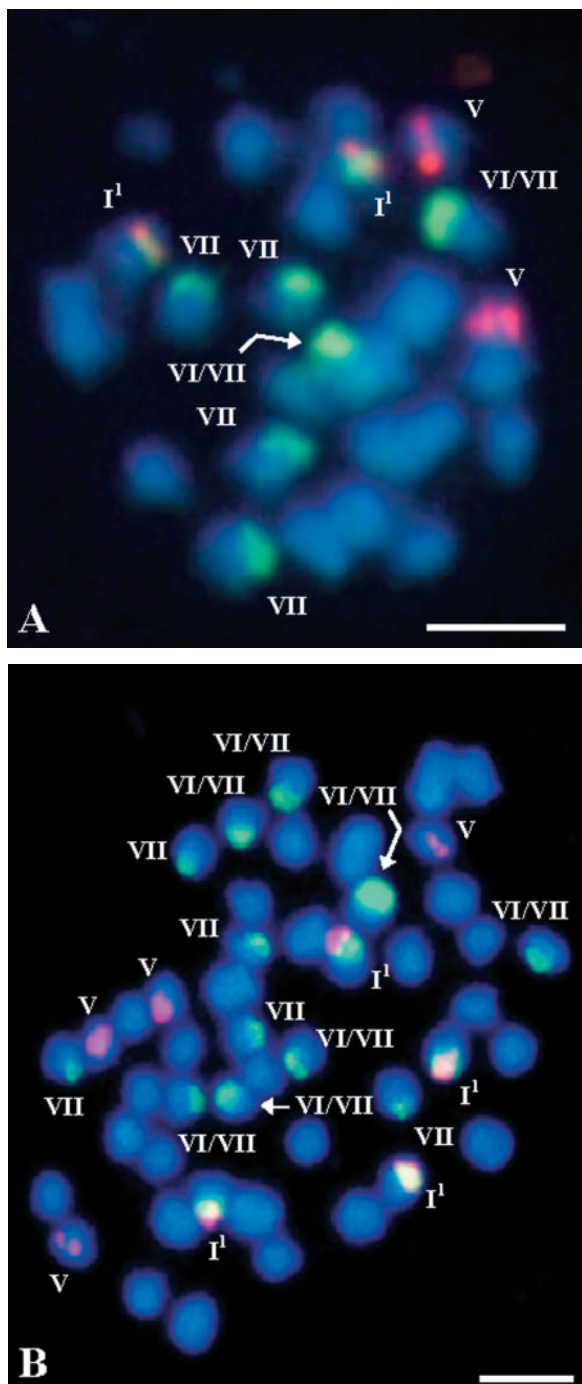


FIG. 5. FISH of 5S and 25S rDNA probes to somatic metaphase chromosomes of (A) *S. alba* subsp. *dissecta*, (B) *S. alba* '774' (autotetraploid).

stability of this site. Apart from the occurrence of type I<sup>1</sup> in some of the A genome-containing allotetraploids (i.e. in *B. juncea* '685'; Fig. 2C), the distal rDNA sites of type I in the A genome never undergo any marked change in number, implying the loss of the entire locus. This is not the case in type VI and VII chromosomes of the B, C and C<sub>r</sub> genomes, where such distal loci may be polymorphic through their presence or absence both in diploids (Fig. 1B vs C) and more

commonly in allopolyploid species of *Brassica* (e.g. Fig. 2A vs B, and 3A and B vs C and D).

The results of the present analyses based on several forms of both diploid and allopolyploid B or/and C genome-containing species suggest that probably the same chromosome can be either type VI or type VII, depending upon the appearance of its short arm (Table 3). As the phenomena of distended/non-distended secondary constriction, presence/absence of satellite, or variation in satellite size were consistent and in many cases form-specific, it is supposed that this may largely reflect some differences in the activity of individual loci (Flavell, 1986). An analogous situation might pertain to chromosome type I, where in some forms of *B. juncea* it possibly appeared as type I<sup>1</sup> (e.g. Fig. 2C and Table 3).

Interindividual polymorphism was investigated in detail in two accessions of *B. carinata*, 's-67' and 'Yellow Dodolla'. The number of 45S rDNA sites was recorded for 20 individuals in each accession and ranged from eight to ten, while four sites of 5S rDNA were consistently found in both materials (Fig. 2A and B). Regardless of accession, the individuals with eight or ten sites of 45S rDNA in total represented 90% of the population, while the remaining 10% were the individuals with nine sites. Interindividual polymorphism in the number and/or distribution of 45S rDNA sites was also occasionally observed in *B. cretica*, some accessions of *B. oleracea*, *B. juncea* and *B. napus* (Table 1), but no detailed investigations were made.

An interesting case of chromosome polymorphism concerns type IV of the C genome. This type is characterized by the presence of two adjacent 5S rDNA sites near the centromere on the long arm and was recorded in earlier investigations of *B. oleracea*, *B. napus* and *B. carinata* (Armstrong *et al.*, 1998; Hasterok *et al.*, 2001; Kulak *et al.*, 2002; Snowdon *et al.*, 2002). That the 5S rDNA genes in this chromosome may be detectable as one signal instead of two in *B. oleracea* 'No. 4003' (Hasterok *et al.*, 2005) was further encountered in the *B. oleracea* accessions: 'Delikates Biala' and 'Kissendrup Swe'. Analysis of both metaphase and prometaphase chromosome spreads suggests that this phenomenon is more likely to be caused by a significant decrease in copy number, or complete loss, of 5S rDNA at one locus, rather than differences in the extent of chromatin condensation influencing mapping resolution.

The origin of chromosome type IV<sup>1</sup> (Table 3), found in some individuals of *B. oleracea* 'No. 4003' (Fig. 1D) and 'Delikates Biala', is debatable. Its origin is connected with the presence of a highly polymorphic additional site of 25S rDNA which is localized interstitially in the short arm. In most accessions of *B. oleracea* studied so far, this locus is absent. The existence of such an interstitial locus of 25S rDNA on the 5S rDNA-bearing chromosome of the C genome has been reported by Howell *et al.* (2002) in another cultivar of *B. oleracea* var. *alboglabra*, but in this case the locus was additional to those on chromosomes types VI and VII. In the present study on the other hand, the existence of type IV<sup>1</sup> chromosomes was always linked with an absence of a 25S locus on type VI or VII chromosomes.



TABLE 2. Selected unreported distributions of rDNA chromosomal sites observed in 18 accessions of the 13 Brassicaceae species studied

Taxon (genome)	2n	rDNA-bearing chromosomal types								Fig.	
		I	II	III	IV	V	VI	VII	(VI/VII)*		VIII
<i>Brassica fruticulosa</i> (F) subsp. <i>fruticulosa</i>	16	2 <sup>1†</sup>	–	2	–	–	–	–	2	–	1A
<i>B. cretica</i> (C <sub>r</sub> )	18	–	–	–	2 <sup>1</sup>	–	–	2	–	–	1B
	18	–	–	–	2 <sup>1</sup>	–	2	–	1	–	1C
<i>B. oleracea</i> (C) ‘No. 4003’	18	–	–	–	2 <sup>1</sup>	–	–	–	2	–	1D
<i>B. oxyrrhina</i> (O)	18	2	–	2	–	–	–	–	2	–	1E
<i>B. rapa</i> (A) ‘Schneebeal’	20	2	2	–	–	2	–	–	–	2	1F
<i>B. tournefortii</i> (T) ‘1412’	20	2	–	–	–	2	–	–	–	–	1G
<i>B. carinata</i> (BC) ‘Yellow Dodolla’	34	–	–	2	2	–	6	1	2	–	2A
	34	–	–	2	2	–	6	2	2	–	2B
<i>B. juncea</i> (AB) ‘685’	36	2 <sup>1</sup>	2	2	–	2	1	3	–	4	2C
<i>B. napus</i> (AC) ‘429’	38	2	3	–	2	2	–	2	–	3	3A
	38	2	5	–	2	2	–	2	–	2	3B
‘Brukiew Wilhelmburska’	38	2	4	–	2	2	–	2	2	4	3C
‘Bpl 5-1’ (rapid-cycling form)	38	2 + 2 <sup>1‡</sup>	4	–	–	2	–	2	2	2	3D
<i>Diplotaxis muralis</i> (D <sub>m</sub> ) ‘690’	42	2 <sup>1</sup>	–	2 + 2	–	–	2	2	–	–	4A
<i>Eruca vesicaria</i> (E) subsp. <i>sativa</i>	22	2	–	–	–	–	2	3	–	–	4B
<i>Raphanus sativus</i> (R) ‘186’	18	2	–	2	–	–	–	–	–	4	4C
‘Opolanka’	18	2 + 1 <sup>1</sup>	–	2	–	–	–	–	–	2	4D
‘Agata’ (autotetraploid)	36	4	–	4	–	–	–	–	–	4	4E
<i>Sinapis alba</i> (S <sub>al</sub> ) subsp. <i>dissecta</i>	24	2 <sup>1</sup>	–	–	–	1 + 1	–	4	2	–	5A
‘774’ (autotetraploid)	48	4 <sup>1</sup>	–	–	–	2 + 2	–	5	7	–	5B

\*Types VI and VII are considered as one group when not distinguishable.

† Superscript numbers indicate the variant types I<sup>1</sup>, I<sup>2</sup> and IV<sup>1</sup>.

‡ Two values are given when distinct and consistent differences in size of signal occur on chromosomes of the same type.

Two chromosomes type IV<sup>1</sup> were also observed in all analysed individuals of *B. cretica* (Fig. 1B and C), the wild species that contains the C<sub>r</sub> genome, which in number and morphometric characteristics of its chromosomes, as well as distribution of rDNA sites, closely resembles the C genome. This seems to confirm the hypothesis that *B. cretica* could be the putative ancestor of *B. oleracea* (Prakash and Hinata, 1980).

Further comparisons between the rDNA distribution patterns documented in the diploid species studied (Table 1) may be considered in a phylogenetical context. Based on traditional taxonomy and molecular analyses, primarily on ribosomal internal transcribed spacer (ITS) sequences, it has been shown that *B. oleracea* and *B. rapa* belong to the same clade, while *B. nigra* and *Sinapis arvensis* belong to another clade, and *Raphanus* is a ‘hybrid’ between these two clades (Yang *et al.*, 1999; Koch *et al.*, 2003; Warwick and Sauder, 2005). The present FISH results highlight the low number of sites of a less variable rDNA in *B. oleracea* in contrast to the high number of sites of a more variable rDNA in *B. rapa*, considered to be in the same clade. This may necessitate a re-evaluation of the interrelationships between these two species. Whether the deviation or divergence of rDNA patterns reflects possible differences in the evolutionary ages of these taxa or not remains to be substantiated. The number and variation in rDNA patterns of *R. sativus* represents an intermediate position between the invariable *B. nigra* and the variable *B. rapa*. *Brassica fruticulosa* and *B. oxyrrhina* with their nearly identical rDNA FISH patterns, and *B. tournefortii* with a slightly deviating pattern, are all closely

related according to the ITS analysis (Warwick and Sauder, 2005). It is to be remembered that one should consider the ribosomal DNA FISH and molecular ITS data with caution when inferring phylogenies, due to limitations such as possible lack of homology, difficulties in defining orthologues from paralogues, and prevalence of non-functional rDNA pseudogenes (Alvarez and Wendel, 2003; Bailey *et al.*, 2003; Dobigny *et al.*, 2004).

#### Number of rDNA sites and polyploidization

It was shown by Maluszynska and Heslop-Harrison (1993) that the number of 45S ribosomal DNA sites in the genomes of the three allotetraploid crop species of *Brassica* was lower than the sum of the sites observed in their putative diploid ancestral species. The number of loci that were absent from chromosome complements of the allotetraploids was either two (*B. carinata* and *B. juncea*) or four (*B. napus*). In a later study, Snowdon (1997) reported an identical situation for *B. juncea* and *B. napus*, while the number of rDNA sites in the *B. carinata* genome equalled the sum of loci observed in its diploid ancestors. The decrease in the expected number of both 5S and 45S ribosomal DNA sites during polyploid evolution was also observed in other plant species, such as *Scilla autumnalis* (Vaughan *et al.*, 1993), *Nicotiana tabacum* (Volkov *et al.*, 1999) and in species of *Sanguisorba* (Mishima *et al.*, 2002). This phenomenon is usually explained as a possible consequence of diploidization, i.e. a consequence of transient genome imbalance following hybridization. The ribosomal

TABLE 3. The base and variant types of chromosomes bearing or lacking 5S rDNA (red) and 25S rDNA (green) loci in the genomes of Brassicaceae species

Base chromosomal types	I	II	III	IV	V	VI	VII	VIII
Genomes	A	A	B	C	A	B,C	B,C	A
	D <sub>m</sub> ,E,F,O,R,S <sub>al</sub> ,T	C,D <sub>m</sub> ,F, O,R,S <sub>ar</sub>			S <sub>al</sub> ,T	C <sub>r</sub> ,D <sub>m</sub> ,E, O,S <sub>ar</sub>	C <sub>r</sub> ,D <sub>m</sub> ,E,F, O,S <sub>al</sub> ,S <sub>ar</sub>	R
Genomes								
Variants	I <sup>1</sup>	I <sup>2</sup>		IV <sup>1</sup>				
Genomes	A,D <sub>m</sub> ,F, R,S <sub>al</sub> ,T	S <sub>ar</sub>	A	C,C <sub>r</sub>		B,C,C <sub>r</sub>	B,C,C <sub>r</sub>	A,R
Genomes								

Differences in size of these chromosome types and in size of rDNA signals were observed between chromosomes of the same type within and between accessions; thus a type can refer to different chromosomes.

The size of the satellite in types I and VI may differ between accessions.

Non-distension of the secondary constriction and/or smaller size or loss of the satellite possibly produce type I<sup>1</sup> from type I. Alternatively, type I<sup>1</sup> may represent a different chromosome. Type I<sup>2</sup>, probably a variant of type I or I<sup>1</sup>, represents a chromosome variant observed so far only in the genome S<sub>ar</sub>.

Arrows indicate possible variation in the appearance of a certain chromosome type that leads to its manifestation as another type.

RNA genes contributed from the two ancestral genomes could constitute an excess of these genes and, as a consequence, precipitate the elimination of some dispensable loci (Dvorak, 1990).

The current observations do not support the above conclusion. It seems that there is no clear tendency to reduce or increase the number of rDNA loci in the allotetraploid species of *Brassica*. This was the case in the resynthesized *B. napus* 'K-151' × 'No. 4003' in which both numbers of 5S rDNA and 25S rDNA sites were exactly the sum of what was observed in the parental types (Table 1). The variation in rDNA patterns in the current material is most likely caused by distinct intervarietal and interindividual polymorphism in the number of ribosomal DNA sites observed in both diploid and allotetraploid species of *Brassica*.

Of interest is the comparison of the number and chromosomal localization of rDNA sites between the diploid (Fig. 4C and D) and allotetraploid (Fig. 4E) accessions of *R. sativus* (Tables 1 and 2). A clearly visible deficit of 25S rDNA loci in the allotetraploid is caused by a lower than expected number of type VIII chromosomes, or an

absence of extra type I<sup>1</sup> chromosomes. It can be quite difficult, however, to unambiguously interpret this observation. One possible explanation could be the absence of two pairs of type VIII chromosomes in the allotetraploid. On the other hand, the presence of two such pairs in the diploid which contributed to the allotetraploid cannot be ruled out. The number and distribution of ribosomal DNA sites observed earlier in diploid *R. sativus* (Schrader *et al.*, 2000) seem to suggest that the latter explanation is more likely.

In contrast to *R. sativus*, the numbers of both kinds of ribosomal DNA sites observed in the allotetraploid accession of *S. alba* (Fig. 5B) were exactly twice the numbers in the three diploid accessions, which may imply lack of highly polymorphic rDNA sites in the S<sub>al</sub> genome. Also, this may suggest that in spite of both the higher numbers of chromosomes in the complement in general and 25S rDNA sites in particular, no detectable chromosome rearrangements involving ribosomal DNA-containing regions have occurred in the allotetraploid form of *S. alba*. Noticeably, changes in chromosome structure were reported for some other species, e.g. in the colchicine-induced allotetraploid

of *A. thaliana*, which was explained in terms of a diploidization feature (Weiss and Maluszynska, 2000).

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