Theor Appl Genet (1998) 96 : 251–265

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Production and cytogenetics of the intergeneric hybrids *Brassica juncea* × *Orychophragmus violaceus* and *B. carinata* × *O. violaceus*

Received: 4 February 1997 / Accepted: 29 July 1997

Abstract Intergeneric hybrids between *Brassica juncea* (2n = 36), *B. carinata* (2n = 34) and *Orychophragmus violaceus* (2n = 24) were produced when *B. juncea* and *B. carinata* cultivars were used as female parents. The hybrids between *B. juncea* and *O. violaceus* had an intermediate morphology except for petal colour and were partially fertile. The hybrids between *B. carinata* and *O. violaceus* had a matroclinous morphology and were nearly fertile. Cytological analysis of the hybrids and their progenies gave the following results. (1) In the hybrids between *B. juncea* and *O. violaceus*, the somatic tissues of the roots, leaves and styles were mixoploid (2n = 12–42), and cells with 24, 30 or 36 chromosomes were the most frequent. Based on the recorded numbers and behaviour of the mitotic and meiotic chromosomes, complete and partial separation of the parental genomes was proposed to have occurred during mitosis. This resulted in the occurrence of cells with possibly complete and incomplete complements of the parental species and cells with parental complements and some additional chromosomes from the other parent. (2) Pollen mother cells (PMCs) possibly with both parental chromosome complements, only *B. juncea* chromosomes or a complete *B. juncea* complement with additional *O. violaceus* chromosomes were more competitive in entering meiosis. The majority of fertile gametes were deduced to have been produced by PMCs with a *B. juncea* complement with or without additional *O. violaceus* chromosomes. (3) The progeny plants from selfed hybrids between *B. juncea* and *O. violaceus* were morphologically either of a *B. juncea*, hybrid or variable type. Cytologically they were grouped into six types according to the frequencies of cells with various chromosome numbers. All of the plants except 2 which constituted two types, were mixoploids, composed of cells with various chromosome numbers, mainly in a certain serial range. (4) The hybrid plants between *B. carinata* and *O. violaceus* were mixoploids with chromosome numbers in the range of 12–34, and cells with 2n = 34 were the most frequent. The main categories of PMCs with 17 bivalents at metaphase I and 17 : 17 segregations at anaphase I contributed to the high fertility of the hybrids and the fact that their progeny after selfing were mainly plants with 2n = 34. Somatic and meiotic separation of the parental genomes was proposed to have occurred in the hybrids between *B. carinata* and *O. violaceus*. (5) Mitotic and meiotic elimination of what could be *O. violaceus* chromosomes might also have contributed to the observed mitotic and meiotic cell types in the two kinds of hybrids studied. Finally, the possible mechanisms behind these cytological observations and their potential in the production of *Brassica* aneuploids were discussed.

Key words *Brassica juncea* · *Brassica carinata* · *Orychophragmus violaceus* · Intergeneric hybrids · Genome separation

Communicated by G. Wenzel

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Introduction

Cytogenetic research on *Brassica* has been conducted for more than half a century, and excellent results have been obtained by various workers such as Fukushima, Karpechenco, Mizushima, Morinaga, U and others

(Prakash and Hinata 1980). Included in the *Brassica* material studied were the three cultivated diploid species *B. campestris* L. (AA, 2n = 20), *B. oleracea* L. (CC, 2n = 18) and *B. nigra* (L.) Koch (BB, 2n = 16). Three other cultivated *Brassica* species are allotetraploids, which evidently have originated as amphidiploids from crosses between pairs of the three diploid species. These allotetraploid species are *B. napus* L. (AACC, 2n = 38), *B. juncea* (L.) Czern. & Coss. (AABB, 2n = 36) and *B. carinata* A. Braun (BBCC, 2n = 34). The cytogenetic relationship between the six cultivated *Brassica* species was established by U (1935).

The placement of *Orychophragmus* in the tribe *Brassiceae* has been questioned (Al-Shehbaz 1985), and Gomez-Campo (1980) suggested that the genus be excluded from the tribe. However, a recent study on isozyme variation in the tribe supported the inclusion of this genus (Anderson and Warwick 1995). In the intergeneric hybrids between *B. napus* and *O. violaceus* (L.) O. E. Schulz (OO, 2n = 24), it was inferred that the genomes of the two parental species separated from each other during the mitotic divisions of some cells (Li et al. 1995b). Thus, the hybrid is mixoploid in nature, containing both cells with presumably haploid and diploid chromosome complements of *B. napus* and *O. violaceus* and cells with the expected chromosome number (2n = 31). Mainly two kinds of plants, *B. napus* and hybrids, were produced by the hybrid, since gametes with n = 12 or 19 might have been formed by cells with either *O. violaceus* or *B. napus* chromosome complements, respectively. No *O. violaceus* plants were found among the progeny. The cytological findings on the hybrid also indicated that these two genera are distantly related.

In pollen mother cells (PMCs) with 2n = 31 of the hybrid *B. napus* × *O. violaceus* all 31 chromosomes sometimes appeared as univalents which segregated into two groups of 12 and 19 chromosomes, respectively (Li et al. 1995b, 1996). Subsequently, the chromosomes underwent the second division giving rise to two small and two large daughter groups, with 12 and 19 chromosomes each, respectively. It was proposed that such a chromosome segregation pattern indicates meiotic separation of the parental genomes. Thus, the same kinds of gametes as those produced by cells with supposedly diploid chromosome complements of *B. napus* or *O. violaceus* resulting from the somatic separation of genomes were also possibly formed by the separation of the parental genomes during the meiotic division of cells with a hybrid chromosome complement. This provided further support for the observed fertility and plant types in the progeny of the hybrid. The formation of unreduced gametes with 31 chromosomes was also observed, which would explain the origin of the progeny plant with 2n = 50 (19 + 31, AACCO) (Li et al. 1995b).

Because of the well-known cytogenetical relationship between the three cultivated *Brassica* tetraploids and the availability of the *B. napus* × *O. violaceus* hybrid, it was considered worthwhile to obtain the other intergeneric hybrids with *B. juncea* and *B. carinata* and to investigate the incidence of genome separation in these hybrids. The present paper reports on (1) the production and morphology of the intergeneric hybrids *B. juncea* × *O. violaceus* and *B. carinata* × *O. violaceus* and (2) the cytogenetics of these hybrids and their progenies.

Material and methods

Plant material

Eight cultivars or lines of *Brassica juncea* (designated GJ), and three of *B. carinata* (designated GO), all from Huazhong Agricultural University, were used. *Orychophragmus violaceus* was obtained from the Department of Biology, Sichuan University (now, Sichuan Union University) (Luo et al. 1991). Six of the *B. juncea* cultivars or lines (GJ04, GJ07, GJ11, GJ19, GJ20 and GJ21) had yellow seeds and 2 (GJ01 and GJ22) had brown seeds. GO-11 was morphologically characterized by having light-yellow petals, dark-green leaves and a brown seed coat, but the other 2 lines (GO-7 and GO-9) had yellow petals, light-green leaves and a yellow seed coat. The materials were grown in the field, and crosses between the two *Brassica* species and *O. violaceus* were made by hand emasculation and pollination in the spring of 1995. Embryo rescue was undertaken only when needed since most crosses promoted normal seed development. Crosses between *B. juncea* and *O. violaceus* were repeated in the following year for confirmation, but results only from hybrid plants obtained during the first year are presented here.

Half of the seeds obtained from the cross *B. juncea* × *O. violaceus*, harvested in May 1995, were sown immediately in the field at the Qinghai Academy of Agricultural and Forestry Science in Xining City of Qinghai Province. The rest of the seeds were stored at −10°C and sown in the campus field in Wuhan in October 1995, together with seeds harvested from the hybrid plants grown in Qinghai. The plants obtained after embryo rescue were also transferred to the field in October and November 1995.

The seeds obtained from the crosses *B. carinata* × *O. violaceus* were stored at −10°C before planting in October 1995. The seeds harvested from the hybrid plants in May 1996 were sown in Qinghai for studies on F_2 progeny plants.

F_1 and F_2 seeds obtained from crosses and cultivated hybrids were put on wet filter paper for germination at 22°C. Germinated seeds were then planted in nutritional soil in small plastic cups. The seedlings with three to four leaves were later transplanted to the campus field in Wuhan.

Cytological methods

Chromosome numbers in hybrids were determined at three developmental stages by using root tips, leaves and styles from seedlings, embryo-rescued young plants grown on tissue culture medium and flowering plants, respectively. All three types of tissues were studied in the hybrids *B. juncea* × *O. violaceus*, and only styles were studied in the hybrids *B. carinata* × *O. violaceus*. A relatively high frequency of mitotic divisions in styles from young flower buds, which were abundant in each plant, made styles the tissue of choice for determining chromosome numbers in hybrids and their progenies. Fewer mitotic divisions were found in preparations made from root tips of the hybrid materials.

Root tips and leaves were treated with 2 m*M* 8-hydroxyquinoline for 4 h, and the styles for 5 h at 22°C, and then fixed in Carnoy's

solution. Preparations were made according to the method of Li et al. (1995b).

For meiotic analysis flower buds were fixed in fresh Carnoy's solution for 24 h and then stored in 70% ethanol. The anthers were dissected out, cut in half and the PMCs squeezed out in a drop of 10% modified carbol fuchsin (Liu and Jiang 1987).

Pollen stainability was determined as the percentage of pollen grains stained with 1% acetocarmine. More than 300 pollen grains from two flowers were counted for each plant. Normal pollen grains were fully round and densely stained, and they were easily distinguished from shrunken and lightly stained or small pollen grains.

Results

Production and morphology of the hybrids (F_1)

B. juncea × *O. violaceus*

There were marked differences between the genotypes of the maternal *B. juncea* cultivars with respect to their crossability with *O. violaceus* (Table 1). Hybrid plants were produced from seeds harvested after *B. juncea* was pollinated by *O. violaceus*. Thus, it was easy to obtain hybrids between these two species, especially in crosses involving GJ07, GJ19 and GJ20. In the reciprocal cross, with *O. violaceus* as the female parent, no hybrids were obtained, and even no swelling of the ovaries was observed.

The hybrid plants obtained from seeds in all eight crosses, both in Wuhan and Xining, were morphologically intermediate between their parents except for petal and seed coat colour (Fig. 1a–f, Table 2). All hybrid plants showed the yellow flower character of *B. juncea*, not the purple colour of *O. violaceus*. The seed coat of the hybrid plants was brown in contrast to the yellow or brown seed coat colour of the parental *B. juncea* cultivar.

The hybrid plants showed hybrid vigour and were easily distinguished from *B. juncea* by having leaves similar to those of *O. violaceus* (Fig. 1a) and petals and flower buds larger than those of the *B. juncea* parent (Fig. 1b,c). The branching of the stem in hybrid plants started at a lower level than in the *B. juncea* parent, and the *O. violaceus* character of basal clustering of stems was observed in some hybrid plants (Fig. 1f). The developmental duration of the hybrid plants was longer than in *B. juncea*. *Orychophragmus violaceus* matures later than *B. juncea*.

The flower of all hybrid plants opened normally and had anthers containing many pollen grains. There were marked differences in the size and morphology of the pollen grains. The hybrid plants were partially fertile and produced many seeds when selfed and following open pollination. The seed set after selfing was lower than after open pollination. In spite of the variation in seed size, most of the seeds were well developed and larger than those of *B. juncea*. About one-third of these seeds germinated after soaking in water, and most of the remaining seeds succeeded in developing plants only after removal of the seed coat.

Table 1 Results of intergeneric reciprocal crosses of *B. juncea* (GJ) and *B. carinata* (GO) with *O. violaceus*

Cross	Number of flowers pollinated (A)	Number of hybrid plants obtained (B)	B/A × 100
GJ01 × O.v.[a]	132	1	0.76
GJ04 × O.v.	150	1	0.67
GJ07 × O.v.	300	10	3.33
GJ11 × O.v.	162	1	0.62
GJ19 × O.v.	360	10	2.78
GJ20 × O.v.	120	4	3.33
GJ21 × O.v.	330	2	0.61
GJ22 × O.v.	90	1	1.11
O.v. × GJ[b]	400	0	0
GJ-7 × O.v.	420	3	0.71
GJ-9 × O.v.	300	2	0.67
GJ-11 × O.v.	450	7	1.56
O.v. × GO[b]	300	0	0

[a] O.v. = *O. violaceus*

[b] Crosses involving all *B. juncea* or *B. carinata* cultivars

B. carinata × *O. violaceus*

Hybrid plants were produced from seeds harvested after *B. carinata* was pollinated by *O. violaceus* in all three crosses (Table 1), but none were obtained from the reciprocal crosses with *O. violaceus* as the female parent.

Morphologically, all the hybrid plants in the three crosses were mainly matroclinous (Table 2). However, the leaves were of a larger size and had a different shape from those of *B. carinata*, exhibiting the more obvious zigzag edge appearance of the leaves of *O. violaceus* (Fig. 2). Only 1 hybrid plant exhibited the character of basal branching of the stem characteristic of *O. violaceus* (Fig. 2c–e). The hybrid plants from the cross GO-11 × *O. violaceus* had yellow petals and light-green leaves and not the light-yellow petals and dark-green leaves of the parental *B. carinata* plants.

The flowers of the hybrid plants in all crosses were about the same size as those of *B. carinata*, opened normally and had anthers containing plenty of stainable pollen grains. When selfed or left for open pollination, the hybrid plants developed nearly normal pods with many seeds (Table 2). The seeds were yellow in the hybrid plants involving GO-7 and GO-9, and thus similar in colour to the seeds of the parental *B. carinata* plants. However, the seeds of the hybrid plants in the cross GO-11 × *O. violaceus* were yellow, thus deviating from the brown colour of both parental types (Table 2).

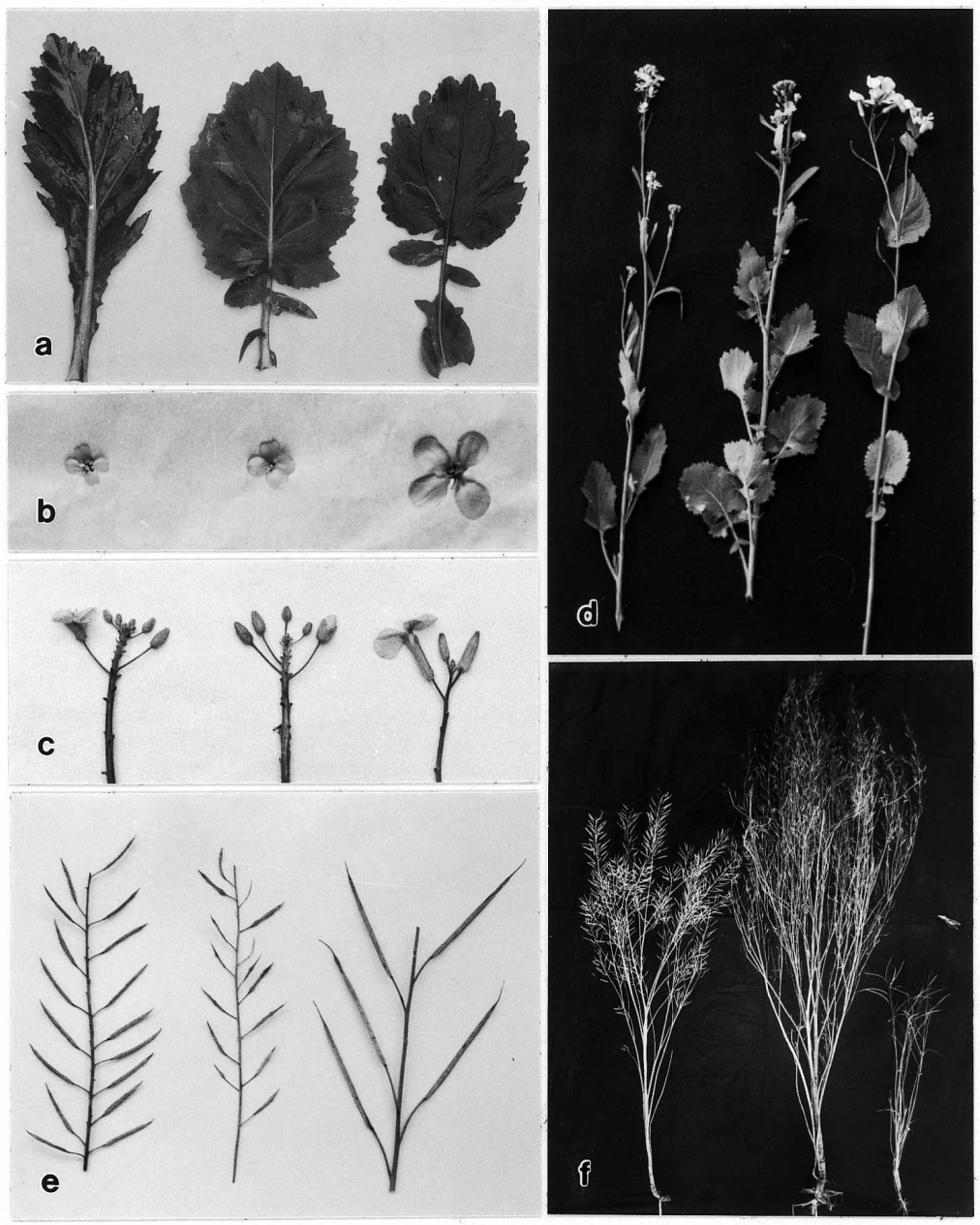

Fig. 1a–f Morphology of *B. juncea* GJ19 (*left*), the hybrid GJ19 × *O. violaceus* (*middle*) and *O. violaceus* (*right*). **a** Leaves, **b** flowers, **c** flower buds, **d** flowering inflorescences, **e** siliques, **f** mature plants. The hybrids displayed the *O. violaceus* character of basal clustering of stems and produced many siliques containing some seeds

Cytology of the hybrids (F_1)

B. juncea × *O. violaceus*

Somatic tissues Mixoploidy prevailed in all of the root, leaf and style tissues studied, with chromosome numbers ranging between 12 and 42 (Table 3, Fig. 3a–f). In roots, mainly three kinds of cells were found with 24, 30 and 36 chromosomes (Fig. 3a, b, d). Cells with 36 chromosomes were the most frequent (37.5%), while those with chromosome numbers other than 24, 30 and 36 accounted for 22.5% of the population (Fig. 3a, c). In the leaves, the percentage of cells with 36 chromosomes was again the highest (50%), followed by cells with 30 (Fig. 3e, f), 18 and 24 chromosomes. In styles there was a higher variation in chromosome number (Table 3). Cells with 36 chromosomes were still the most common, but they appeared less frequently (27.1%) than in roots and leaves. Next, in a decreasing order of frequency, were cells with 30, 34, 37, 32 and 33 chromosomes.

The chromosome number 2n = 30 was the second most common number encountered in all of the somatic

Table 2 Botanical characters of *B. juncea*, *B. carinata* and *O. violaceus* and their intergeneric hybrids[a,b]

Material	Plant height (cm)	Position of first branching (cm)	Number of first branchings	Number of pods on main stem	Length of pod (cm)	Seeds per pod	Weight of 1000 seeds (g)	Colour of petal	Colour of seed coat
O.v.	72.1 ± 5.6	0	13.4 ± 3.8	13.8 ± 4.5	8.4 ± 0.6	37.1 ± 2.8	2.54	Purple	Brown
GJ07	132.3 ± 3.1	20.4 ± 2.4	10.6 ± 2.2	20.5 ± 2.7	3.2 ± 0.6	15.6 ± 1.7	1.53	Yellow	Yellow
Hybrid	134.2 ± 8.3	16.5 ± 3.4	12.3 ± 4.5	5.7 ± 7.2	2.1 ± 0.9	1.4 ± 0.5	2.48	Yellow	Brown
GJ19	141.4 ± 2.9	15.5 ± 3.2	11.4 ± 3.4	22.7 ± 2.1	3.5 ± 0.5	16.7 ± 1.9	1.44	Yellow	Yellow
Hybrid	147.3 ± 7.8	10.2 ± 4.8	14.6 ± 4.6	7.4 ± 5.6	2.3 ± 0.7	1.7 ± 0.7	2.57	Yellow	Brown
GO-7	151.2 ± 2.9	25.7 ± 3.1	11.2 ± 2.1	15.4 ± 3.1	5.2 ± 0.6	17.8 ± 2.1		Yellow	Yellow
Hybrid	154.6 ± 3.2	19.3 ± 5.4	13.6 ± 3.2	15.1 ± 3.9	4.9 ± 0.7	15.2 ± 3.2		Yellow	Yellow
GO-11	146.1 ± 3.5	19.9 ± 3.7	12.4 ± 1.6	13.6 ± 2.8	4.6 ± 0.5	18.3 ± 2.6		Slightly yellow	Brown
Hybrid	147.3 ± 4.1	18.6 ± 4.9	12.7 ± 2.7	13.2 ± 4.4	4.8 ± 0.8	16.2 ± 3.7		Yellow	Yellow

[a] Twenty plants of the parental types and 5 hybrid plants from each cross were studied for each character; only 3 hybrid plants from the cross GO-7 × *O. violaceus* were studied
[b] Standard errors are given

tissues studied. It is the number expected if the hybrid has received the A and B genomes from *B. juncea* and O genome from *O. violaceus*. In spite of the generally small size of the chromosomes, they obviously covered a wide range of sizes (Fig. 3b–f). In some cells, a group of 12 relatively large and more darkly stained chromosomes could be differentiated (Fig. 3f).

Lagging and the exclusion of chromosomes from daughter nuclei were observed at anaphase and telophase, respectively.

Meiosis The PMCs were classified into five types according to their chromosome numbers (Table 4): (1) cells with 30 chromosomes, which is the expected chromosome number in the hybrid; (2) cells with 36 chromosomes that showed equal segregation of 18 : 18 at anaphase I; (3) cells with 36 chromosomes showing unequal segregations at anaphase I; (4) cells with more than 36 chromosomes; (5) cells with fewer than 36 chromosomes, excluding those representing Type I.

At metaphase I (MI) of Type I PMCs, it was possible to differentiate some large and more darkly stained chromosomes from the others (Fig. 4a). In most cells about 12 darkly stained and relatively large chromosomes that appeared as univalents and bivalents could be easily identified. The MI pairing configurations in PMCs of Type I are listed for three crosses in Table 5. The main five kinds of pairing configurations were 5–9 bivalents and 12–20 univalents for the first two crosses, and 3–7 bivalents and 16–24 univalents for the third cross, accounting for 78%, 74% and 79% of the total cells, respectively. Seven bivalents and 16 univalents was the most frequent configuration in the three crosses. Up to 6 bivalents and a variable number of univalents lagged between the two daughter groups at anaphase I/telophase I (AI/TI).

PMCs of Type III (Table 4) showed different AI segregation patterns of chromosomes such as 20 : 16, 21 : 15, 22 : 14 and 13 : 23. In some PMCs it was possible to differentiate one or two darkly stained chromosomes either lagging or included in the two polar anaphase groups.

In Type IV PMCs, the chromosome numbers ranged from 37 to 42. Although many patterns of chromosome segregation were observed at AI, the majority of the segregations had 18–24 chromosomes in each polar group (Fig. 4b–d). Some large and darkly stained chromosomes, either included in the two polar groups or lagging, were often found in these cells (Fig. 4b, c). The differences in the staining density of chromosomes were still noticable during the second meiotic division and microspore development (Fig. 4e, f). Darkly stained entities of condensed chromatin and micronuclei were observed in the nucleus and cytoplasm of microspores, respectively (Fig. 4f).

Chromosome numbers in Type V PMCs ranged from 26 to 35. One or two of the chromosomes were often identifiable by being large in size and darkly stained.

B. carinata × *O. violaceus*

Somatic tissues Various chromosome numbers were recorded in the style cells of the hybrids obtained from the crosses of GO-7 and GO-11 with *O. violaceus* (Table 6, Fig. 5a, b). All the hybrid plants were mixoploids consisting of cells with 12–34 chromosomes, with majority of cells having 34 chromosomes. The

Fig. 2a–e Morphology of the hybrids *B. carinata* × *O. violaceus* and their parents. **a, b** Leaves and flowering branches of GO-11, the hybrid and *O. violaceus* (from *left* to *right*), **c–e** flowering plants of GO-7 (**c**), the hybrid with basal branched stems (**d**) and *O. violaceus* (**e**). For comparison of actual plant heights (**c–e**), see Table 2

second most frequent chromosome number was 29 (Fig. 5a), which is the expected chromosome number if the B and C genomes of *B. carinata* and O genome of *O. violaceus* were contributed to the hybrid. Nearly 30% of the cells had 30–33 chromosomes. No cells with more than 34 chromosomes were observed.

Lagging chromosomes were frequently observed at anaphase/telophase. They often showed signs of chromatid separation and were generally excluded from the daughter anaphase groups. Micronuclei formed by the lagging chromosomes were observed at telophase.

Meiosis In PMCs of hybrids obtained from two cross combinations, the majority of the diakinesis and MI cells had 17 bivalents (Table 7). At AI, 17 : 17 segregations (Fig. 6) were the most frequent. The number of PMCs with other chromosome numbers (Fig. 6) or with lagging chromosomes was low (Table 7). A few PMCs with nearly 29 chromosomes showed an approximate 12 : 17 segregation at AI (Fig. 6). The fertility of the hybrids inferred from pollen stainability was very high, which was expected to be the case judging from the kind of PMCs most frequently encountered (Table 7).

Table 3 Numbers and percentages of different kinds of cells in the hybrids between *B. juncea* GJ19 and *O. violaceus* at three developmental stages[a]

Growth stages		Number and percentage of cells with chromosome numbers																				Total
		12	16	18	20	22	24	26	27	28	29	30	31	32	33	34	35	36	37	38	40	42	
Roots	Number of cells	1	2	2	3	1	14	3		2		34		1	1	2		45	2	1	6		120
	Percentage	0.8	1.7	1.7	2.5	0.8	11.7	2.5		1.7		28.3		0.8	0.8	1.7		37.5	1.7	0.8	5.0		100
Leaves	Number of cells	5	1	11	1		8	1	2			30		1				62			2		124
	Percentage	4.0	0.8	8.9	0.8		6.5	0.8	1.6			24.2		0.8				50.0			1.6		100
Styles	Number of cells	1		4	5	5	4	3	1	4	1	35	3	18	13	31	9	62	19	7	3	1	229
	Percentage	0.4		1.7	2.2	2.2	1.7	1.3	0.4	1.7	0.4	15.3	1.3	7.9	5.7	13.5	3.9	27.1	8.3	3.1	1.3	0.4	100

[a] Data obtained from roots of 10 germinating seeds, leaves of plantlets raised from 3 cultured embryos and styles of 5 flowering plants

Morphology and cytology of the hybrid progeny (F_2)

B. juncea × *O. violaceus*

Great variations in plant morphology were observed in F_2 populations of the hybrids. The progeny plants could be roughly classified morphologically into three groups: *B. juncea* type, hybrid type and a group with variable morphology. The majority of the plants belonged to the second and third groups. Except for 1 plant of the *B. juncea* type, all of the surviving plants were partially fertile and produced seeds when selfed or after open pollination. Seed setting differed among the progeny plants.

Depending on the number of the chromosomes and the frequencies of cells with various numbers, progeny plants could be separated into six types (Table 8, Fig. 7). Except for 1 plant with 30 chromosomes (Type I) and 1 plant with 36 chromosomes (Type III), all plants were mixoploid with numbers varying within the range 30–42 and mainly comprised the serial numbers 30–35 (Type II), 30–36 (Type IV-1), 33–36 (Type IV-2), 34–40 (Type V) and 36–42 (Type VI). The maximum serial chromosome number of 35 in plants of Type II was less than the chromosome number of the parental *B. juncea*. Plants of Type IV had a maximum serial number of 36 and were subdivided into two kinds on the basis of the ranges of their serial chromosome numbers (30–36 and 33–36). More than 75% of the plants were included in Type V, which mainly consisted of cells with serial chromosome numbers of 34–37, 34–38, 34–39 and 34–40. Cells with 34 and 35 chromosomes (fewer than 2n = 36 of *B. juncea*) constituted 15.6% of the cells found in these ranges. Plants with a minimum chromosome number of 36 harbouring the serial chromosome numbers 36–41 or 36–42 were included in Type VI.

Marked differences in chromosome size and staining density were frequently apparent between different groupings of chromosomes in the metaphase spreads (Fig. 7).

B. carinata × *O. violaceus*

Two kinds of plants were cytologically differentiable in two crosses (Table 9). The first kind had predominantly a high number of cells with 2n = 34 (98–99%) and a few cells with 2n = 32 or 33. The second kind had mainly cells with 2n = 34 (70–75%) but also a relatively high frequency of cells with 2n = 29–33 (22–28%). The first kind appeared by far most frequently being represented by 94–96% of the plants. The two kinds of plants were morphologically very similar to the *B. carinata* parent and could be distinguished only cytologically. More morphological variation was observed in the progeny plants from the cross GO-11 × *O. violaceus*. Plants with

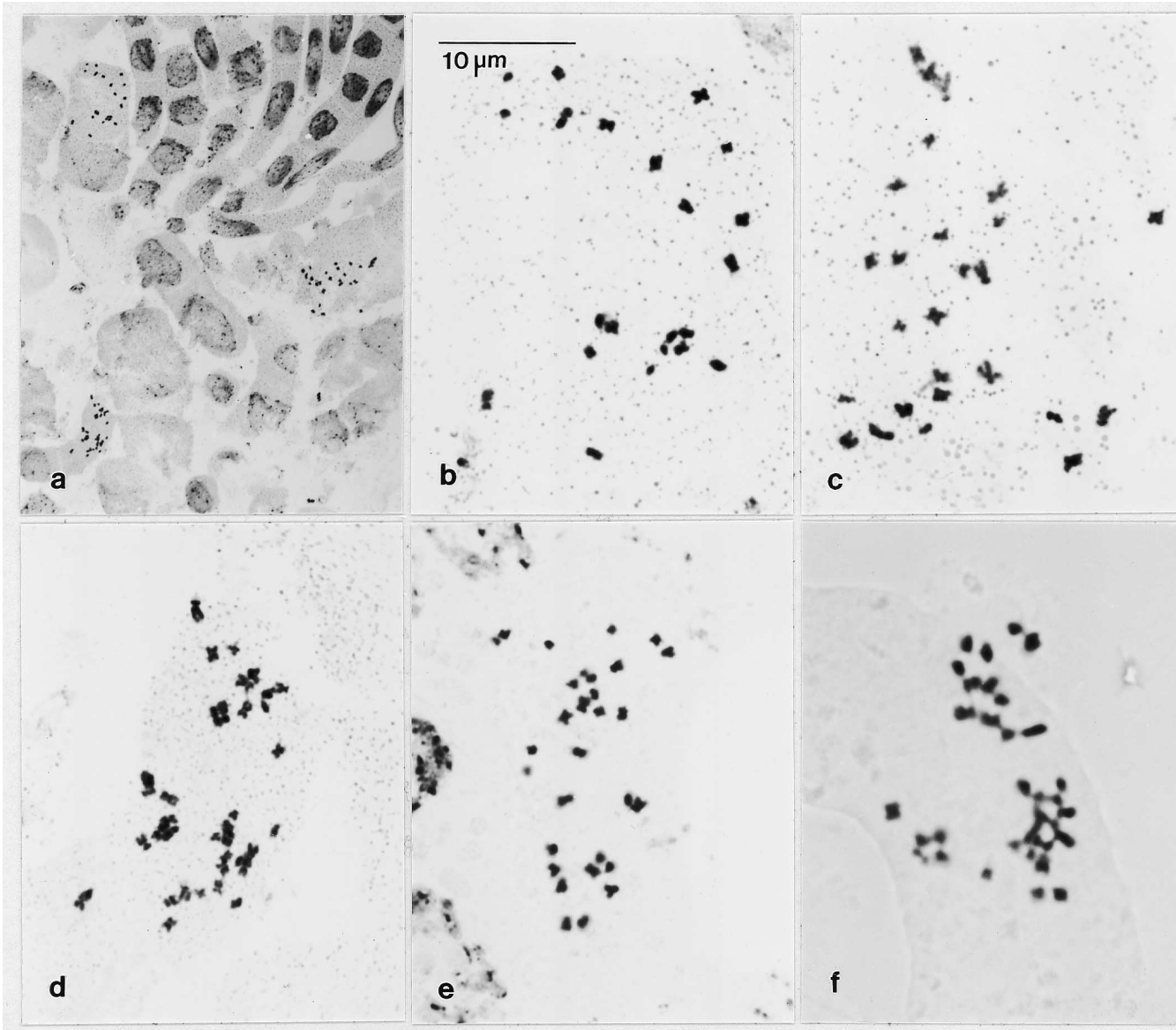

Fig. 3a–f Examples of the different chromosome numbers recorded in mitotic metaphases of the hybrids GJ19 × *O. violaceus*. **a–d** Three mitotic root cells in the same field (**a**), higher magnifications of the cell at *top left* with 2n = 24 (**b**), cell at *middle right* with 2n = 26 (**c**) and cell at *bottom left* with 2n = 36 (**d**) chromosomes. **e, f** Thirty chromosomes in leaf cells, **f** a group of 12 chromosomes (*above*) are larger in size and more darkly stained than the other 18 chromosomes (*below*)

yellow or brown seeds were found among this progeny.

Discussion

Crossability

The crossability of both *B. juncea* and *B. carinata* with *O. violaceus* was higher than that of *B. napus* with *O. violaceus* (Li et al. 1995b). That the intergeneric hybridizations were successful only when *B. juncea* or *B. carinata* were used as female parents is in accordance with the results obtained from crosses between *B. napus* and *O. violaceus* (Li et al. 1995b). Also in crosses of *B. campestris* and *B. oleracea* with *O. violaceus*, hybrids were obtained only when the *Brassica* species were used as the female parent (Li et al., unpublished).

Complete and partial separation of the parental genomes

Similar to the situation in the *B. napus* × *O. violaceus* hybrids (Li et al. 1995b), mixoploidy was a characteristic feature in both mitotic and meiotic divisions of the newly obtained hybrids *B. juncea* × *O. violaceus* and *B. carinata* × *O. violaceus*. More variability in

Table 4 Different kinds of PMCs (see text) and pollen stainability in the hybrids *B. juncea* × *O. violaceus*

Cross	Kinds of PMCs Chromosome number	I 30	II 36	III 36	IV > 36	V < 36[a]	Total	Pollen stainability (%)
GJ07 × O.v.	Number of PMCs	94	2	5	53	4	158	38.1
	Percentage	59.5	1.3	3.2	33.5	2.5	100	
GJ19 × O.v.	Number of PMCs	114	1	3	51	2	171	40.2
	Percentage	66.7	0.6	1.8	29.8	1.1	100	
GJ20 × O.v.	Number of PMCs	102	4	9	59	3	177	39.5
	Percentage	57.6	2.3	5.1	33.3	1.7	100	

[a] Excluding PMCs with 30 chromosomes (Type I)

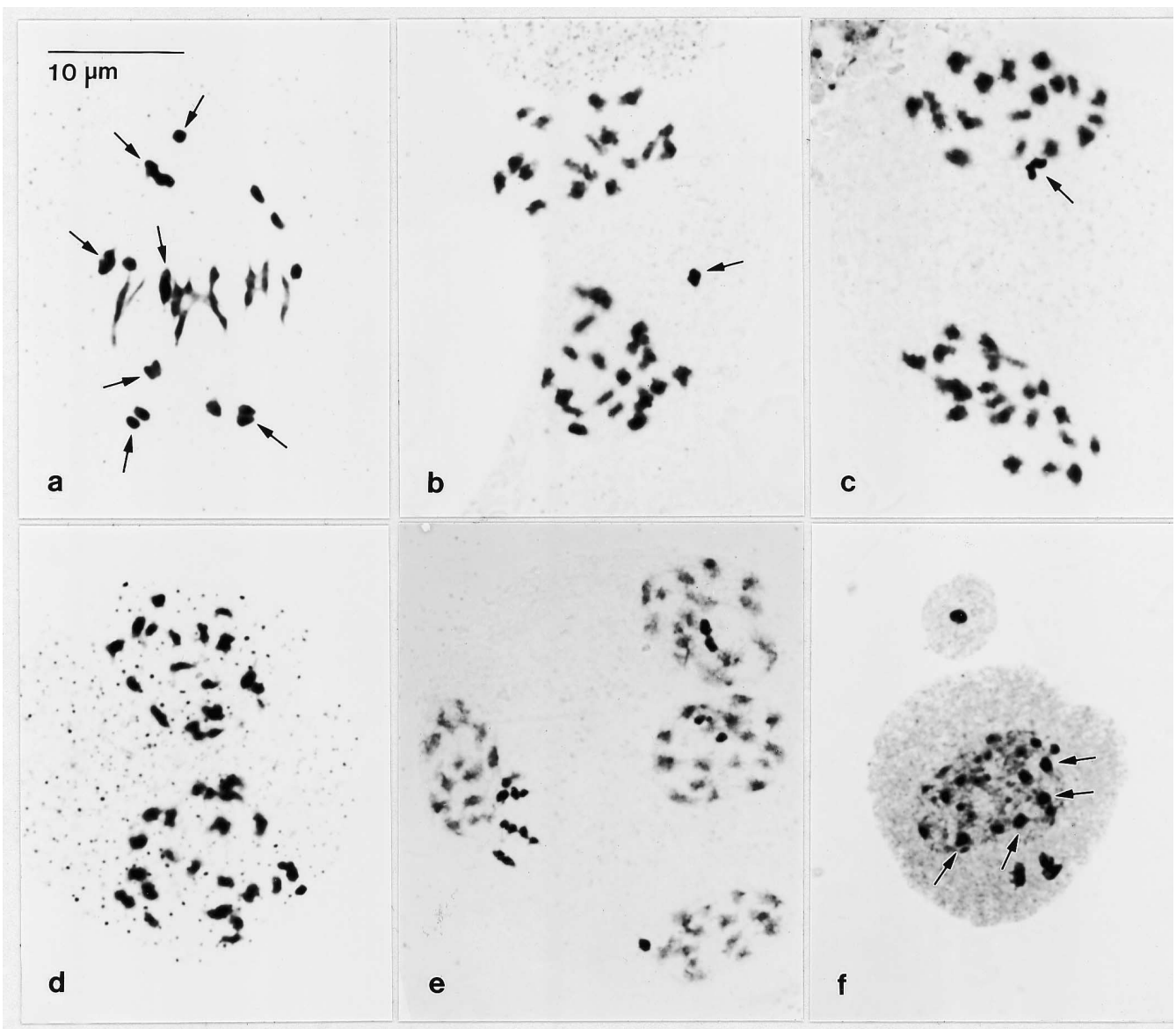

Fig. 4a–f Meiotic behaviour in PMCs of the hybrid GJ19 × *O. violaceus*. **a** Metaphase I. Ten chromosomes in the form of three bivalents and four univalents (*arrows*) are of a relatively larger size and darker colour, and supposedly originate from *O. violaceus*. **b** Anaphase I with 38 chromosomes segregating as 18 (*above*): 19 (*below*) and a lagging darkly stained chromosome (*arrow*). **c** Anaphase I with 39 chromosomes segregating as 19 (*above*): 20 (*below*). One darkly stained chromosome is included in one polar group (*arrow*). **d** Anaphase I with 42 chromosomes segregating as 18 (*above*): 24 (*below*). **e** Anaphase II showing differences in the staining density of some chromosomes in the four daughter chromosome groups. Also note the unequal segregations of the darkly stained chromosomes. **f** One microspore with darkly stained and condensed chromatin in the nucleus (*arrows*), 3 darkly stained chromosomes in the cytoplasm, and a minor microspore with a micronucleus

Table 5 Chromosome pairing at metaphase I of PMCs with 2n = 30 in the hybrids between *B. juncea* and *O. violaceus*

Cross	Number of plants	Number of cells	Means of different chromosome configuration			
			I	II	III	IV
GJ07 × O.v.	3	65	15.85 (6–24)[a]	6.77 (3–12)	0.12 (0–2)	0.06 (0–1)
GJ19 × O.v.	3	72	16.67 (12–26)	6.46 (2–9)	0.08 (0–2)	0.04 (0–1)
GJ20 × O.v.	3	57	17.96 (10–24)	5.58 (3–10)	0.11 (0–2)	0.05 (0–1)

[a] Figures between brackets are ranges

Table 6 Number and percentage of different style cells in the hybrids *B. carinata* × *O. violaceus*

Cross	Number of plants observed	Number of cells with chromosome numbers										Total
		12	17	24	25–28	29	30	31	32	33	34	
G0-7 × O.v.	3	3	1	6	23	36	5	20	24	19	81	218
		1.4	0.5	2.8	10.6	16.5	2.3	9.2	11.0	8.7	37.2	100
G0-11 × O.v.	7	3	8	12	39	49	20	18	35	25	128	337
		0.9	2.4	3.6	11.6	14.5	5.9	5.3	10.4	7.4	38.0	100
Total	10	6	9	18	62	85	25	38	59	44	209	555
		1.1	1.6	3.2	11.2	15.3	4.5	6.8	10.6	7.9	37.7	100

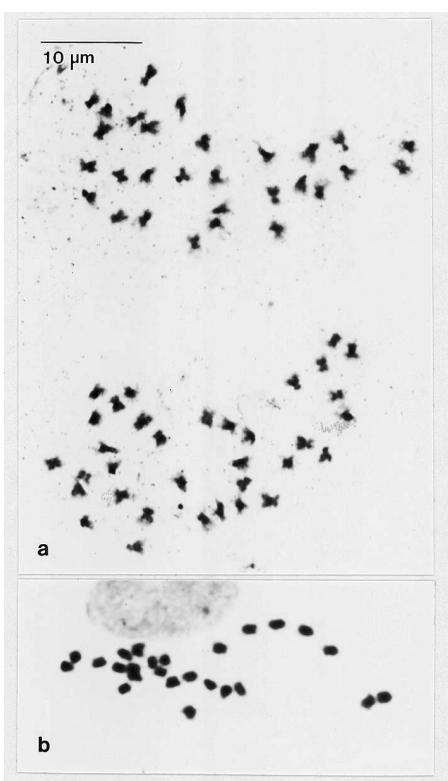

Fig. 5a, b Examples of different chromosome numbers found in the hybrids between *B. carinata* and *O. violaceus*. **a** Two metaphase spreads in the same field (2n = 29, *above*, and 2n = 34, *below*). **b** A metaphase spread with 25 chromosomes

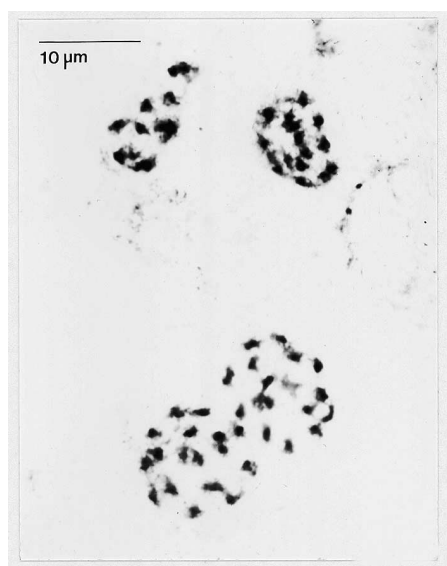

Fig. 6 Meiosis in PMCs of the hybrid *B. carinata* × *O. violaceus*. Two anaphase I configurations in one field, one (*above*) with nearly 29 chromosomes segregating as 12 (*left*): 17 (*right*); the other (*below*) with 34 chromosomes segregating as 17 : 17

chromosome number occurred in these hybrids than in hybrids involving *B. napus*.

In both the mitotic and meiotic divisions of the hybrid *B. napus* × *O. violaceus* the parental chromosome numbers of 38 and 24 were next in frequency after

Table 7 Number of various categories of PMCs, and pollen stainability of the hybrids *B. carinata* × *O. violaceus*

Cross	Kinds of PMCs	I[a]	II[b]	Total	Stainability (%)
GO-7 × *O. violaceus*	Number	515	9	524	96.3
	Percentage	98.3	1.7	100	
GO-11 × *O. violaceus*	Number	420	4	424	98.2
	Percentage	99.1	0.9	100	

[a]I: With 17 bivalents or 17:17 segregation
[b]II: With other MI pairing configurations, AI segregations or lagging chromosomes at AI

Tables 8 Plant types and their chromosome numbers determined in the styles of progeny of the hybrids GJ19 × *O. violaceus*

Plant types	Number of plants	Number of cells with chromosome numbers													Others	Total
		30	31	32	33	34	35	36	37	38	39	40	41	42		
I	1	18													0	18
II	5	13	15	14	32	54	24								23	175
III	1							15							0	15
IV-1	4	12	7	18	22	28	14	39							9	149
IV-2	4				10	24	12	48							14	108
V	86					196	178	502	453	503	252	59			256	2399
VI	10							29	29	35	36	41	71	28	24	293
Total	111	43	22	32	64	302	228	633	482	538	288	100	71	28	326	3157

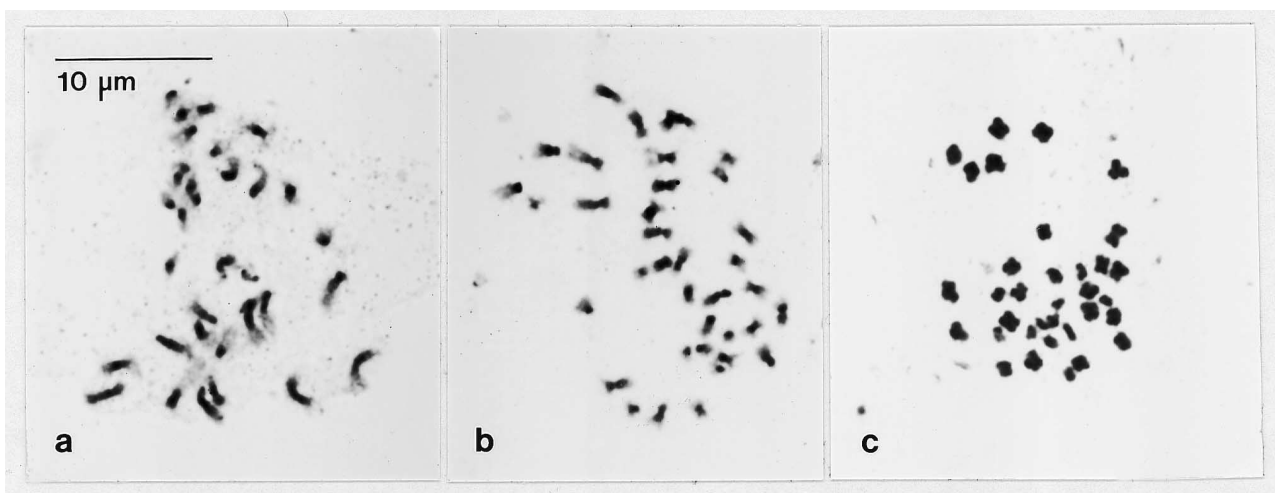

Fig. 7a–c Metaphase spreads in style cells of progeny plants from the hybrid GJ19 × *O. violaceus*. **a** One cell with 32 chromosomes. Most of the chromosomes in the *lower half* of the spread have larger sizes than those *above*. **b** A cell with 37 chromosomes. Several large and more densely stained chromosomes are in the *upper part* of the spread. **c** A cell with 34 chromosomes. Most of the chromosomes in the *top* and *left* parts of the spread are large and more darkly stained

the expected number of 31. This combined with the fact that *B. napus* plants were among the progeny of this hybrid (Li et al. 1995b) suggests that a closer look be directed towards chromosome numbers and eventual differentiation between the parental chromsomes in the two new types of hybrids obtained.

The expected chromosome number of 2n = 30 in the hybrid *B. juncea* × *O. violaceus* was the most frequent number in PMCs and the second most frequent number encountered in somatic tissues. Cells with the parental chromosome number 36 (*B. juncea*) were, however, the most frequent in somatic tissues, and cells with more than 36 chromosomes were the second most frequent among PMCs. The reason for the more frequent appearance of cells with more than 36 chromosomes during meiosis than mitosis remains to be

Table 9 Plant types and their chromosome numbers in the progeny obtained after selfing the hybrids *B. carinata* × *O. violaceus*

Cross	Number and percetange of plants	Number of cells with chromosome numbers						Others	Total
		29	30	31	32	33	34		
GO-7 × O.v.	72				17	2	1042		1061
	96.0				1.6	0.2	98.2		100
	3	6	3	2	4		52	2	69
	4.0	8.7	4.3	2.9	5.8		75.4	2.9	100
GO-11 × O.v.	66				11		894		905
	94.3				1.2		98.8		100
	4	15	3	4	24		116	3	165
	6.7	9.1	1.8	2.4	14.6		70.3	1.8	100

clarified. Cells with the parental chromosome number 24 (*O. violaceus*) were also frequent, mainly in the root tips of seedlings. It is most likely that the expected number of 2n = 30 was the original number from which other chromosome numbers arose. It remains to be shown if the parental chromosome numbers encountered also represent parental chromosome constitutions as was inferred in the *B. napus* × *O. violaceus* hybrid (Li et al. 1995b).

From the chromosome numbers recorded in styles (Table 3) and PMCs (Table 4) and chromosome segregations in PMCs with more than 36 chromosomes (Fig. 4b–d), we deduced that incomplete separation of the parental genomes might have occurred in some cells with 30 chromosomes in the hybrid *B. juncea* × *O. violaceus*. Thus, 1–3 chromosomes of *O. violaceus* might have been included together with the 18 *B. juncea* chromosomes during the process of genome separation, resulting in the formation of cells with 19, 20 or 21 chromosomes and, subsequently after chromosome duplication, cells with 38, 40 and 42 chromosomes. In this way 1–3 chromosome pairs from *O. violaceus* would be added to the *B. juncea* chromosome complement. The additional chromosomes might be successively eliminated during mitosis (this will be discussed later in more detail), and cells with odd chromosome numbers of 37, 39 and 41 would thus arise. The easy differentiation in PMCs of a few darkly stained chromosomes, possibly of *O. violaceus* origin, from the other, less darkly stained chromosomes, possibly of *B. juncea* origin (Fig. 4b, c), is in support of our hypothesis.

In a similar way, 1 or 2 chromosomes of *B. juncea* may move with 12 *O. violaceus* chromosomes during genome separation, thus producing hypoploid cells with 32 and 34 *B. juncea* chromosomes, and cells with 26 and 28 chromosomes comprising an *O. violaceus* chromosome complement and 2 or 4 extra *B. juncea* chromosomes. If style cells with 32 or 34 chromosomes and with 26 or 28 chromosomes actually comprise incomplete *B. juncea* and complete *O. violaceus* complements, respectively, then one would conclude that the first category, which occurs at a higher frequency, is more competitive (Table 3). The inclusion of 1 to several *O. violaceus* chromosomes with separated *B. juncea* chromosomes or the exchange of some chromosomes between separated complements would result in the production of additional chromosome constitutions.

The obvious differences in the frequencies of the different cell types (Table 3 and 4) are in support of the possible occurrence of both complete and incomplete genome separation in the *B. juncea* × *O. violaceus* hybrids. About 58–67% of the PMCs and only 15% of the style cells had the expected 2n = 30 for the hybrid. Cells with more than 36 chromosomes were also more frequent in PMCs than in mitotic cells of styles. On the contrary, PMCs with 36 chromosomes showing equal or unequal segregations were not frequent. This probably indicates that only a minority of the cells with 36 chromosomes had a euploid complement of *B. juncea* produced through complete genome separation and that the majority of these cells were *B. juncea* complements substituted with 1 to several *O. violaceus* chromosome pairs resulting from incomplete genome separation and chromosome elimination. The results in Table 4 also show that cells with a presumed hybrid chromosome complement (2n = 30) and cells with a presumed *B. juncea* complement with additional *O. violaceus* chromosomes were more competitive than other types of cells by entering meiosis. Most of the cells with fewer than 36 chromosomes, excepting those with the hybrid chromosome number of 2n = 30, failed to undergo meiosis.

Knowledge concerning genome separation during the mitotic divisions of the *B. napus* × *O. violaceus* hybrids could possibly contribute to the interpretation of the results observed in the hybrids between *B. carinata* and *O. violaceus*. That the most frequently encountered mitotic cells (37–38%) had the diploid chromosome number of *B. carinata* might imply that these cells also contain the chromosome complement of this species. If these cells arose through genome separation, one would expect that cells with chromosome numbers 17, 12 and 24 would represent haploid *B. carinata* cells and haploid and diploid cells of *O. violaceus*, respectively. The abundance of cells with 34 chromosomes which

presumably have a *B. carinata* chromosome complement might be due to their relatively faster rate of division and stronger growing ability (Li et al. 1995b).

Cells with chromosome numbers close to those of the parental diploids (e.g. 2n = 30–33 and 2n = 25–28) could result from incomplete genome separation, as in the hybrids between *B. juncea* and *O. violaceus*, implying the lack of some chromosomes from a parental complement and their inclusion in the other parental complement. Chromosome substitution and addition during mitotic genome separation and the successive elimination of alien chromosomes would produce cells with a variety of chromosome numbers. The cells with presumable *B. carinata* chromosomes (2n = 34), their hypoploids (2n = 30–33) and cells with the expected hybrid chromosome number (2n = 29) were more competitive during plant growth than the others (Table 6).

The cytological observations indicated that chromosome elimination frequently took place during the mitotic divisions of the hybrids between *B. carinata* and *O. violaceus*. The chromosomes eliminated were assumed to be of *O. violaceus* origin as inferred from the cell types found in the hybrid plants (Table 6). This apparently contributed to the low frequency of cells with the hybrid chromosome number 2n = 29 (15–17%). Thus, the presumed mitotic separation and elimination of the *O. violaceus* genome provided an explanation for the somatic components (Table 6) and meiotic configurations (Table 7) observed in the hybrids. The data in Table 7 also shows that cells with 2n = 34 were more competitive: they underwent normal meiosis and produced fertile gametes (n = 17).

The majority of the offspring obtained from the *B. carinata* × *O. violaceus* hybrids had 2n = 34 in 99% of their cells, suggesting that they might have a *B. carinata* chromosome constitution. Whether the origin of the offspring plants with 2n = 34 in only 70–75% of their cells was a hybrid chromosome constitution (2n = 29, BCO) needs further investigation. More effort should be made to further define different plant categories in the progeny of these hybrids.

On the basis of the segregation pattern 12 : 19 during the first meiotic division, Li et al. (1995a, 1996) proposed that meiotic genome separation occurred in the hybrid between *B. napus* and *O. violaceus*. Such chromosome behaviour might also occur in the hybrids between *B. carinata* and *O. violaceus* (Fig. 6). No observations substantiated such a mode of chromosome behaviour in the hybrids between *B. juncea* and *O. violaceus*.

As the chromosomes of *O. violaceus* are larger than those of *Brassica napus*, it was possible to roughly differentiate between the chromosomes of the two parental species in the hybrid *B. napus* × *O. violaceus*, particularly in cells showing somatic genome separation (Li et al. 1995b). Larger and more darkly stained chromosomes, inferred to be of *O. violaceus* origin, were observed in the hybrids *B. juncea* × *O. violaceus* (Figs. 3f, 4a–c, e, 7a–c). These features are useful in the chromosome analysis of these hybrids.

Possible mechanisms of somatic genome separation

The suspected reasons for chromosome elimination, a phenomenon often encountered in wide hybridizations of plants (Schulz-Schaeffer 1980), are the formation of multipolar spindles with subsequent elimination of resulting unbalanced cells (Rieger and Michaelis 1958) and the difference in duration of the somatic cell cycles of the two parents involved (Gupta 1969; Lange 1977; Subrahmanyam and Kasha 1973). The initial event behind the observed variation in chromosome numbers in the *Brassica* × *Orychophragmus* hybrids studied might be somatic genome separation, as has been proposed for the *B. napus* × *O. violaceus* hybrid (Li et al. 1995b). In favour of this interpretation is the high frequency of cells with the chromosome number of the parental *Brassica* species in the hybrids. The lagging and subsequent elimination of individual chromosomes that are more densely stained than others in cells with aneuploid chromosome numbers around 2n = 36 in *B. juncea* × *O. violaceus* hybrid might reflect the tendency of losing individual *O. violaceus* chromosomes from a predominantly *B. juncea* chromosome background.

Chromosome behaviour in hybrids between the three cultivated diploid *Brassica* species and *O. violaceus* may help in explaining the possible occurrence of somatic genome separation in the hybrids between the three amphiploids and *O. violaceus* because of the well-documented interrelationships between cultivated *Brassica* diploids and amphiploids. The hybrid between *B. oleracea* and *O. violaceus*, with somatic cells and PMCs having the expected chromosome number of 21, was totally sterile and expressed the character of purple petals from *O. violaceus* (Li et al. unpublished). However, the hybrid between *B. chinensis* (a form of *B. campestris*) and *O. violaceus* was mixoploid and produced many seeds (Li et al. unpublished). Another hybrid between *B. chinensis* and *O. violaceus* was also partially fertile (Wu et al. 1996). Thus the A genome might be responsible for the occurrence of somatic genome separation in the hybrid between *B. napus* and *O. violaceus*. The production and cytological analysis of the hybrid between *B. nigra* and *O. violaceus* is expected to further elucidate chromosome behaviour and interrelationships in the hybrids involving *B. juncea* and *B. carinata*.

Genomic in situ hybridization (GISH) has been used to provide a direct visual method of distinguishing entire parental genomes in both intergeneric hybrids (Schwarzacher et al. 1989; Anamthawat-Jónsson et al. 1990; Leitch et al. 1991) and interspecific hybrids

(Schwarzacher et al. 1992a; Parokonny et al. 1992) and of identifying alien chromosomes or chromosome segments in wheat (Heslop-Harrison et al. 1990; Mukai and Gill 1991; Schwarzacher et al. 1992b). The possible occurrence of parental genome separation in the hybrids between the three *Brassica* amphiploids and *O. violaceus* inferred from the cytogenetical observations argues for the great value of studying the mitotic and meiotic divisions of the hybrids by applying GISH.

A new approach to produce *Brassica* aneuploids

Limited genome transfer into crop Brassicas from distantly related *Brassiceae* might be achieved via several routes. Depending on the route taken, the transfer of information from the nuclear genome may involve single genes, chromosome segments or whole chromosomes. One approach used to promote the transfer of partial genomes is somatic hybridization between untreated and X- or γ-irradiated protoplasts (Gupta et al. 1984; Bates et al. 1987). Another approach is gametosomatic hybridization, which was attempted in the *Brassiceae* to produce alien addition and substitution lines of *B. napus* (Wiegand et al. 1995).

In conventional breeding, aneuploids are produced by synthesizing alloploids or backcrossing interspecific hybrids. In the polyploid cereals, extensive use has been made of nullisomic, tetrasomic and alien chromosome addition and substitution lines to promote the integration of novel genes into crops from distantly related species. These aneuploid lines have also contributed to studies of chromosome homoeology, chromosome pairing and genome analysis. The polyploid nature of cereals (e.g. *Triticum aestivum* L.) makes them tolerate the loss of individual chromosomes from homologous pairs, thus facilitating the development of aneuploid lines. The three cultivated *Brassica* allotetraploids should also be able to tolerate the manipulation of chromosome numbers.

That complete or partial genome separation is inferred to occur in the hybrids between *B. juncea* and *O. violaceus* makes it feasible that aneuploids with a complete and partial *B. juncea* chromosome complement and one to several *O. violaceus* chromosomes are found among the progenies of the hybrids. Based on the chromosome numbers recorded in the progeny of the pentaploid plant (2n = 50, AACCO) derived from the *B. napus* × *O. violaceus* hybrid, we were able to deduce the number of *O. violaceus* chromosomes in each progeny plant from the distribution of cells with serial chromosome numbers, since the *B. napus* chromosomes are cytologically stable while those of *O. violaceus* are successively eliminated (Wu et al. 1997). In a similar way, the numbers of chromosomes of the different parental types can be deduced roughly in the plants of Types II and IV–VI (Table 8), always assuming that the *B. juncea* chromosomes are stable while those of *O. violaceus* are liable to be lost during mitosis. For example, the chromosomes from *B. juncea* are possibly 30 and 36, and the ones from *O. violaceus* are up to 5 and 6 in plants of Types II and VI, respectively. Thus, plants of Types II, IV and V possibly contain a partial *B. juncea* complement and additional *O. violaceus* chromosomes, and plants of Type VI contain a complete *B. juncea* complement and additional *O. violaceus* chromosomes. The 2 plants of Types I and III would have 30 and 36 *B. juncea* chromosomes, respectively. Such a deduction is supported by the cytology of the F_3 populations of the hybrids (Li et al. in preparation). The exact numbers of chromosomes of different parental types will be determined by applying GISH.

Although the majority of the progeny plants from the hybrids between *B. carinata* and *O. violaceus* were of a *B. carinata* type, some plants had a category of cells with 2n = 32 and 33 in addition to the principle cells with 2n = 34. Morphological deviations from the *B. carinata* plants were expressed by the mixoploid plants. Whether chromosome substitution had occurred during genome separation in this type of hybrid needs further research.

Thus, complete and partial genome separation in the *Brassica* × *Orychophragmus* hybrids provides a new approach for producing aneuploids for plant genetic research and breeding.

Acknowledgements We wish to express our most sincere thanks to Prof. R. R-C. Wang, Utah State University for his visit to Wuhan and for the constructive discussion with the first author. The help of Kerstin Brismar with the final preparation of the photographs is greatly appreciated. The study was supported by a grant to Dr. Zaiyun Li from the National Natural Science Foundation of P.R. China and a grant from The Nilsson-Ehle Foundation of The Royal Physiographic Society of Sweden.

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