Theor Appl Genet (1999) 99:694–704

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Production and cytogenetics of intergeneric hybrids between the three cultivated *Brassica* diploids and *Orychophragmus violaceus*

Received: 8 February 1998 / Accepted: 12 March 1999

Abstract It has been proposed that both complete and partial separation of the parental genomes during mitosis and meiosis occurs in the intergeneric hybrids between *Orychophragmus violaceus* (2n=24) and the three cultivated *Brassica* tetraploids (*B. napus*, *B. carinata* and *B. juncea*). The hypothesis has been that this and the variations in chromosome numbers of these hybrids and their progenies result from the different roles of the A, B and C genomes originating from *Brassica.* To test this hypothesis, we produced hybrids between *O. violaceus* and the cultivated *Brassica* diploids. The hybrids with *B. oleracea* (2n=18, CC) had an intermediate morphology, but their petals were purple like those of *O. violaceus.* They were sterile and had the expected chromosome number (2n=21) in their mitotic and meiotic cells. The hybrid with *B. campestris* (2n=20, AA) was morphologically intermediate, except for its partial fertility and its yellow petals, which were similar to those of *B. campestris.* It was mixoploid (2n=23–42), and cells with 2n=34 were most frequent. Partial separation of parental genomes during mitosis, leading to the addition of *O. violaceus* chromosomes to the *B. campestris* complement, was proposed to explain the findings in the mitotic and meiotic cells of the hybrid and its progeny. In crosses with *B. nigra* (2n=16, BB), the majority of the F_1 plants were of the maternal type (2n=16), a small fraction had *B. nigra* morphology but were mixoploids (2n=16–18), predominantly with 2n=16 cells and three plants, each with a specific morphology, were mixoploids consisting of cells with varying ranges of chromosome numbers (2n=17–26, 11–17 and 14–17). The origin of these different types of plants was inferred to be a result of the complete and partial separation of parental genomes and the loss of *O. violaceus* chromosomes. Our findings in the three crosses suggest that the A genome was more influential than the C genome with respect to complete genome separation during mitosis and meiosis of the hybrids with *B. napus.* Possible complete and partial genome separation during mitotic divisions of the hybrids with *B. carinata* was mainly attributed to the role of the B genome. The combined roles of the A and B genomes would thus contribute to the most variable chromosome numbers of mitotic and meiotic cells in the hybrids with *B. juncea* and their progenies. The possible cytological mechanisms pertaining to these hybrids and the potential of genome separation in the production of *Brassica* aneuploids and homozygous plants are discussed.

Key words Cultivated *Brassica* diploids · *Orychophragmus violaceus* · Intergeneric hybrids · Genome separation · Cytogenetics

Introduction

Most aspects of chromosome behaviour are considered to be under genetic control, as are most morphological characteristics (Rees 1961). For example, genetic control has been demonstrated for asynapsis (Beadle 1930, 1933), desynapsis (Li et al. 1945; Ramage and Hernandez-Soriano 1972), homoeologous chromosome pairing (Riley and Chapman 1958; Sears and Okamoto 1958), chiasma frequency (Rees 1955; Rees and Thompson 1956), chromosome morphology (Beadle 1932; Wellwood and Aandolph 1957) and chromosome elimination (Ho and Kasha 1975).

Only with *Orychophragmus violaceus* (L.) O.E. Schulz (2n=24, genomes OO) as male parent has it been possible to obtain intergeneric hybrids in crosses with the three cultivated *Brassica* tetraploids *Brassica napus*

Communicated by H. Becker

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L. (2n=38, AACC), *B. juncea* (L.) Czern. & Coss (2n=36, AABB) and *B. carinata* A. Braun (2n=34, BBCC). All the hybrids were mixoploids. Most of the variation in chromosome numbers was recorded in somatic tissues and pollen mother cells (PMCs) of the hybrids with *B. juncea* and their progenies. Cytogenetical analysis of the three types of hybrids and their progenies indicated that complete separation of the parental genomes occurred during mitosis and meiosis in the hybrids with *B. napus* (Li et al. 1995, 1996), that both complete and partial separation of genomes occurred during mitosis in the hybrids with *B. juncea* and *B. carinata* and that meiotic separation of the genomes occurred in the hybrids with *B. carinata* (Li et al. 1998). Obvious differences in chromosome behaviour prevailed among the three types of hybrids.

It is well established that the three cultivated *Brassica* tetraploids originated as amphidiploids from crosses between pairs of the three cultivated diploids *B. campestris* L. (2n=20, AA), *B. oleracea* L. (2n=18, CC) and *B. nigra* (L.) Koch (2n=16, BB) (U 1935; Prakash and Hinata 1980). A closer phylogenetical relationship has been revealed between *B. campestris* and *B. oleracea*, while *B. nigra* was found to be closer to *Sinapis arvensis* (e.g. Song et al. 1990). As to the phylogenetical position of *O. violaceus*, its inclusion within the tribe Brassiceae was questioned by Al-Shehbaz (1985) but supported by Anderson and Warwick (1995).

In this report we document the production of intergeneric hybrids between *O. violaceus* and the three cultivated *Brassica* diploids and compare the cytology of these hybrids with that of the hybrids with the three cultivated *Brassica* tetraploids (Li et al. 1995, 1996, 1998). Chromosome number and behaviour in hybrids with the *Brassica* diploids might elucidate the possible roles of the A, B and C genomes as to complete and partial genome separation in the hybrids with the three tetraploids.

Materials and methods

Plant materials

The following *Brassica* materials were useed in reciprocal crosses with *Orychophragmus violaceus*: *B. oleracea* var 'capitata' cv 'Niuxinbaocai' and *B. alboglabra* Bailey (a form of *B. oleracea*) (No. 4003); *B. campestris* cvs 'Tianmenyoucai', 'Qixinjian', 'Yellow Sarson' (K-151), 'Sv 88-39339' and 'Tyko' , *B. campestris* L. ssp. *chinensis* L. cvs 'Chuanyou 8', 'Shanghaiqing' and 'Aijuehuang'; *B. nigra* cv 'Giebra'. *Brassica campestris* cvs. 'K-151', 'Sv 88-39339' and 'Tyko', *B. alboglabra* and *B. nigra* were supplied by the department at Svalöv, and all the others by the department at Wuhan. The cultivar 'Niuxinbaocai' is widely cultivated as a vegetable in the Wuhan area of China.

The Chinese and Swedish materials were grown in the campus field at Wuhan and in the greenhouse at Svalöv, respectively, and crosses were made by hand emasculation and pollination. *Orychophragmus violaceus* was vernalized for 6 weeks at 8°C to induce flowering in the greenhouse. Embryo rescue was applied in the cross with *B. oleracea* cv 'Niuxinbaocai'. Plantlets with roots were transferred to the field in October or November. The detailed procedures were as reported earlier (Li et al. 1995).

The seeds obtained from crosses with *B. campestris* were germinated on wet filter paper at 22° C. After taking root tips for chromosome counts, we planted the germinating seeds in small plastic cups with nutritional soil. The seedlings with three to four leaves were then transplanted to the field. The seeds obtained from crosses with *B. nigra* and from selfing or backcrossing the plants raised from these seeds were put on wet filter paper for germination at room temperature. Root tips were taken for determination of the chromosome number, and the germinating seeds were potted in the greenhouse. Root tips from 3- to 4-week-old plants were collected for a further analysis of chromosome number.

Cytological methods

Root tips from seeds and young plants and styles from young flower buds were used to determine the chromosome number of the hybrids and their progenies. The root tips and styles were treated with 2 m*M* 8-hydroxyquinoline for 4–5 h at 22° C and then fixed in Carnoy's solution. Chromosome preparations were made according to our method (Li et al. 1995).

For meiotic analysis flower buds were fixed for 24 h in fresh Carnoy's solution 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. Dividing anther-wall cells in young flower buds were also studied when encountered in meiotic preparations.

Results

Crosses were made between *Orychophragmus violaceus* and *Brassica oleracea*, *B. campestris* and *B. nigra*. The morphology, cytology, pollen stainability and seed setting of the F_1 plants obtained were recorded. Similar data were collected from the F_2 plants resulting from selfing of the F_1 progeny or backcrossing it to the *Brassica* parent. A scheme summarizing these findings is presented in Fig. 1, and the details are described as follows.

B. oleracea×*O. violaceus*

Two hybrid plants were obtained after embryo rescue when *B. oleracea* cv 'Niuxinbaocai' was used as female parent, and no hybrids were produced in the reciprocal crosses and in crosses with *B. alboglabra* (Table 1). When *B. oleracea* or *B. alboglabra* were used as female parent, most of the ovaries were elongated after pollination, but few of them contained embryos.

The young hybrid plants were morphologically intermediate between the two parents. They had hairy leaves and petioles as in *O. violaceus*. The leaves tended to curl as those of *B. oleracea*, but no cabbage head was formed. The character of serrated leaves of *O. violaceus* was shown by the hybrids (Fig. 2a). The morphology of the mature hybrid plants (Fig. 2c) was closer to that of *B. oleracea* except for petal colour, which was purple as in the male parent *O. violaceus* (Fig. 2b). The purple colour of the petals was lighter than that of *O. violaceus*, and the colour turned to nearly white after the flowers were open for some time. The purple colour of *O. violaceus*

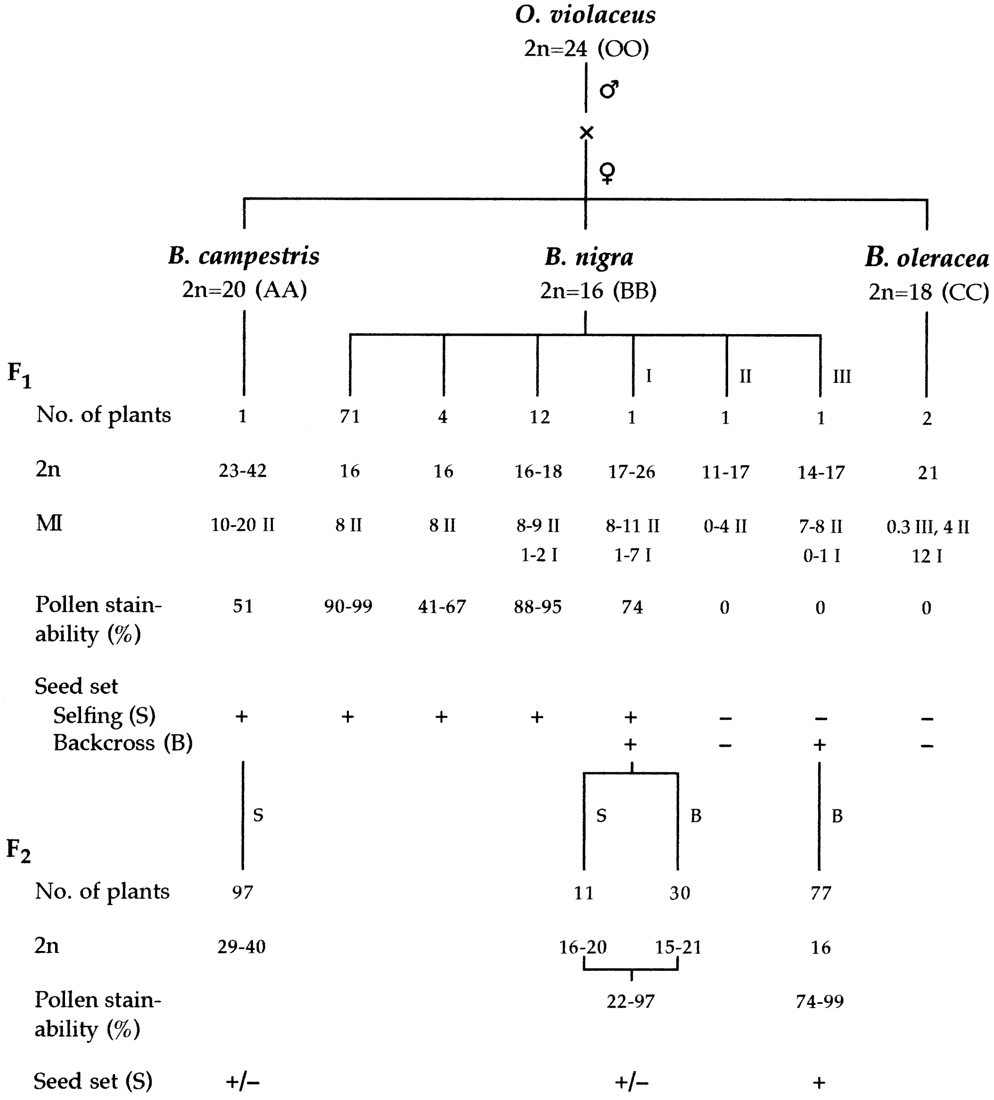

Fig. 1 A scheme summarizing the main results obtained in the crosses made between *O. violaceus* and the three cultivated diploid *Brassica* species and in the F_2 obtained after selfing or backcrossing of F_1 plants to the *Brassica* parent. For details see the text

Table 1 Results of intergeneric reciprocal crosses of *B. oleracea*, *B. campestris* and *B. nigra* with *O. violaceus*

Cross	Number of flowers pollinated (A)	Number of hybrid plants obtained (B)	B/A×100
B. oleracea×*O.v.*[a]	550	2	0.36
O.v.×*B. oleracea*	300	0	0
B. alboglabra×*O.v.*	300	0	0
B. campestris L. ssp. *chinensis* L. cv Aijuehuang×*O.v.*	300	1	0.33
B. campestris×*O.v.*	270[b]	0	0
O.v.×*B. campestris*	300[c]	0	0
B. nigra×*O.v.*	1050	15	1.43

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

[b] Total number of flowers pollinated with all other *B. campestris* cultivars

[c] Total number of flowers pollinated with all *B. campestris* cultivars

was slightly expressed in petioles, leaf veins and ovaries of the hybrid plants. Some abnormal pollen grains were produced in the thin anthers of the hybrids which were totally sterile. No seeds were obtained after selfing or pollination by the two parents.

All observed prometaphase and metaphase cells in styles and anther walls had the expected chromosome number of 21 (Fig. 3a). In PMCs at metaphase I (MI) there were on average 0.3 III+4 II+12 I (Table 2, Fig. 3b). Multivalents comprising 4–7 chromosomes were also encountered. A maximum chromosome pairing of 3 III+4 II+4 I was recorded. The maximum numbers of trivalents and bivalents in a cell were 4 and 8 respectively, and the number of univalents per cell varied between 4 and 21. The chromosomes appeared in groups in some PMCs with 21 univalents. The majority of PMCs at anaphase I (AI) had lagging chromosomes. Frequently several daughter groups of anaphase chromosomes were formed (Fig. 3c) which gave rise to several microspores instead of tetrads. Six to ten nuclei of different sizes were usually produced by each PMC at telophase II

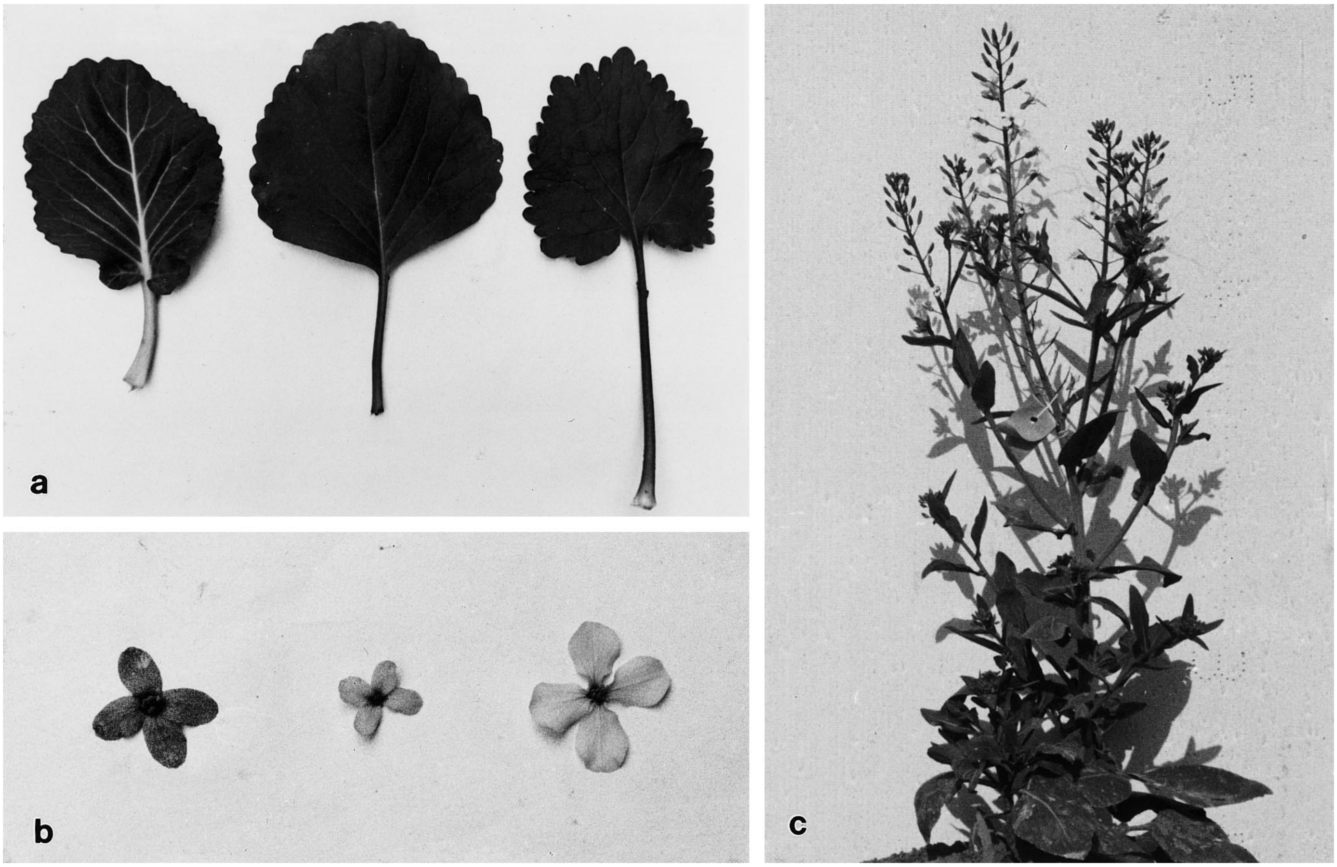

Fig. 2a–c Morphology of the *B. oleracea*×*O. violaceus* hybrid and its parents. **a**, **b** Leaves and yellow, pale violet and violet flowers of *B. oleracea*, the hybrid and *O. violaceus* (from *left* to *right*). **c** A flowering hybrid plant

(TII), which resulted in the formation of unstainable pollen grains.

B. campestris×*O. violaceus*

Of all the crosses with *B. campestris* cultivars, only 1 putative hybrid plant was obtained when *B. campestris* L. ssp. *chinensis* L. cv ‘Aijuehuang’ was used as the female parent (Table 1). The young hybrid could be distinguished from ‘Aijuehuang’ plants by the morphology of its first leaf which was serrated as in *O. violaceus*. At a later stage of development the petioles of the serrated leaves were also similar to those of *O. violaceus* (Fig. 4a). The flowers were yellow and the petals were nearly as large as those of ‘Aijuehuang’, but the styles were longer than those of ‘Aijuehuang’. The hybrid also showed the character of basal clustering of stems, a characteristic of *O. violaceus* (Fig. 4b). The

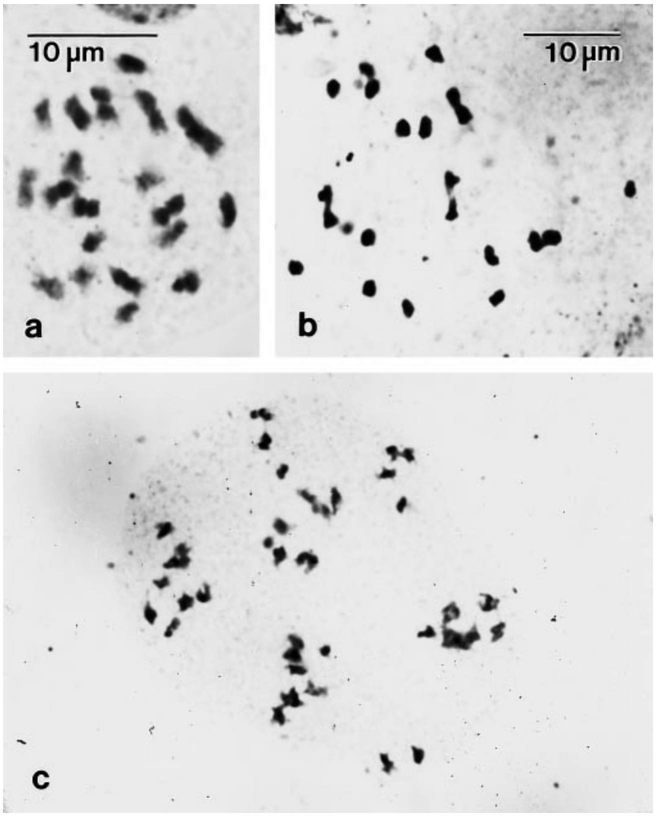

Fig. 3a–c Mitotic and meiotic cells of the *B. oleracea*×*O. violaceus* hybrids. **a** Mitotic chromosomes in a style cell (2n=21), **b** MI with 4 II+13 I, **c** one AI PMC with several daughter groups of chromosomes

Table 2 Frequency of different meiotic configurations in the hybrids between *B. oleracea* and *O. violaceus*

Number of plants	Number of PMCs	Means of different chromosome configurations						
		I	II	III	IV	V	VI	VII
2[a]	196	11.80 (4–21)[b]	3.93 (0–8)	0.31 (0–4)	0.03 (0–1)	0.02 (0–1)	0.02 (0–1)	0.01 (0–1)

[a] Plants from two rescued embryos

[b] Figures between brackets are ranges

Fig. 4a–d Morphology of *B. campestris* L. ssp. *chinensis* L. cv 'Aijuehuang', the 'Aijuehuang'×*O. violaceus* hybrid and F_2 progeny. **a** Young plants of 'Aijuehuang' (*left*) and the hybrid (*right*), **b** a flowering hybrid plant showing basal clustering of stems, **c**, **d** Two young F_2 seedlings with four cotyledonary leaves, having four separate petioles (**c**) or two separate and two joint petioles (**d**). The first leaf in one seedling (**c**) is serrated and hairy as in *O. violaceus*. See **Fig. 2a** or **Fig. 6a** for the leaf morphology of *O. violaceus*

purple colour from *O. violaceus* was expressed to a minor degree on leaves and stems. Pollen grains were abundant, but stainability was 51.3%. When selfed or left for open pollination, some siliques with 1–4 seeds were formed. About 400 seeds of different sizes were harvested.

The hybrid plant was a mixoploid composed of cells with 23–42 chromosomes. Cells with 34 chromosomes were most frequent in styles (Table 3). No cells with the expected chromosome number (2n=22) were found. Obvious differences in chromosome size were observed in mitotic cells with different chromosome numbers (Fig. 5a, b). Lagging chromosomes were observed at anaphase and telophase.

It was difficult to determine the meiotic configurations at diakinesis and MI because of the uncertainty regarding chromosome numbers. The number of bivalents per cell varied from about 10 to 20. Some large and darkly stained univalents and bivalents were differentiated, and the chromosomes composing such bivalents showed a delayed separation. PMCs with or without lagging chromosomes were observed at AI/TI. Some large and darkly stained chromosomes could be easily recognized. They were included in, or lagged between, the polar chromosome groups at AI/TI (Fig. 5c, d) or AII/TII. The chromosome numbers recorded at AI were mainly in the range 36 to 40, being higher than the most frequent numbers recorded in style cells (Table 3).

Among 97 F_2 plants, 14 had three and 2 had four cotyledonary leaves (Fig. 4c, d). No seeds were produced by 47 plants when selfed or left for open pollination in the greenhouse, while 50 plants showed variable fertility. The F_2 plants were morphologically classified into three types: a type similar to *B. campestris* L. ssp. *chinensis* L., a hybrid type and a group with various other kinds of morphology, amounting to 11, 10 and 76 plants, respectively. The purple colour characteristic of *O. violaceus* was expressed to different extents in leaves, petioles, stems and pods of the majority of the F_2 plants. Some plants showed the *O. violaceus* character of basal clustering of stems. Eighteen F_2 plants were studied cytologically. They were mixoploids, consisting of cells with chromosome numbers in different ranges, maximally between 29 and 40.

Table 3 Frequency of cells with various chromosome numbers in styles and AI PMCs of the hybrid between *B. campestris* L. ssp. *chinensis* L. cv 'Aijuehuang' and *O. violaceus*

Tissue	Number of cells with chromosome numbers:																		
	23	24	26	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	Total
Style	1	2	1	1	2	3	8	9	14	25	12	18	11	8		2		1	118
PMCs		1	1			1	1	1	2	2	2	3	4	7	5	3	2	1	36

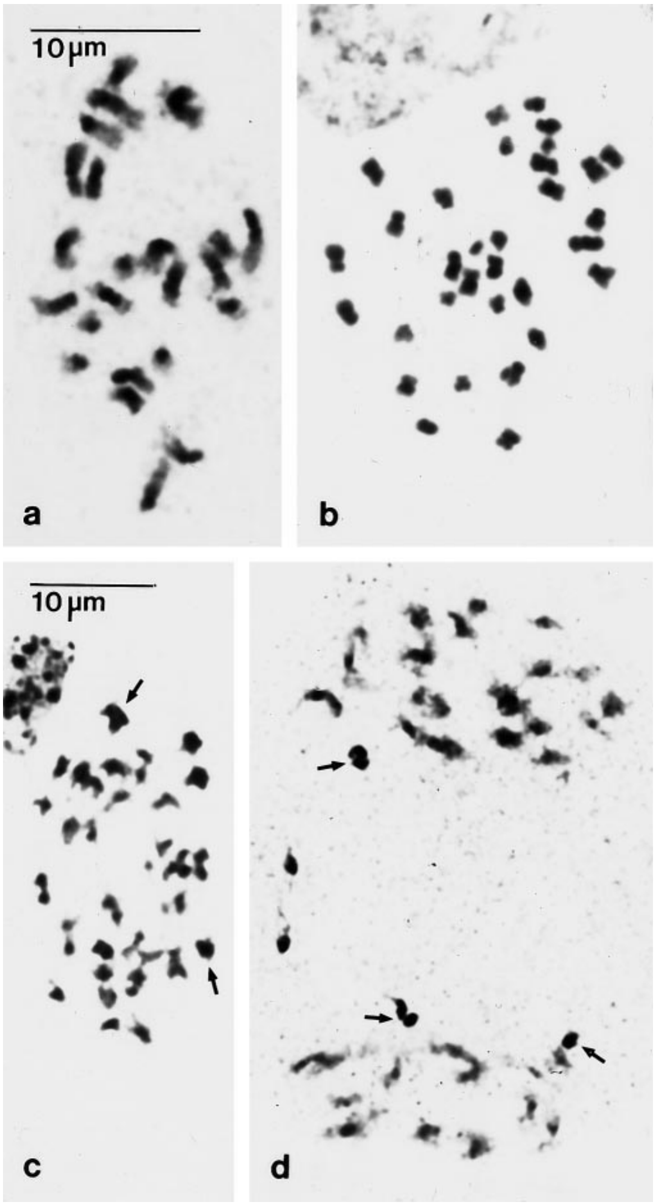

Fig. 5a–c Mitosis and meiosis of the *B. campestris* L. ssp. chinensis L. cv 'Aijuehuang'×*O. violaceus* hybrid. **a**, **b** Style cells at prometaphase (**a**) and metaphase (**b**) with 2n=23 and 2n=32, respectively, showing differences in chromosome size. **c** AI with some large and darkly stained chromosomes (*arrows*) in the two polar groups. **d** TI with some darkly stained chromosomes/chromatids included in (*arrows*), or lagging between, the two daughter nuclei

B. nigra×O. violaceus

When *B. nigra* was pollinated by *O. violaceus*, a relatively high seed set of 1.43% was obtained (Table 1). The harvested seeds were of different sizes, and most of them germinated readily on wet filter paper, but for some it was necessary to remove the seed coat to promote germination. All germinated seeds produced plants.

The majority of the 90 F_1 plants obtained were morphologically similar to *B. nigra* before flowering, except for some plants which had serrated or lightly green leaves. After flowering, 3 plants were distinguished from the others by the deviating shape, size, number and disposition of their petals. The greatest variation among the F_1 plants was in pollen stainability, being 0 (no pollen grains) – 99%.

The F_1 plants were cytologically classified into three groups. (1) In 75 plants, all mitotic cells had 2n=16 and all PMCs had 8 II at diakinesis and MI and 8:8 segregations at AI. All these plants had the same morphology as *B. nigra*, and most of them showed high percentages of stained pollen (>90%), but 4 plants had low percentages (41–67%). The low pollen stainability (44%) of 1 plant may be attributed to heterozygosity for a chromosome inversion, because a bridge and a fragment were observed at AI in this plant. No cytological reasons for the low percentages of stainable pollen in the other plants were found.

(2) In 12 plants, the mitotic cells predominantly had 2n=16 and occasionally 2n=17 or 18, and the majority of PMCs had 8 II at diakinesis and MI and 8:8 segregations at AI, while a few PMCs had 8 II+1 I or 9 II at diakinesis and MI and 8:9 segregations and occasional lagging of 1 or 2 chromosomes at AI/TI. Lagging chromosomes or chromatids were also observed during the second meiotic division. The frequency of PMCs with lagging chromosomes varied. The plants were morphologically non-distinguishable from those in the first group, but some deviated from *B. nigra* by having round, serrated and lightly green leaves. The plants in this group showed high pollen stainability (88–95%).

(3) Three plants (I, II, III) had distinct morphological and cytological characteristics. Plant I had serrated leaves (Fig. 6a) of the *O. violaceus* type, the largest petals among all F_1 plants, and a petal shape and disposition (Fig. 6b) also similar to that of *O. violaceus*. The purple colour of *O. violaceus* was expressed at the joint between petiole and stem. The anthers were well developed, and abundant pollen grains of different sizes and morphology (74% stainability) were produced. The flowering period was the longest among all F_1 plants.

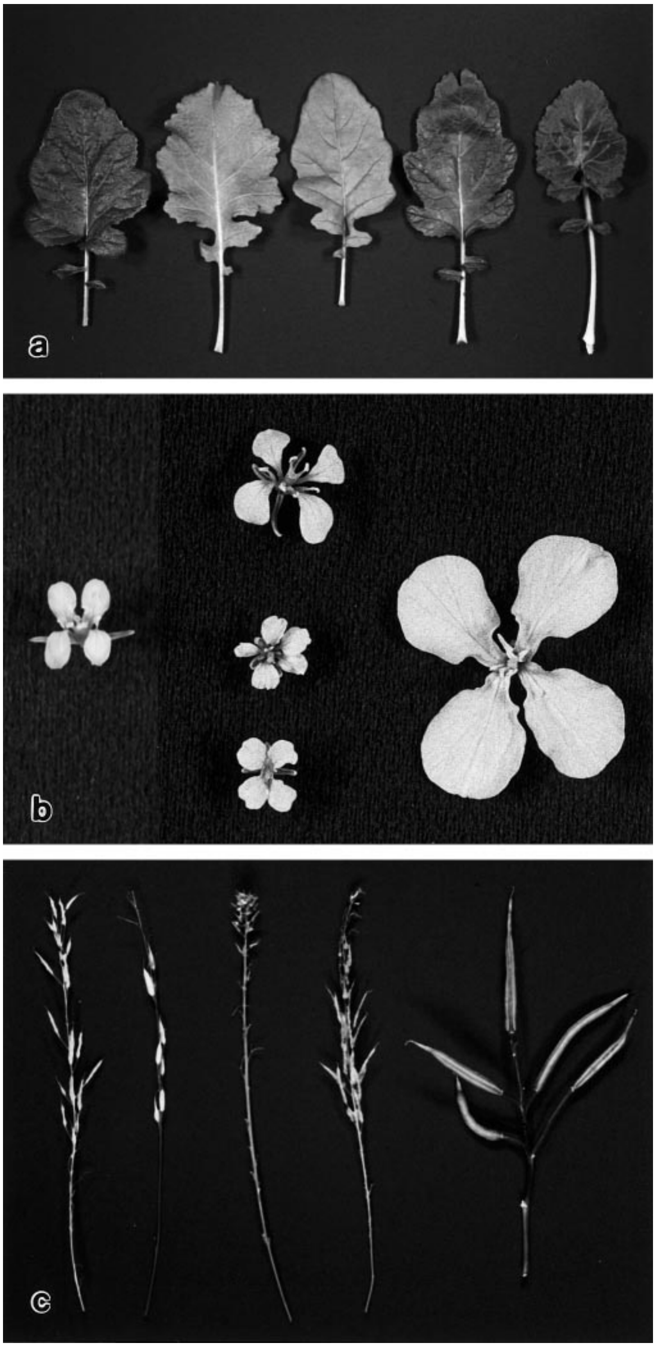

Fig. 6a–c Morphology of *B. nigra* (*left*), the 3 *B. nigra*×*O. violaceus* hybrid plants I, II and III (*middle three*) and *O. violaceus* (*right*). **a** Leaves, **b** flowers (from *top* to *bottom* for plants I, II and III) **c** mature inflorescences

Many seeds were produced after selfing (Fig. 6c) or reciprocal backcrossing to *B. nigra*. A higher seed-set was achieved by backcrosses. Plant II had smaller and lighter green leaves than those of *B. nigra* (Fig. 6a). Its petals were slightly smaller than those of *B. nigra*, but their shape was similar to that of *O. violaceus* and the number was occasionally five (Fig. 6b). Plant III had the same morphology as *B. nigra* except for its petals, which were similar to those of *O. violaceus*, but wrinkled (Fig. 6a, b). Large numbers of immature pollen grains were observed in plants II and III, but in opened flowers only very low numbers of nonstainable and shrunken pollen grains remained in the tiny anthers of plant II, and no pollen grains were found in the very weakly developed anthers of plant III. When pollinated by *B. nigra*, plant II produced no seeds, but plant III showed nearly normal seed-set and produced many seeds (Fig. 6c).

In plant I, cells with 18–25 chromosomes were found and those with 2n=24 were most frequent. A wider range of chromosome numbers occurred in PMCs than in somatic tissues (Table 4). Various pairing configurations with 8–11 bivalents and 1–7 univalents were observed at diakinesis and MI. It was possible to differentiate some large and darkly stained univalents and bivalents in some PMCs at MI. Various AI segregations were observed in cells with similar or different chromosome numbers, and the number of chromosomes per polar group ranged between 7 and 15, which gave rise to AII polar groups with different numbers of chromosomes (Fig. 7a). The densely stained chromosomes lagged between, or were included into, polar groups or daughter nuclei at AI/TI or AII/TII (Fig. 7b). Besides the large microspores, minor microspores with micronuclei were formed.

The plants obtained after selfing plant I and backcrossing it to *B. nigra* were mixoploids, except for 4 plants acquired from backcrosses which had 2n=16 in both mitotic and meiotic cells (Table 5). Except for 1 plant with 2n=15–16 from a backcross which produced PMCs showing 7:8 or 8:8 segregations at AI, all other mixoploid progeny plants had mitotic and meiotic cells with chromosome numbers varying between 16 and 17–21 (Table 5). For example, 1 selfed plant had PMCs with 8 II, 8 II+1–4 I, 9 II+1–3 I and 10 II at diakinesis and MI, and PMCs at AI with segregations of 8:8–11, 9:9–11 and 10:10. One backcross plant contained PMCs with 8 II, 8 II+1–3 I, 9 II and 9 II+1 I at diakinesis and MI, and PMCs at AI with segregations of 8:8–11 and 9:9–10 (Fig. 7c). In PMCs of these mixoploid plants, large and more darkly stained chromosomes appearing as univalents and bivalents were usually differentiated at diakinesis and MI. The more darkly stained chromosomes lagging between, or included into, polar groups or daughter nuclei were observed at AI/TI or AII/TII. One plant, obtained after selfing, had cells with 33 chromosomes and much larger petals and flower buds than all the other progenies.

The progenies of plant I showed discernible variations in leaf morphology, stem branching pattern and petal size and shape, while exhibiting the main morphological features of *B. nigra*. A wider morphological variation was found after selfing than in backcrosses. The purple colour of *O. violaceus* was observed at the joint between petiole and stem in some plants. Four plants from backcrosses had two stems at the cotyledonary node. The plants obtained after selfing and backcrosses had 22–97% pollen stainability and varied in fertility. A wide range of pollen stainability (29–96%) was shown by the backcross plants with 2n=16, 17. One such plant

Table 4 Frequency of cells with various chromosome numbers in styles and PMCs at diakinesis, MI and AI of 3 F_1 plants between *B. nigra* and *O. violaceus*

Plant	Tissue	Number of cells with chromosome numbers:																
		11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	Total
I	Style								1		2	5	3	5	17	2		35
	PMCs							1	1	4	17	17	21	10	13	5	2	91
II	Style		1			6	6											13
	PMCs	1	3	6	39	46	66	2										163
III	Style					5	8	2										15
	PMCs				1	1	41	3										49

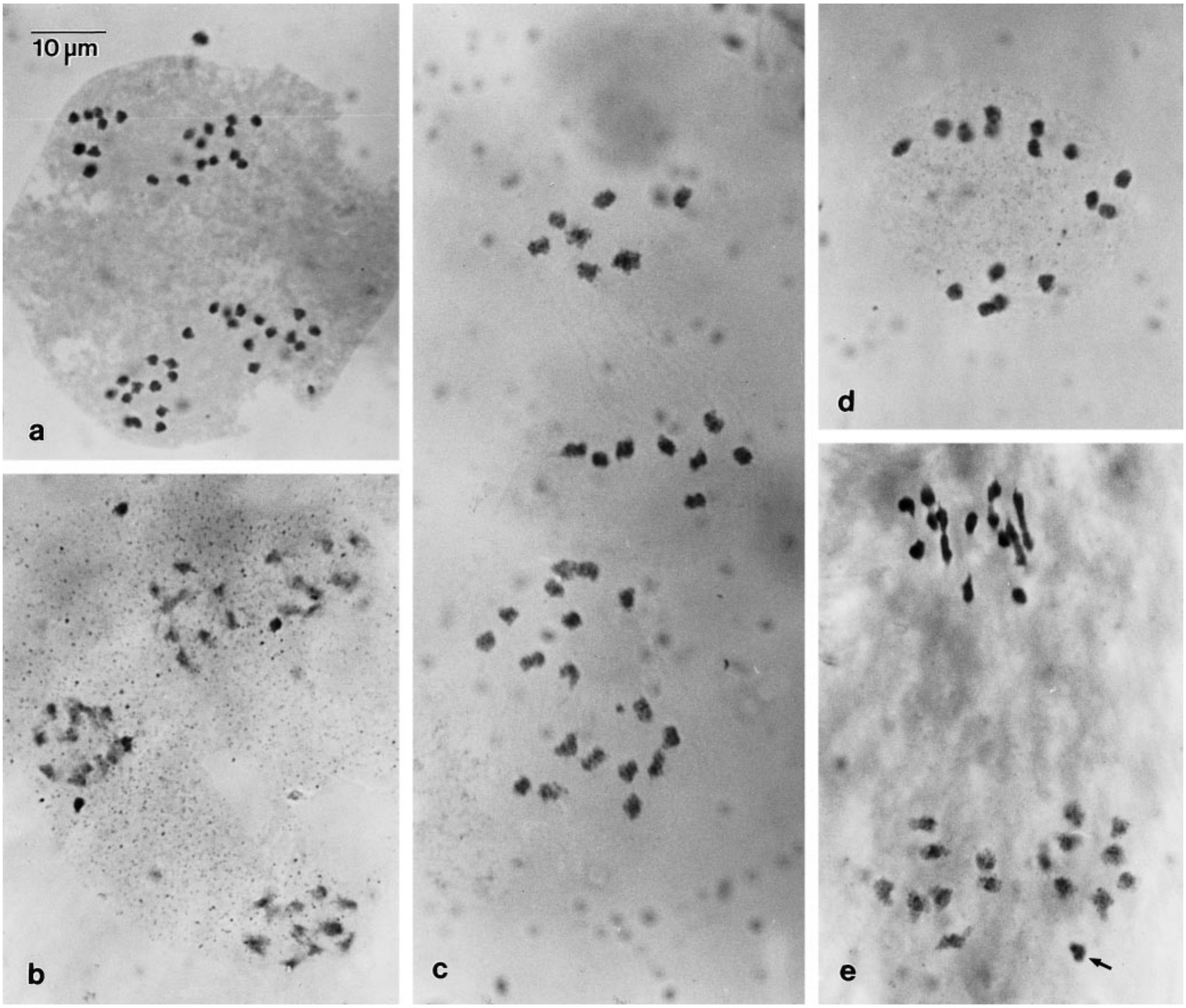

Fig. 7a–e Meiosis of the hybrid plants I, II and III from *B. nigra*×*O. violaceus* crosses. **a**, **b** AII with different numbers of chromosomes in the four daughter groups (**a**) and TII with darkly stained chromosomes included in, or lagging between, daughter groups (**b**), from plant I. **c** Two AI configurations in one field showing 8:8 (*above*) and 9:9 (*below*) segregations, from 1 progeny plant obtained after backcrossing plant I. **d** MI or AI with 16 chromosomes, from plant II. **e** Two cells in one field, one at MI (above) with 8 II and the other at AI (below) showing 8:9 segregation with 1 darkly stained chromosome (*arrow*), from plant III

Table 5 Frequency of F_2 and backcross (with *B. nigra* as female parent) plants with different chromosome numbers obtained from F_1 plant I of *B. nigra*×*O. violaceus* crosses

Population	Number of plants with cells having chromosomes in ranges:								
	15–16	16	16–17	16–18	16–19	16–20	16–21	33	Total
F_2			3	2	4	1		1	11
BC	1	4	17	4	2	1	1		30

with 88% pollen stainability had a portion of exceptionally large pollen grains (8.9%) and produced no seeds after selfing and open pollination.

Plant II had cells mainly with 2n=15–16 and PMCs with 2n=11–17 (Table 4, Fig. 7d). The number of bivalents was 0–3 for cells with 2n=14 and 0–4 for cells with 2n=15 and 16, and no multivalents were observed. Various segregations with 5–10 chromosomes in polar groups were shown by cells with similar or different chromosome numbers. Some univalents and bivalents could be differentiated by being larger and more darkly stained.

In plant III, mitotic and meiotic cells had 2n=14–17 (Table 4). The majority of PMCs had 8 bivalents at diakinesis and MI (Fig. 7e) and showed 8:8 AI segregations. A few PMCs had 8 II+1 I and 8:9 or 8:8:1 laggard segregations at AI (Fig. 7e). The chromosome appearing as a univalent at diakinesis and MI, and lagging between or included into polar groups at AI/TI, was usually identifiable by its larger size and darker colour (Fig. 7e). Some PMCs with 7 II and 7 II+1 I and the 7:8 segregations were observed.

Among 77 progeny plants obtained after pollinating plant III by *B. nigra*, 23 had three and 2 had four cotyledonary leaves. All plants were morphologically very similar to *B. nigra*, but a few had deeply purple petioles and leaf veins or serrated leaves, and some had larger petals than those of *B. nigra*. Their pollen stainability was generally high, being 74–99%, and seeds were obtained after selfing. Among 47 plants whose chromosome numbers were successfully determined in roots, 45 had all cells with 2n=16, and the remaining 2 plants were mixoploids with 2n=14–16 and 26–32.

Discussion

Chromosome behaviour in hybrids and role of the *Brassica* genome

Among the hybrids of *O. violaceus* with the three cultivated *Brassica* diploids, only those with *B. oleracea* had the expected chromosome number (2n=21) in mitotic and meiotic cells. The hybrids between *O. violaceus* and *B. alboglabra* obtained by Yin et al. (1998) also had 2n=21 and were totally sterile. As has been proposed for the hybrids of *O. violaceus* with *B. juncea* and *B. carinata* (Li et al. 1998), partial separation of the parental genomes and chromosome doubling during mitosis might explain our results observed in the sole mixoploid hybrid plant (2n=23–42) obtained from all crosses with *B. campestris*. The addition of a partial chromosome complement of *O. violaceus* to a *B. campestris* complement might have occurred during embryo development or early stages of plant growth, since no cells with the expected chromosome number (2n=22) were observed in the style and PMCs. That mixoploidy in the hybrid was the outcome of the loss of *O. violaceus* chromosomes in an embryo with 2n=44 as a result of fusion between unreduced gametes or the doubling of zygote chromosomes cannot be excluded. The types and chromosome numbers of progeny plants obtained after selfing this hybrid, and particularly the recovery of *B. campestris*-type plants, showed that fertile gametes with a *B. campestris* complement with or without additional *O. violaceus* chromosomes were produced. The inclusion of *O. violaceus* chromosomes in the hybrid and its progeny was well evidenced by their morphology and cytological behaviour. Large and darkly stained chromosomes (Fig. 5c, d) were conceived as being of *O. violaceus* origin.

The complete and partial separation of parental genomes during mitosis, inferred to occur in the hybrids of *O. violaceus* with *B. juncea* and *B. carinata* (Li et al. 1998), might occur as well in the *B. nigra*×*O. violaceus* hybrids. The majority of the F_1 plants (the first two groups, 87 out of 90 plants) very likely resulted from the more frequent occurrence of complete rather than partial genome separation, followed by the elimination of cells with complete or partial *O. violaceus* complements and the loss of most of the additional *O. violaceus* chromosomes in cells with a complete *B. nigra* complement. A gynogenetic origin of the *B. nigra*-like plants cannot be excluded, especially with respect to the first group of F_1 plants (75 out of 90 plants). That these plants arose through selfing can be excluded, however, since the flowers were emasculated in these crosses.

The results obtained from F_1 plants I, II and III from the *B. nigra*×*O. violaceus* crosses possibly reflect different situations relating to the proposed complete and partial genome separation. The somatic chromosome numbers and meiotic behaviour of plant I and its progenies are suggestive that 1–4 *O. violaceus* chromosomes were added to the 8 *B. nigra* chromosomes, probably during the process of genome separation, resulting in the formation of cells with 9–12 chromosomes and, upon subsequent chromosome duplication, of cells with 18, 20, 22 and 24 chromosomes. The additional chromosomes might be lost successively during mitosis, and cells with odd chromosome numbers of 17, 19, 21 and 23 would arise.

In plant II, a reciprocal inclusion of parental chromosomes during genome separation possibly gave rise to cells with a partial *B. nigra* complement and some additional *O. violaceus* chromosomes. In plant III, mitotic and meitoic cells with 2n=16 were the most frequently observed, and these were possibly produced by complete genome separation; fewer cells with 2n=14, 15, 17 were observed, and these may have been produced by the loss of individual *B. nigra* chromosomes and the addition of individual *O. vialaceus* chromosomes during partial genome separation. The finding of PMCs with 7 II+1 I and a 7:8 segregation and obtaining mixoploid plants from backcrosses to *B. nigra* showed that the substitution of individual *B. nigra* chromosomes by *O. violaceus* chromosomes might occur. The reason for the male sterility of plant III remains unknown. Further studies should be made to characterize more progeny plants and to determine whether *O. violaceus* chromosomes do occur in progeny plants with 2n=16 which show some *O. violaceus* characters. It cannot be excluded that homoeologous pairing between *Orychophragmus* and *Brassica* chromosomes might occur and thus lead to the introgression of *O. violaceus* genetic material in the *B. nigra* offspring plants.

Intergeneric hybrids of the six cultivated *Brassica* species with *O. violaceus* have been obtained and their cytogenetics documented (Li et al. 1995, 1996, 1998; present study). The chromosome behaviour in the hybrids of the three diploids could explain chromosome behaviour in the hybrids of the three tetraploids. In the hybrids with *B. oleracea* there were no indications of genome separation, suggesting that the C genome might have a passive role in genome separation. In the present experiments, only one mixoploid hybrid plant was obtained from crosses with different *B. campestris* cultivars. In crosses with *B. campestris* L. ssp. *chinensis* L. cv ‘Chuanyou 8’, hybrids with the expected chromosome number were reported, and possible meiotic separation of parental genomes resulted in the partial fertility of the hybrids and the production of F_2 plants of both parental and hybrid types (Wu et al. 1996). Thus, the *B. campestris* genotype might be a factor that determines chromosome behaviour in the hybrids. Apparently, the A genome is more influential than the C genome regarding the complete genome separation encountered in the hybrids with *B. napus.* The cytological features inferred to occur in the hybrids with *B. juncea* (Li et al. 1998) would also be valid for interpreting chromosome behaviour in the hybrids with *B. nigra.* Thus, complete and partial genome separation, the addition of some *O. violaceus* chromosomes to the *Brassica* complement, the loss of some chromosomes from the *Brassica* complement and reciprocal inclusion or exchange of some chromosomes between parental complements probably occur in both types of hybrids. The combined roles of the A and B genomes in hybrids with *B. juncea* might result in the most complicated type of cytological behaviour among the hybrids of the three tetraploids. In hybrids with *B. carinata*, complete and partial genome separation would consequently be attributed to the presence of the B genome.

All the hybrids of *O. violaceus* with *Brassica* species were obtained only when *O. violaceus* was used as the male parent, and all the reciprocal crosses (the cross with *B. nigra* was untried) were unsuccessful. The high crossability of *B. nigra* with *O. violaceus* might contribute to the higher crossability of *B. juncea* amd *B. carinata* with *O. violaceus* than that of *B. napus.* It should be pointed out that seed-set was also high in crosses with *B. juncea* and *B. carinata* and that most of the F_1 plants were of the *B. juncea* or *B. carinata* types. As in crosses with *B. nigra*, progeny plants of parental types were probably produced by complete genome separation. To determine whether genome separation occurred during embryo development in the crosses with *B. nigra*, we have studied some embryos but failed to get dividing cells.

Possible cytological mechanisms for genome separation and chromosome elimination

As pointed out above, the cytogenetics of the hybrids of *O. violaceus* with the six *Brassica* species was possibly related to the presence and roles of different *Brassica* genomes. Of relevance in this context might be the chromosome behaviour of the different genomes during cell division. The chromosomes of both *B. campestris* and *B. nigra* exhibited a characteristic differential chromatin condensation and staining pattern at diakinesis (Cheng et al. 1994; Heneen et al. 1995; Mackowiack unpublished), but the chromosomes of both *B. oleracea* (Cheng et al. 1994; Heneen et al. 1995) and *O. violaceus* (Li et al. 1995; Li and Liu 1995) condensed and stained relatively homogeneously. During the meiotic divisions of the hybrids of *O. violaceus* with *B. campestris* and *B. nigra* and their progenies, a marked difference in chromosome stainability existed, with the chromosomes possibly from *O. violaceus* being much more darkly stained (Figs. 5c, d and 7b, e), but in the hybrids with *B. oleracea*, such a difference was not observed.

The chromosome behaviour in *Brassica* hybrids with *O. violaceus* might be related to the chromosome condensation and staining patterns of the parental *Brassica* species. In all hybrids with the six *Brassica* species, the expected chromosome number was only shown by the hybrids between *B. oleracea* and *O. violaceus*, whose chromosomes showed a homogeneous condensation and staining pattern. In the hybrids with *B. campestris* and *B. nigra*, the different patterns of chromosome condensation and staining of the parental species might have contributed to genome separation and the subsequent elimination of *O. violaceus* chromosomes. Even in hybrids and amphidiploids between the two closely related species *B. campestris* and *B. alboglabra*, a later AI separation was shown by the larger *B. alboglabra* chromosomes (Heneen et al. 1995).

The different patterns of chromosome condensation and staining might also contribute to the differences in stainability and chromosome behaviour in the hybrids with the three *Brassica* tetraploids. Among the hybrids with the three tetraploids, the most frequent occurrence of differences in stainability of the parental chromosomes was in the hybrids with *B. juncea* (Li et al. 1998) and their progenies (Li et al. unpublished). No obvious difference in chromosome stainability was observed in the hybrids with *B. napus* and *B. carinata*, which harboured homogeneously condensed chromosomes of the

B. oleracea and *O. violaceus* genomes and differentially condensed chromosomes of either the A or B genomes.

A new approach for producing *Brassica* aneuploids and homozygous plants

As indicated for the crosses of *O. violaceus* with *B. juncea* and *B. carinata* (Li et al. 1998), it might be feasible to produce *Brassica* aneuploids comprising complete or partial *Brassica* complements with or without additional *O. violaceus* chromosomes through genome separation in crosses with *B. campestris* and *B. nigra*. The mixoploid plants with a morphology similar to that of *B. campestris* obtained among the F_2 progeny of hybrids with *B. campestris* probably contained a complete *B. campestris* complement and some *O. violaceus* chromosomes. The results from the crosses with *B. nigra* showed that plants with complete and partial *B. nigra* complements and additional *O. violaceus* chromosomes were produced in F_1, F_2 and backcross progenies. Mixoploids with 2n=16–17 (possibly BB and BB+1 chromosome from *O. violaceus*) were easily obtained and could be useful for the study of the different chromosomes of the *O. violaceus* genome. Mixoploids (2n=16, 17) with various pollen stainabilities among the backcross progeny of plant I might contain different *O. violaceus* chromosomes. That hybrid plant II probably had a partial *B. nigra* complement and additional *O. violaceus* chromosomes is also suggestive that *B. nigra* might be a secondary polyploid (Röbbelen 1960) that tolerates chromosome loss.

The production of plant haploids by chromosome elimination in interspecific hybridizations has been exploited as a means to obtain homozygous lines in barley (Kasha and Kao 1970) and wheat (Barclay 1975). The *Brassica* plants produced after possible complete genome separation in hybridizations between *Brassica* species and *O. violaceus* are expected to be homozygous. In crosses with *B. nigra*, *B. juncea* and *B. carinata*, homozygous *Brassica* plants could be easily obtained among the F_1 plants, but in crosses with *B. napus* (Li et al. 1995) and *B. campestris* (Wu et al. 1996), maternal plants were more accessible among the F_2 progeny.

Acknowledgements We wish to express our most sincere thanks to Kerstin Brismar for her help with the figures. The study was supported by grants to Dr. Zaiyun Li from The Hubei Provincial and National Natural Science Foundation of P.R. China and from The Nilsson-Ehle Foundation of The Royal Physiographic Society of Sweden.

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