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Phylogenetic studies in *Papaver* section *Oxytona:* cytogenetics of the species and interspecific hybrids*

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Summary. Chromosome behaviour at meiosis was studied in the F_1, F_2, and backcross generations, in the three species of *Papaver* section *Oxytona,* and in artificially induced autopolyploids of *P. bracteatum.* Close homology was found between the genome of *P. bracteatum* and that of the two polyploid species, *P. orientale* and *P. pseudo-orientale,* suggesting that the *P. bracteatum* genome is present in both polyploid species. A genetic mechanism controlling bivalent pairing in the polyploid species is suggested. Further study is needed for finding out the breeding potential of interspecific hybridization in section *Oxytona.*

Key words: *Papaver bracteatum* – *Papaver orientale* – *Papaver pseudo-orientale* – Hybrids – Polyploidy

Introduction

The genus *Papaver* has about 100 species, some of which are used as ornamentals (oriental poppies) and others as medicinal plants, mainly *Papaver somniferum* L. from section *Mecones.* Another species, *Papaver bracteatum* Lindl. of the section *Oxytona,* is also known as a potential medicinal plant of economic importance (Böhm 1981). The capsules and the roots of this plant contain a high concentration of thebaine which is readily converted to codeine by industrial processes. Thebaine is also a starting material for naloxone and naltroxone, synthetic compounds used as morphine antagonists, and for other medicinal purposes (McNicholas and Martin 1984).

During the past few years there has been an increase in a worldwide interest in this species because of its potential as a crop which might supplant the traditional opium poppy as a source of raw material for the pharmaceutical industry (Fairbairn 1976).

Considerable confusion has prevailed in the bio and chemo-systematics of the section *Oxytona,* resulting in insufficient characterization of the species of this section. Goldblatt (1974) reviewed the taxonomy, chemistry and ecology of the section *Oxytona* and included three species in it: *P. bracteatum, P. orientale* and *P. pseudo-orientale.* So far, very few studies of the cytogenetics of the species of this section have been conducted. In all of them, only F_1 hybrids were examined and no further segregating generations were studied, due to the sterility of the hybrids (Yasui 1936; Böhm and Nixdorf 1983; Roussi and Ojala 1984). The present work is part of a study on the chemical and phylogenetic relationships of the species of section *Oxytona.* We report here on the chromosomal behaviour of the hybrids between the species of this section and of the induced autopolyploids, as well as the analysis of F_2 and/or backcross generations. The implications of the results on the possibility of transferring genetic material from one species to another are discussed.

Materials and methods

The seeds of *Papaver orientale* L. (=PO, $2n=28$) (P.I. 376815) and *Papaver pseudo-orientale* L. (=PPO, $2n=42$) (P.I. 375952) were obtained from the Introduction Service of the U.S.D.A. at Beltsville; for *Papaver bracteatum* Lindl. (=PB, $2n=14$), seeds of an early flowering breeding line were used (Levy et al. 1981). The three species were sown in a nethouse and then transplanted onto an experimental farm in Jerusalem. At flowering, controlled reciprocal crosses were made between the three species in all possible combinations. The backcrosses

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between the F_1 hybrids and the parental species were done a year later. The *P. bracteatum* × *P. pseudo-orientale* hybrids were selfed to obtain the F_2 generation. The *P. bracteatum* × *P. orientale* hybrids were relatively sterile and gave only nine plants when pollinated with *P. bracteatum*. The backcross of *P. pseudo-orientale* × *P. orientale* to the parents was unsuccessful in setting seed. The parental species and the hybrids from each cross were grown during the next year in the same experimental plot. For the study of chromosome pairing at meiosis in the parental lines and the hybrids, anthers were collected from flowering buds, fixed in 3 : 1 absolute ethanol : acetic acid for 18 h, and then stored in 70% ethanol at 4 °C. Anthers were stained with 2% acetocarmine and PMC's at metaphase I were analysed. The chiasma per chromosome value was calculated as:

$$\frac{\text{total chiasma in the cell} \times 2}{\text{chromosome number}}$$

Doubling of the chromosome number was attempted by colchicine treatment: 700 seedlings of *P. bracteatum* at the six true leaves' stage were soaked for 5 h in an aqueous solution of 0.1% colchicine and 2% DMSO at 22 °C. After rinsing with water, the seedlings were planted in the field. Chromosome number was scored in PMC's. One plant with $2n=28$ was identified. The autotetraploid plant was pollinated with normal pollen ($n=7$) and gave autotriploid plants $2n=21$.

Results

In most cases *P. bracteatum* had three or four ring bivalents per cell (Fig. 1 a). In the autopolyploids, the number of chiasma per chromosome decreased in comparison with the diploid, and about 23% of the chromosomes were involved in multivalents. The number of chiasma per chromosome decreased further in the autotriploid, mainly because of the high number of univalents; however, in this case about 32% of the chromosomes paired as trivalents (Table 1; Fig. 1 d, e, f). In the two other species the frequency of ring bivalents was lower: for *P. orientale* only one or two per cell, and in *P. pseudo-orientale* most cells had only rod bivalents,

Table 1. Chromosome configurations and chiasma frequency in metaphase I in diploid ($2n=2x=14$), autotriploid ($2n=3x=21$) and autotetraploid ($2n=4x=28$) plants of *Papaver bracteatum*

Ploidy level	Chromosome no. (2n)	No. of cells analysed	No. per cell					Chiasma/ chromosome
			I[a]	II rod	II ring	III	IV	
Diploid	14	20	–	3.4 (1–5)	3.6 (2–6)	–	–	1.54
Triploid	21	37	4.7 (0–7)	2.8 (2–5)	1.9 (1–3)	2.3 (0–6)	–	1.04
Tetraploid	28	38	1.9 (0–4)	5.8 (5–10)	3.9 (1–6)	1.4 (0–3)	0.6 (0–2)	1.33

[a] I = univalent, II = bivalent, III = trivalent, IV = quadrivalent

Table 2. Chromosome configurations and chiasma frequency in metaphase I of *Papaver bracteatum*, *Papaver orientale*, *Papaver pseudo-orientale* and their hybrids

Parents or cross	Chromosome no. (2n)	No. of cells analysed	No. per cell				Chiasma/ chromosome
			I[a]	II rod	II ring	III	
Species							
PB	14	20	–	3.4 (1–5)	3.6 (2–6)	–	1.54
PO	28	25	0.6 (0–4)	13.1 (10–14)	0.6 (0–3)	–	1.08
PPO	42	31	0.4 (0–4)	20.8 (19–21)	–	–	1.00
Hybrids							
PB×PO	21	43	4.9 (4–8)	4.7 (4–7)	0.9 (0–2)	1.6 (0–3)	0.85
PB×PPO	28	34	2.2 (0–6)	12.9 (11–14)	–	–	0.89
PPO×PO	35	27	8.4 (7–15)	13.3 (10–14)	–	–	0.77

[a] See Table 1

Table 3. Chromosome configurations and chiasma frequency in metaphase I of backcross *P. bracteatum* × *P. orientale* to *P. bracteatum*

Cross and chromosome no.	No. of cells analysed	No. per cell				Chiasma/ chromosome
		I[a]	II rod	II ring	III	
(PB×PO)×PB	27	9.4 (7–14)	4.2 (3–5)	1.5 (0–3)	–	0.69
2n=21	23	7.0 (6–8)	5.4 (4–7)	1.6 (0–3)	–	0.82
	23	5.7 (5–7)	6.2 (5–7)	0.5 (0–2)	0.7 (0–2)	0.82
	29	6.3 (4–7)	4.9 (4–6)	1.7 (1–3)	0.5 (0–2)	0.89
	21	6.3 (4–7)	4.5 (4–6)	1.8 (1–3)	0.7 (0–2)	0.92

[a] See Table 1

each with a terminal chiasma (Figs. 1 b, c). In these two species one or two pairs of univalents per cell were found occasionally, probably as a result of chiasma failure or early terminalization.

The reciprocal crosses *P. bracteatum* × *P. orientale* and *P. bracteatum* × *P. pseudo-orientale* were successful and seed set was high in both directions. In the cross *P. pseudo-orientale* × *P. orientale*, seeds were obtained only when *P. pseudo-orientale* was the female parent.

The hybrid between *P. orientale* and *P. bracteatum* had trivalents, bivalents and univalents. In the other hybrids, *P. bracteatum* × *P. pseudo-orientale* and *P. pseudo-orientale* × *P. orientale*, only bivalents and univalents were formed. (Table 2; Fig. 1 i, j, k). The number of chiasma per chromosome in the hybrids involving *P. bracteatum* was higher than that found in the *P. pseudo-orientale* × *P. orientale* hybrid.

Nine plants resulted from the backcross of *P. bracteatum* × *P. orientale* to *P. bracteatum*. The five plants examined had 21 chromosomes. Three of these plants had one or two trivalents per cell and the other two plants formed only bivalents and univalents (Table 3; Fig. 1 h). From the 64 plants of the F_2 *P. bracteatum* × *P. pseudo-orientale*, all the plants analysed had 28 chromosomes. Most of the chromosomes paired in bivalents and number of chiasma per chromosome averaged 0.9 (with S.D. of 0.05). One plant formed 10 bivalents per cell and had a lower chiasma frequency, 0.72 (with S.D of 0.10) (Table 4).

The backcrosses to the *P. bracteatum* parent gave 117 plants with 21 chromosomes. All the plants examined had trivalents, bivalents and univalents. The average frequency of trivalents per cell varied in different plants from 0.2 to 2.3 with a chiasma frequency of 0.73 to 0.98, respectively. Only four of the 55 plants which originated from the backcross to the *P. pseudo-orientale* parent were analysed cytogenetically. Chromosome configurations and chiasma frequency in these plants are given in Table 4 and Fig. 1 g, l.

Discussion

Cytologically the polyploid species of the section *Oxytona* behave as allopolyploids since only bivalents were formed at meiosis. In the polyploid series of this section the number of chiasma per chromosome decreased while polyploidy increased. In the hexaploid *P. pseudo-orientale* only rod bivalents and occasional univalents were observed. Ring bivalents were rare in the tetraploid *P. orientale* but quite common in the diploid *P. bracteatum*. The potential of *P. bracteatum* to form two associations per chromosome is maintained in the artificial polyploids. In the autotriploid the trivalent frequency was as expected (Jackson and Casey 1980), but in the autotetraploid the multivalent frequency was lower than expected (Driscoll et al. 1979). This feature

Fig. 1 a–l. Chromosome associations at metaphase I in: **a** *P. bracteatum* 2n = 14 (5 ring II + 2 rod II); **b** *P. orientale* 2n = 28 (1 ring II + 13 rod II); **c** *P. pseudo-orientale* 2n = 42 (4 I + 19 II); **d** *P. bracteatum* autotriploid 2n = 21 (1 I + 1 II + 6 III); **e** *P. bracteatum* autotetraploid 2n = 28 (3 I + 9 II + 1 III + 1 IV); **f** *P. bracteatum* autotetraploid 2n = 28 (14 II); **g** *P. bracteatum* × *P. pseudo-orientale* backcross to *P. pseudo-orientale* 2n = 35 (16 I + 8 II + 1 III); **h** *P. bracteatum* × *P. orientale* backcross to *P. bracteatum* 2n = 21 (7 I + 7 II); **i** *P. pseudo-orientale* × *P. orientale* 2n = 35 (7 I + 14 II); **j** *P. bracteatum* × *P. orientale* 2n = 21 (7 I + 5 II + 1 III); **k** *P. bracteatum* × *P. pseudo-orientale* 2n = 28 (14 II); **l** *P. bracteatum* × *P. pseudo-orientale* backcross to *P. bracteatum* 2n = 21 (4 I + 4 rod II + 1 ring II + 2 III)

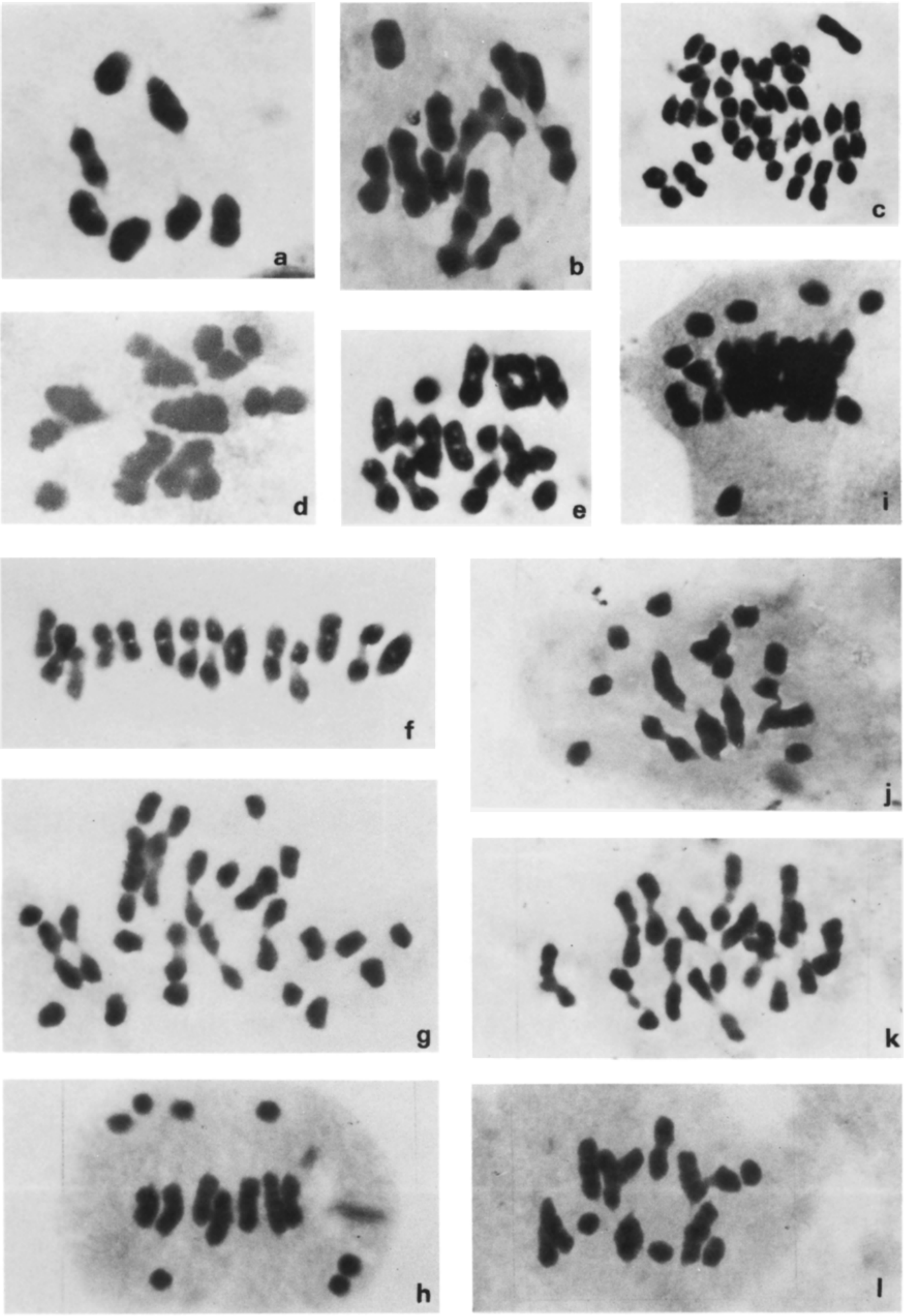
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Table 4. Chromosome configurations and chiasma frequency in metaphase I of F_2 and backcross generations of *P. bracteatum* × *P. pseudo-orientale*

Cross and chromosome no.	No. of cells analysed	No. per cell				Chiasma/ chromosome
		I[a]	II rod	II ring	III	
F_2(PB × PPO) 2n = 28	26	8.0 (6–18)	10.0 (6–11)	–	–	0.72
	31	2.4 (0–8)	12.8 (11–14)	–	–	0.91
	28	3.8 (0–10)	12.1 (9–14)	–	–	0.86
	25	2.5 (0–4)	12.7 (12–14)	–	–	0.91
	31	2.0 (0–4)	12.6 (11–14)	0.4 (0–2)	–	0.95
	29	6.4 (2–12)	10.3 (7–13)	0.4 (0–1)	0.4 (0–1)	0.85
(PB × PPO) × PB 2n = 21	25	6.7 (6–8)	6.1 (5–7)	0.5 (0–1)	0.2 (0–1)	0.71
	28	7.5 (5–9)	5.9 (4–6)	0.4 (0–2)	0.3 (0–2)	0.73
	28	6.1 (5–7)	5.8 (4–7)	0.3 (0–1)	0.9 (0–2)	0.78
	31	6.5 (5–9)	5.5 (4–6)	0.7 (0–2)	0.7 (0–2)	0.79
	30	6.2 (5–7)	4.9 (4–6)	0.4 (0–1)	1.4 (0–2)	0.81
	29	6.2 (4–8)	5.4 (4–7)	0.8 (0–1)	0.8 (0–1)	0.82
	29	6.5 (5–9)	5.2 (4–6)	1.1 (1–2)	0.6 (0–2)	0.82
	30	6.7 (4–9)	4.1 (2–6)	1.2 (0–2)	1.2 (0–3)	0.84
	29	6.3 (4–9)	4.6 (2–6)	1.1 (0–2)	1.1 (0–3)	0.86
	32	5.7 (5–7)	4.8 (4–6)	0.9 (0–3)	1.3 (0–2)	0.88
	34	5.4 (4–7)	4.1 (2–7)	1.3 (0–3)	1.6 (0–3)	0.94
	30	4.7 (3–6)	3.7 (2–6)	1.0 (0–2)	2.3 (1–4)	0.98
(PB × PPO) × PPO	27	9.8 (6–12)	13.1 (12–14)	–	–	0.72
2n = 36[b]	28	8.0 (6–12)	14.0 (12–14)	–	–	0.78
	32	7.4 (8–14)	13.1 (11–14)	–	–	0.72
2n = 35	39	8.7 (6–14)	12.6 (10–14)	0.1 (0–1)	0.3 (0–1)	0.76

[a] See Table 1

[b] Pollinated with trisomic PPO (2n = 43)

has been reported for several autopolyploid species (Riley and Law 1965). The difference in number of chiasma per chromosome between species of the section *Oxytona* should be borne in mind in any attempt to establish phylogenetic relationships.

Chromosome association in the triploid *P. bracteatum* × *P. orientale* is compatible with the possibility that the diploid species was involved in the formation of *P. orientale*. The deviation of the pairing pattern from 71 + 711 can be attributed to chromosomal rearrangements since the formation of the tetraploid. The interpretation of the pairing patterns of the tetraploid and pentaploid hybrids in the *Oxytona* section is more complex. In both hybrids only univalents and bivalents were formed, but more important, the number of bivalents in these hybrids was much greater than expected, if the species of the lower ploidy is the progenitor of the species with the higher ploidy. In other words, the chromosomes of the polyploid species and particularly of *P. pseudo-orientale* are capable of autosyndesis upon interspecific hybridization. This type of synapsis is apparently typical of the genus *Papaver* and has been reported also in the hybrid between *P. nudicale* (2n = 14) and *P. striatocarpum* (2n = 70) (Ljungdahl 1924). Thus, the formation of 14 bivalents in some P.M.C.s, and up to six univalents in others, in the tetraploid *P. bracteatum* × *P. pseudo-orientale* can be interpreted as if the *P. bracteatum* genome was present in the hexaploid species. Autosyndesis of *P. pseudo-orientale* chromosomes is indicated from the 14 bivalents in the tetraploid hybrid. Moreover, the 2n = 28 chromosome number in all progenies of that hybrid and the 2n = 21 in the backcross products to the *P. bracteatum* parent, strengthen this assessment. It can be further said that the chromosomes that underwent autosyndesis apparently were considerably homologous to one another or random segregation in anaphase I could not have produced viable gametes. If this is so, one might expect multivalent associations at meiosis in *P. pseudo-orientale.* The fact that only bivalents were formed can be taken as an indication that in this species bivalent pairing is under genetic control. The nature of that control is not clear but we tend to speculate that it is performed through formation of only one chiasma per chromosome.

Some relationships between the *P. bracteatum* chromosomes and those of the two *P. pseudo-orientale* genomes that are capable of autosyndesis can be inferred from the pairing pattern in the backcross generation. In all the plants examined (2n = 21), trivalent associations were observed. In one plant the rate of the trivalent formation was as high as in the autotriploid of *P. bracteatum.* It seems also, that two doses of *P. bracteatum* genome can suppress the bivalent pairing control of *P. pseudo-orientale.* Chromosome associations in *P. bracteatum* × *P. orientale* and *P. bracteatum* × *P. pseudo-orientale* hybrids suggest that the *P. bracteatum* genome is present in both polyploid species. It is not yet clear whether or not *P. orientale* is the tetraploid progenitor of *P. pseudo-orientale,* as suggested by Goldblatt (1974). The up to 14 bivalents observed in the pentaploid hybrid could be the result of association between chromosomes of the *P. bracteatum* genome that both polyploids share, and autosyndesis between the remaining chromosomes of *P. pseudo-orientale.* The formation of only rod bivalents in the pentaploid indicates, however, that the control of bivalent pairing remained effective in that hybrid.

The interspecific hybrids between the species of the section *Oxytona* showed some degree of fertility (up to 80% pollen stainability) and viable seeds were obtained from their selfing or backcrosses to the parents. The transfer of genetic material between these species is therefore possible and can be used in breeding programs of cultivars with the desirable alkaloid spectrum and agronomical characters, such as the early and profuse flowering during the first year of growing, and the lodging – resistance of the polyploid species.

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