Folia Geobotanica 41: 183–201, 2006

POSSIBLE PATHWAYS OF THE GENE FLOW IN *TARAXACUM* SECT. *RUDERALIA*

Lenka Mártonfiová

P.J. Šafárik University, Botanical Garden, Mánesova 23, SK-043 52 Košice, Slovakia; fax +421 55 6337 353, e-mail lenkam@upjs.sk

Abstract: Reproductive behaviour and the pathways of gene flow among ploidy levels were studied experimentally in *Taraxacum* sect. *Ruderalia*. Diploid, triploid and tetraploid individuals were sampled from mixed diploid – polyploid natural populations. 136 experimental hybridizations between the plants of different ploidy levels were performed. Seeds resulting from these crosses, those obtained from isolated anthodia as well as from open pollinated anthodia (both from cultivated and wild plants) were subjected to the flow-cytometric seed screening (FCSS) to determine ploidy levels in the progeny and to infer breeding behaviour of maternal plants. Three possible pathways of the gene flow were studied: (A) fertilization of sexuals by pollen of apomicts, (B) B_{III} hybrid formation, (C) facultative apomixis. Diploid maternal plants when experimentally crossed with triploid pollen donors produced diploids and polyploid progeny, while when pollinated with a mixture of the pollen of diploids and triploids or insect pollinated, no polyploids were discovered. It seems that in the mixture with the pollen of diploids, the pollen of triploids is ineffective. Tetraploids produce hybrids much easier with diploid mothers and their role in wild populations requires further study. Triploid mothers, even those with subregular pollen did not show traces of facultative apomixis. B_{III} hybrids were present in the progeny of both triploids and tetraploids, in tetraploids in quite high percentages (up to 50% of the progeny in some crosses).

Keywords: *Asteraceae*, B_{III} hybrids, *Compositae*, Dandelion, Facultative apomixis, Flow-cytometry, Gene-flow, Hybridization

INTRODUCTION

Taraxacum WIGG. is a very large, widespread and complicated genus that comprises some 2000 species grouped into over 30 sections (MOGIE & FORD 1988), according to KIRSCHNER & ŠTĚPÁNEK (1996) over 40 sections and new sections are still beeing described (KIRSCHNER & ŠTĚPÁNEK 2004, UHLEMANN et al. 2004). *Taraxacum* sect. *Ruderalia* KIRSCHNER, H. ØLLG. et ŠTĚPÁNEK is one of the largest and phylogenetically youngest ones (DOLL 1982). Like in other *Taraxacum* sections, the species form polyploid series with basic chromosome number x=8. In the section *Ruderalia*, both sexual and apomictic plants are found. Diploids are self-incompatible sexuals. Polyploids are reproducing asexually, via apomixis (diplospory and parthenogenesis, ASKER & JERLING 1992). Male meiosis is reductional. Asexual reproduction leads to progeny of maternal genotype, and variation is usually generated by spontaneous ploidy variation, somatic recombination and mutations (RICHARDS 1997, KIRSCHNER & ŠTĚPÁNEK 1998). Gene flow is always connected with sexual reproduction. Thus avoidance of sexual processes forms a barrier for the gene flow. The gene flow between sexuals and apomicts, however, is not excluded. Several pathways are possible:

(A) Fertilization of sexuals by pollen of apomicts. Since male meiosis is reductional (though irregular) in apomicts, the pollen (or some fraction of the pollen) can fertilize reduced

egg cells of a diploid sexually reproducing individual and form B_{II} hybrids (i.e., hybrids resulting from fertilization of reduced egg cells, ASKER & JERLING 1992). This was experimentally shown by several *Taraxacum* students (e.g. TAS & VAN DIJK 1999). These hybrids are usually polyploids, and the majority of diploids in these crosses is the result of induced self-fertilization (MORITA et al. 1990a). Diploid hybrids are rare (TAS & VAN DIJK 1999).

(B) B_{III} hybrid formation (i.e., hybrids originating from fertilization of unreduced egg cells, ASKER & JERLING 1992) was observed by MAŁECKA (1973) in *Taraxacum*. This pathway always represents gene flow to higher ploidy levels. B_{III} hybrids of triploid apomictic maternal plants and sexual diploid pollen donors are tetraploids. Tetraploids probably played an important role in evolutionary processes in *Taraxacum* (KIRSCHNER & ŠTĚPÁNEK 1996) and seem to be important mediators in the gene flow from diploids to triploids.

(C) Facultative apomixis – the presence of some reduced ovules that require fertilization in polyploid *Taraxacum*. This was reported by RICHARDS (1970b) and MAŁECKA (1973). MAŁECKA (1973) gave cytoembryological evidence of facultative apomixis – she observed both reduced and unreduced ovules in one anthodium in a triploid plant from the *Taraxacum* sect. *Palustria* (H. LINDB.) DAHLST. The reduced ovules required fertilization. RICHARDS (1970b) found a facultatively agamospermous species in *Taraxacum* sect. *Erythrosperma* (H. LINDB.) DAHLST. – the triploid *Taraxacum brachyglossum* (DAHLST.) RAUNK. with subregular pollen. It showed a reduced seed set in the absence of cross-pollination and gave some diploid offspring in the presence of the pollen of diploid. Female meiosis seemed to be synaptic there and it was presumed that unreduced agamospermous eggs are the result of the failure of meiosis (RICHARDS 1997). In the section *Ruderalia*, the facultative apomixis was reported by SORENSEN (1958) in an aneuploid plant (2n=23).

Pathway A was studied by various *Taraxacum* students. These reports often concern intersectional crosses (e.g . RICHARDS 1970a, MORITA et al. 1990a). However, within the section *Ruderalia* some crosses were reported, too (e.g. JENNISKENS 1985, STERK 1987, MORITA et al. 1990a, TAS & VAN DIJK 1999). Crosses between two diploid sexuals usually gave developed seeds with regular seed set. The crosses between different ploidy levels mostly concerned the crosses between diploids as seed parents and triploids. The results were much varied here, from a poor (STERK 1987) to a very good seed set (TAS & VAN DIJK 1999), and from almost diploid progeny (JENNISKENS 1985) to very variable progeny with mixture of ploidy levels (RICHARDS 1970a). Crossings between diploids and tetraploids are reported very rarely (e.g. RICHARDS 1970a, however, only remote crosses were performed here). The induced selfing (MORITA et. al. 1990a) generates the majority of diploid progeny in diploid mother-apomict pollen donor crosses. The pathways B and C are often neglected by *Taraxacum* students.

The chromosome numbers in the progeny were evaluated mostly by means of chromosome counts; later flow-cytometric analyses were applied to determine the ploidy levels of the plants studied. The former method required quite a lot of time, so only a limited number of crossings could be evaluated in this way. Breeding behaviour was traditionally estimated by comparison of the chromosome numbers of parental plants and their progeny. A new method based on flow cytometric analysis of seeds, flow cytometric seed screen (FCSS, MATZK et al.

2000), allows to infer breeding behaviour characterized by the ratio of embryo : endosperm ploidy levels.

The aim of this work is to clarify the particular pathways mediating the gene flow. The analysis of the progeny of different crossings in *Taraxacum* sect. *Ruderalia*, allows us (1) to estimate the role of tetraploids in gene flow between particular ploidy levels; (2) to make an insight into the situation in nature; (3) to search for facultative apomixis, a possible mediator for gene flow from triploid to diploid levels.

MATERIAL AND METHODS

Plants

Mature plants from *Taraxacum* sect. *Ruderalia* were collected in the city of Košice, Slovakia and its vicinity (Table 1) and were transferred to cultivation. Several plants were collected in early March in the Botanical Garden of P.J. Šafárik University in Košice and used in preliminary hybridizations in the spring 2001. The main part of the plant material was collected in April and May 2001, always in mixed diploid-triploid populations. Much disturbed habitats were prefered (because of higher proportion of polyploids), however, others were also visited. The plants were selected on the basis of pollen morphology characteristics (TCHERMAK-WOESS 1949). The search was aimed at two types of plants: (1) Plants with almost regular pollen. In these plants the variation in polen size is only a little higher than that in regular pollen of diploid sexuals (the search for facultative apomixis). (2) Plants with large, sligthly irregular pollen (the search for tetraploids). Other plants were taken in reasonable proportion, too, because of the need for diploids (regular pollen) and apomictic triploids (irregular pollen) for hybridization experiments. In total, 120 plants survived the transfer to the culture. Mature seeds of widely growing diploids (entire anthodia) were taken especially from the localities where an increased proportion of polyploids was found by means of pollen screening (the habitats with disturbed plant cover). A part of each plant was used for the preparation of herbarium specimens that are deposited in the herbarium KO. No attempt was made to identify all plant material down to the microspecies level. The taxonomy of *Taraxacum* sect. *Ruderalia* is as yet unsettled and only a small portion of the material has been identified with the help of specialists. After all, no significant differences in genome size were observed within *T.* sect. *Ruderalia* (ZÁVESKÝ et al. 2005).

Karyology

Chromosome numbers of all 120 plants were determined (Table 1). To determine chromosome numbers, root tip meristems of potted plants were used. They were pre-treated in 0.1 or 0.05% aquaeous solution of colchicine for about 90 minutes. The roots were fixed in acetic acid : ethanol mixture (ratio 1 : 3) for 2–24 h, washed in distilled water, macerated in 1N HCl at the temperature 60 °C for 5 minutes and washed in distilled water. The squashes were made using cellophane squares (MURÍN 1960), stained in the 10% solution of Giemsa stain stock solution in Sörensen phosphate buffer, washed, dried and observed in the drop of immersion oil.

Table 1. The list of localities of the collections of plant material (*Taraxacum* sect. *Ruderalia*) used in crosses and chromosome numbers of particular plants. All of the localities are situated in Slovakia.

	diploids 2n=16	triploids 2n=24	tetraploids2n=32
Košice, Botanical Garden of P.J.Šafárik University, III.2001	13, 42, 50, 52, 57, 64, 68, 76, 86, 94,107, 127,154,	69, 181, 230, 257	
Košice, Botanical Garden of P.J. Šafárik University, 11.IV.2001	t1	t3	
Košice, area near East Slovakian Printing House, grass plot, 15.IV.2001	t9		
Košice, Botanical Garden of P.J. Šafárik University, 18.IV.2001	t12	t11	
Košice, crossroads of Moldavská and Trieda SNP streets, lawn, 19.IV.2001		t16	
Košice, Trieda SNP streeet, near Ferrocentrum, lawn, 19.IV.2001		t17	
Jasov, railway station, grass plot, 19.IV.2001	t20		
Jasov, area opposite Monaster, grass plot, 19.IV.2001		t23	
Košice, Watsnova streeet, lawn, 20.IV.2001	t26	t24	t25
Košice, Hlinkova street, lawn, 23.IV.2001		t32	
Kavečany, bob-sleigh course, meadow, 23.IV.2001		t40	
Košice, railway station Košice-Suburb, lawn, 25.IV.2001		t50	t52
Košice, area near former East-Slovakian Dairy, unused field, 26.IV.2001		t65	t60
Košice, Botanical Garden of P.J. Šafárik University, lawn, 26.IV.2001		t67, t69	
Košice, Helsinská street, construction site, 27.IV.2001		t77	
Košice, Hlinkova street, lawn, 27.IV.2001		t78, t79, t80	
Obišovce, garden near sawmill, grass plot, 28.IV.2001			t81
Košice, Watsnova street, lawn, 29.IV.2001	t82		
Košice, Area of Technical University, lawn, 30.IV.2001		t87	t85, t91, t92
Košice, Dolný Bankov, forest clearing, 1.V.2001		t97	
Košice, Botanical Garden of P.J. Šafárik University, 2.V.2001		t101	t100, t102
Košice, Michalovská street, opposite garages, road margin, 3.V.2001		t104	
Košice, Railway station Košice-Suburb, lawn, 3.V.2001	t106	t108	
Košice, Helsinská street, construction site, 7.V.2001	t113		
Kavečany, area near the church, grass plot, 9.V.2001	t118 (2n=17)		
Košice, area near brook, Park Anička, grass plot, 9.V.2001	t122, t123	t119	
Košice, Botanical Garden of P.J. Šafárik University, 10.V.2001	t125, t131, t132		
Košice, area between gardens and former East-Slovakian Dairy, unused fields, 10.V.2001	t134, t136	t139	

Hybridizations

Preliminary hybridizations between diploids and triploids were carried out in 2001. In 2002, the plants collected in 2001 in natural localities were grouped according to chromosome numbers. Gradually the flower buds were isolated by bags made of dense tissue impenetrable for pollen grains (Fig. 1a,b). The cross-pollination was carried by rubbing two anthodia (of approximately the same stage of flowering) together usually in three subsequent days, until one of them became overblown. The following types of crossings were carried out: diploid × triploid with irregular pollen, diploid × triploid with almost regular pollen, diploid × tetraploid, triploid × triploid, triploid × tetraploid, diploid pollinated with the mixture of the pollen of diploid and triploid. As a control, also diploid × diploid crosses were made. On almost each plant, some anthodia were left isolated and unpollinated to verify the breeding system, some anthodia were left open-pollinated.

Mature seeds were collected, the seed set was evaluated in each anthodium. Seeds were classified as developed or undeveloped according to TAS & VAN DIJK (1999). The seed set was classified as regular when more than 100 developed seeds were present in the anthodium.

Flow cytometric seed screen

For flow cytometric analyses 30 seeds or less per sample (if there was lower seed yield from the respective crossing, Table 2) from one anthodium were used. The method of flow cytometric seed screen (FCSS) follows MATZK et al. (2000). Combined extraction and staining was used (buffer: 0.107 g $MgCl_2 \times 6H_2O$, 0.5 g NaCl, 1.211 g Tris, 0.1 ml Triton × 100 in 200 ml aquaeous solution, pH 7.0, DAPI staining). The analyses were made on Partec Ploidy Analyzer PA-II (Partec GmbH, Münster, Germany) in the Botanical Institute of the Academy of Sciences of the Czech Republic in Průhonice. Altogether analyses of 211 anthodia were carried out in the two subsequent years. To estimate the numbers of nuclei of particular ploidy levels, the software FloMax (Partec GmbH, Münster, Germany) was employed.

The FCSS of fresh seed samples (analyzed in June, about one month after harvest) showed large embryo peak and small endosperm peak. After four months of seeds storage (in October) in dry conditions at room temperature no endosperm peak was detectable (56 analyses, including repeated analyses of the same seed samples from June). The only sample with detectable endosperm was the one collected about two weeks before analysis (from autumn flowering). In the following year fresh seed samples were analyzed in spring and the remaining seed samples were kept in freezer and analyzed in autumn. In the both groups the endosperm peak was detectable.

Based on FCSS of fresh seeds of the open-pollinated plants, the proportion between the number of embryonal and endospermal nuclei was estimated. The endospermal nuclei represented about 7 to 12% of the embryonal nuclei number (this was a little higher in diploids, 8–12%, lower in apomicts, 7–8%) the average in diploids was about 10% (that can be also upper limit for apomicts). After the subtraction of the number of endospermal nuclei from the total number of the nuclei of particular ploidy level the final number of diploid, triploid and tetraploid embryonal nuclei was estimated and their percentage calculated. However, this estimation is very rough, since endosperm content varies slightly not only

Fig. 1. Crossing experiments in *Taraxacum* sect. *Ruderalia*. a – isolated anthodium; b – view at a part of the culture. Photo: P. Mártonfi.

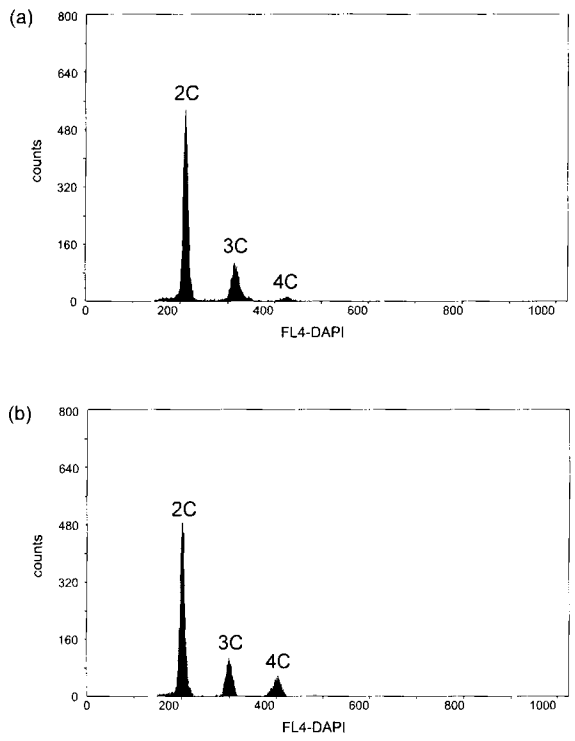

Fig. 2. Flow cytometric analysis of seed sample of diploid plant pollinated by triploid (C-value represents amount of nuclear DNA in unreplicated haploid nucleus). (a) – progeny consisting of diploids and triploids (2C embryo DNA peak, 3C combined embryo and endosperm DNA peak, small 4C endosperm DNA peak); sample size 30 seeds, coefficient of variation for the peaks: 2.52, 2.84, 2.71; (b) – progeny consisting of diploids, triploids, tetraploids (rather high 4C DNA peak represents combined tetraploid embryo DNA and endosperm DNA of triploid); sample size 14 seeds, coefficient of variation for the peaks: 2.66, 2.37, 2.70.

between sexuals and apomicts, but also within one reproduction mode as a result of natural and random variations.

The breeding behaviour of particular maternal plants was evaluated according to embryo : endosperm ploidy ratio (MATZK et al. 2000). In *Taraxacum*, this ratio is 2 : 3 in sexuals (1C ovule + 1C sperm cell for embryo, and 1C + 1C polar bodies + 1C sperm cell for endosperm) and 3 : 6 in apomicts (3C unreduced ovule and 3C + 3C polar bodies in autonomous endosperm). In facultative apomicts the mixture of peaks for both reproduction modes is expected (some of them may be superimposed). Since in *Taraxacum* the number of endosperm cells is much lower than that of embryo, the peaks for embryo and endosperm can be clearly distinguished.

RESULTS

Complete results of particular crossings and FCSS are summarized in the Table 3. Diploid maternal plants produced progeny always in sexual way. In the experimental crosses with triploid pollen donors (as triploid pollen donors, plants with different pollen size variation were employed – from subregular pollen, resembling the pollen of diploids to the plants with high pollen size variation) the progeny consisted of diploids, triploids and tetraploids (Figs. 2a, 2b, Table 4). By means of FCSS, no aneuploids were recorded, however, in some cases the embryo with closely similar DNA content to that of euploid ones may have remained undiscovered by this method. Insect pollinated diploids (3 cultivated and 32 growing plants were examined) produced no polyploid progeny. In most cases (31) diploid embryo and triploid endosperm were detected (Fig. 3a), and in four cases, besides two peaks in (near-)diploid region an asymetric peak for endosperm was recorded (Fig. 3b). The simulation of insect-pollination by pollinating diploid maternal plants with a mixture of triploid and

Table 2. Survey of crossing experiments with diploids as maternal plants. Seed yield is expressed as number of developed seeds. (sl – plant with slightly irregular pollen, regular seed set – more than 100 seeds developed). Seeds from the crosses marked with * were analyzed by FCSS.

diploid × triploid	seed set in diploid	diploid × triploid	seed set in diploid	diploid × tetraploid	seed set in diploid	diploid × mixture of diploid-triploid pollen	seed set in the first diploid
127 × t108*	12 (7%)	t122 × t32	4 (3%)	127 × t91*	regular	127 × t118 × 181 sl*	regular
127 × t20	12 (9%)	t122 × t40	20 (10%)	134 × t52	regular	13 × 94 × T6*	regular
127 × t31	22 (14%)	t122 × t65*	15 (16%)	t1 × t102	regular	154 × 52 × T8*	regular
13 × 69 sl	7 (5%)	t122 × t69 sl	1 (1%)	t1 × t85*	5, rest insect-damaged	52 × 154 × T9*	regular
154 × t77	2 (1%)	t123 × 181 sl	12 (8%)	t106 × t52	30 (19%)	57 × 64 × T7*	regular
50 × 230	27 (18%)	t123 × t17 sl*	3 (2%)	t106 × t60	regular	64 × 57 × T2*	regular
64 × 257	0	t123 × t20	11 (9%)	t106 × t81	0	94 × 13 × T3*	regular
76 × 181 sl	36 (24%)	t123 × t23 sl	5 (3%)	t122 × t91	regular	t1 × t9 × t108*	regular
86 × 69 sl	32 (21%)	t123 × t32*	14 (10%)	t122 × t92*	36 (24)	t106 × t122 × t104	regular
86 × t23 sl	23 (15%)	t123 × t40*	33 (21%)	t123 × t91*	regular	t118 × 127 × 181 sl*	insect damaged
t1 × t119	41 (24%)	t123 × t50*	5 (3%)	t123 × t92	13 (9%)	t118 × t132 × t16*	regular
t1 × t65*	4 (3%)	t131 × t113*	regular	t123 × t92	45 (26%)	t118 × t136 × t31*	56 (37%)
t1 × t80*	3 (2%)	t131 × t97*	61 (41%)	t125 × t92*	21 (15%)	t118 × t87 × t16*	12 (8%)
t106 × 181 sl	41 (27%)	t132 × t11*	64 (38%)	t131 × t91*	regular	t122 × t106 × t104	regular
t106 × t108	0	t132 × t113	18 (18%)	t131 × t92*	96 (64%)	t122 × t123 × t78*	regular
t12 × t50	5 (3%)	t132 × t24 sl*	11 (6%)	t132 × t25	8 (5%)	t122 × t125 × t23 sl	regular
t122 × t11	29 (22%)	t136 × t101	10 (7%)	t132 × t85*	regular	t123 × t122 × t78*	regular
t122 × t24 sl	0	t26 × 181 sl*	46 (23%)	t132 × t85	regular	t125 × t122 × t23 sl*	regular
t122 × t3*	8 (6%)	t26 × t101*	regular	t136 × t85*	4 (3%)	t125 × t122 × t23 sl*	regular
		t26 × t17 sl	47 (30%)	t136 × t85	regular	t131 × t9 × t113*	regular
		t82 × 181 sl*	9, rest insect damaged	t26 × t52	regular	t131 × t9 × t50*	regular
		t82 × 69 sl	19 (12%)	t82 × t81	regular	t132 × t118 × t16	11 (7%)
		t82 × t67*	36 (24%)	t82 × t91	regular	t132 × t67 × t31	73 (49%)
		t82 × t79	28 (15%)	t9 × t100	1 (1%)	t136 × t118 × t31*	regular
		t9 × 181 sl	0	t9 × t81	regular	t26 × t134 × t65	regular
		t9 × 69 sl	19 (13%)	t9 × t91*	regular	t26 × t9 × t101	regular
		t9 × t101	54 (27%)	t9 × t92*	regular	t9 × t1 × t108*	regular
		t9 × t139	0			t9 × t106 × t108*	49 (33%)
		t9 × t20	6 (4%)			t9 × t131 × t113*	regular
						107 × 57 × 42 × T5*	66 (44%)
						107 × 64 × 68 × T4*	regular
						57 × 107 × 42 × T10*	44 (29%)
						64 × 107 × 68 × T1	2 (1%)

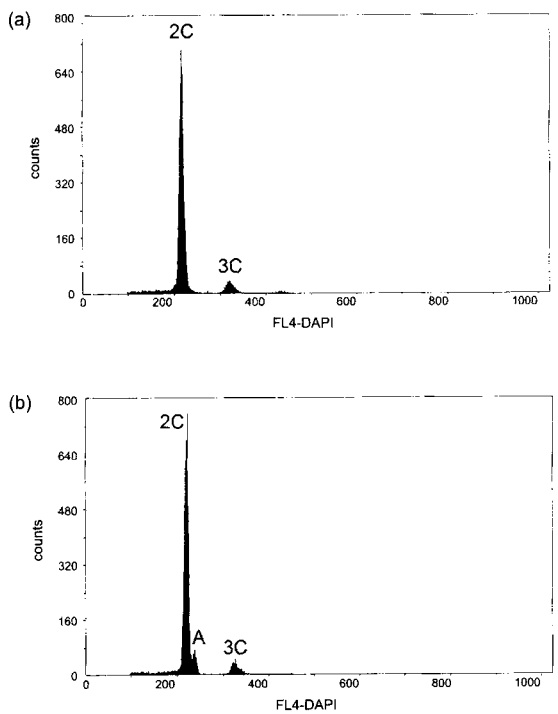

Fig. 3. Flow cytometric analysis of seed sample of open pollinated diploid (C-value represents amount of nuclear DNA in unreplicated haploid nucleus); (a) – progeny consisting of diploids only (2C embryo and 3C endoperm DNA peak); sample size 30 seeds, coefficient of variation for the peaks: 2.10, 2.77; (b) – progeny consisting of diploids (2C) and near-diploids (A); sample size 30 seeds.

diploid pollen gave results strongly resembling insect-pollination (only diploid progeny or, in the case of aneuploid diploid parent near diploid progeny, was recorded). Diploid maternal plants when crossed with tetraploid pollen donors produced seeds much more easily (of 28 crosses 16 yielded more than 100 seeds per anthodium) than in the crosses with triploids. Besides the diploids, triploids and tetraploids were found in the progeny (Fig. 4, Table 5). Anthodia of diploid plants that were left isolated and unpollinated did not develop any seeds, except for one plant. One cultivated diploid plant repeatedly produced viable seeds in sexual way (7 seeds in one anthodium and 33 in the other) in the absence of cross pollination.

Triploid maternal plants developed two types of progeny. The majority of the progeny were triploids developed by apomixis (Fig. 5a). In some cases (12.5% of anthodia pollinated by diploid pollen donor, 16% of anthodia pollinated by tetraploid one, 7% of open pollinated anthodia analyzed) tetraploid B_{III} hybrids were present (about 1–3 per 30 seeds analyzed), see Fig. 5b.

The situation was similar in tetraploid mothers – they produced both progeny formed by apomixis (tetraploids) and B_{III} hybrids (pentaploids, and in the case of triploid pollen donors also hexaploids), Fig 6a, b. However, in the case of cross-pollination with diploid pollen donors they produced B_{III} hybrids with much higher frequency (in 40% of crosses and they represented approximately 6–50% of progeny).

DISCUSSION

Seed set in crossing experiments

In literature, crosses between two diploids are usually reported to result in regular seed set (RICHARDS 1970a; JENNISKENS et al. 1985) and so it was in the present experiment (Table 2). Seed set from the crossings between diploid maternal plants and triploid pollen donors is

Table 3. The results of particular crossing experiments. For each type of crossing experiment the data are given as follows: 1st line – total number of crossings/number of successful crossings (i.e., at least one developed seed obtained)/number of anthodia analyzed by FCSS; next lines – ploidy level of embrya in the resulting seeds, number of anthodia where they were found, estimation of their percentage in these anthodia; S – stands for sexually arisen progeny, A – for progeny arisen by apomixis, B_{III} – for B_{III} hybrids, last line – references to tables and figures. [b] – 25 of them with more than 100 well-developed seeds per anthodium; [c] – 16 of them with more than 100 well-developed seeds per anthodium; [d] – 16 wild + 22 cultivated plants; [e] – participation of aneuploid plant expected; [f] – hyperdiploid 2n=17 used as a pollen parent.

	Mother		
	Diploid (2n=16)	Triploid (2n=24)	Tetraploid (2n=32)
Diploid pollen donor	5/5/5 (5) 2n: 100% S	58/58/32 3n: 32 (93–100%) A 4n: 4 (4–7%) B_{III} Fig 5a,b	28/28/20 4n: 20 (50–100%) A 5n: 8 (6–50%) B_{III} Fig. 6b
Triploid pollen donor	48/44/14 2n: 14 (33–100%) S 3n: 10 (9–67%) S 4n: 4 (3–20%) S Table 4; Fig. 2a,b	4/4/4 3n: 4 (100%) A	6/6/6 4n: 5 (100%) A 5n and 6n: 1 – B_{III} , together with 4n: A
Tetraploid pollen donor	27/26[c]/11 2n: 6 (6.7–80%) S 3n: 11 (20–100%) S 4n: 4 (3.3%) S Tables 2, 5; Fig.4	12/12/6 3n: 6 (97–100%) A 4n: 1 (3%)	1/1/1 4n: 1 (100%) A
Insect pollinated	50/50/35 2n: 31 S near 2n: 4 S[e] Fig. 3a,b	81/81/38[d] 3n: 38 (92–100%) A 4n: 3 (3–8%) B_{III}	7/7/7 4n: 7 (96–100%) A 5n: 1 (4%) B_{III}
Mixture diploid+triploid	33/33/18 2n: 13 (100%) S near 2n: 5 (100%) S[f]	4/4/4 3n: 4 (100%) A	1/1/1 4n: 1 (100%) A
Isolated	20/2/2 2n: 2 (100%) S	50/50/5 3n: 5 (100%) A	10/10/2 4n: 2 (100%)

variable. In my crossings, 94.4% of diploid plants (91.4% of anthodia used) produced at least some developed seeds (Table 2). JENNISKENS et al. (1985) reported that about 69% of anthodia involved produced some seeds and only 31.5% (in my experiments 86.2%) yielded more than 2% of seeds. Poor seed set was also reported by STERK (1987): five of eight plants tested produced seeds, the seed set was from 0.3 to 11%. Good seed set, however, was reported by TAS & VAN DIJK (1999), where in all of the 11 crosses carried out the seed set was at least 2% of seeds in particular anthodia. The seed set similar to my results was reported by MORITA et al. (1990a) – 82.8% of anthodia employed in crosses. The variability in seed set is

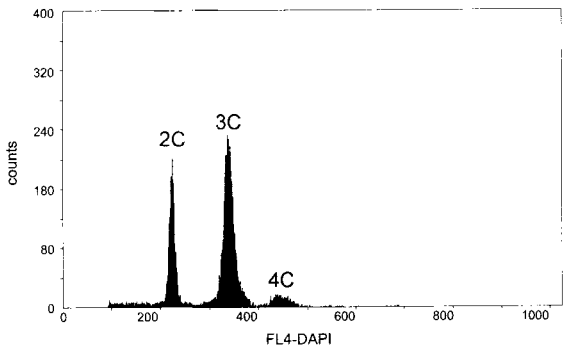

Fig. 4. Flow cytometric analysis of seed sample of diploid pollinated by the pollen of tetraploid: progeny consisting of mixture of diploids and triploids (C-value represents amount of nuclear DNA in unreplicated haploid nucleus); sample size 30 seeds, coefficient of variation for the peaks: 2.62, 3.06, 3.97.

certainly caused by the complex of factors concerning, above all, the material used as parent plants, methods of hybridization employed, but also weather conditions.

In the present series of crosses between diploids and tetraploids the seed set was much higher than that in the diploid-triploid crosses. Diploids crossed with tetraploids gave regular seed set (more than 100 developed seeds per anthodium) in about 60% of treatments and only one treatment gave no seeds.

In my experiments, in the absence of cross pollination, diploids usually produced no viable seeds (this corresponds well with the data reported in literature, e.g. RICHARDS 1970b). However, one plant repeatedly produced viable seeds. In this case, the self-incompatibility must have been broken (for unknown reasons, in the absence of incompatible pollen) since the selfing is the only explanation for this case. This diploid plant gave plenty of seeds also in the crosses with triploids (Table 2). The self-fertility of diploids was reported also by MORITA et al. (1990a). Seed set in polyploids was usually regular.

Pathway A – apomicts as pollen donors

The progeny of diploid maternal plants (2n=16) pollinated with the pollen of triploids or tetraploids seems to be euploid: diploid, triploid and/or tetraploid (Fig. 7). This fact confirms the results of other authors: JENNISKENS et al. (1985), HUGHES & RICHARDS (1988), TAS & VAN DIJK (1999). STERK (1987) reported euploid or near euploid progeny. Since especially the triploid pollen donors show much disturbed male meiosis and the pollen consists of the grains with different chromosome numbers (including the aneuploid ones), the fertilization capacity of aneuploid pollen grains is low, or the aneuploid zygotes die early (TAS & VAN DIJK 1999). This also can explain the low seed set in diploid-triploid crosses. The pollen of tetraploids is often subregular with larger grains, probably containing a higher amount of euploid pollen grains than that of triploids. The presence of a higher amount of viable pollen grains in tetraploids can explain that diploid-tetraploid crosses are much more successful than diploid-triploid ones. In the progeny of former crosses the triploids prevail, which is also in accordance with the expected prevalence of eudiploid pollen grains in the subregular pollen of tetraploids [MAŁECKA (1965) observed this phenomenon in the section *Palustria*]. Some *Taraxacum* researchers have, however, a different experience, e.g. RICHARDS (1970a,b), who reported aneuploids in diploid-triploid crosses, but outside the section *Ruderalia*.

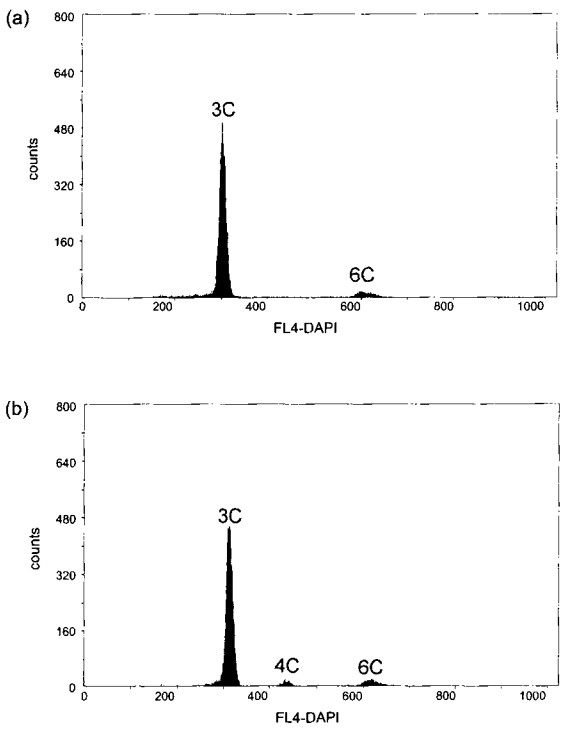

Fig. 5. Flow cytometric analysis of seed sample of triploid pollinated by the pollen of diploid (C-value represents amount of nuclear DNA in unreplicated haploid nucleus). (a) – pure triploid progeny formed by apomixis (3C embryo and 6C endosperm DNA peak); sample size 30 seeds, coefficient of variation for the peaks: 2.40, 2.80; (b) – tetraploid B_{III} hybrid (4C embryo DNA peak) among triploid apomictic progeny; sample size 30 seeds, coefficient of variation for the peaks: 2.29, 2.40, 2,83.

Triploid and tetraploid progeny of my crosses thus had to arise as a result of fertilization of haploid ovule of diploid maternal plants by diploid and triploid sperm cells, respectively. Thus this progeny is always of hybrid origin. The diploid progeny could be of two types: plants that have arisen as a result of self-pollination (after the break--down of self incompatibility by the mentor effect of incompatible pollen, MORITA et al. 1990a) or the hybrids resulting from pollination of haploid ovule by haploid sperm cell. However, it was reported (MORITA et al. 1990a, TAS & VAN DIJK 1999) that the majority of diploid progeny of diploid mother – triploid pollen donor crosses results from selfing. The estimation of percentage of diploid hybrids can be made e.g. according to the study of TAS & VAN DIJK (1999), who reported four hybrid diploid plants (i.e., about 1.8%) out of overall number 223 diploids in the progeny of several diploid-triploid crosses. MORITA et al. (1990a), however, recorded no true diploid hybrids, as all diploid progeny originated from selfing. The knowledge of diploid progeny of diploid mother-tetraploid pollen donor is very poor and requires further study. It can be supposed that most of them are the result of selfing, as well, since the pollen of tetraploids usually does not contain small haploid pollen grains.

Even though hybrids are quite easily produced in experimental crosses between sexual and apomictic individuals (i.e., diploid – polyploid), their occurrence in natural populations is reported very rarely (e.g. FÜRNKRANTZ 1961 reported intersectional hybrids). The identification of natural hybrids in the section *Ruderalia* is also a methodological problem. I pollinated diploid maternal plants with the mixture of pollen of triploid and diploid donors in order to simulate the situation in the wild. The possibility that the insect pollinated diploid mother receives completely triploid pollen at the locality is fairly low [in the majority of

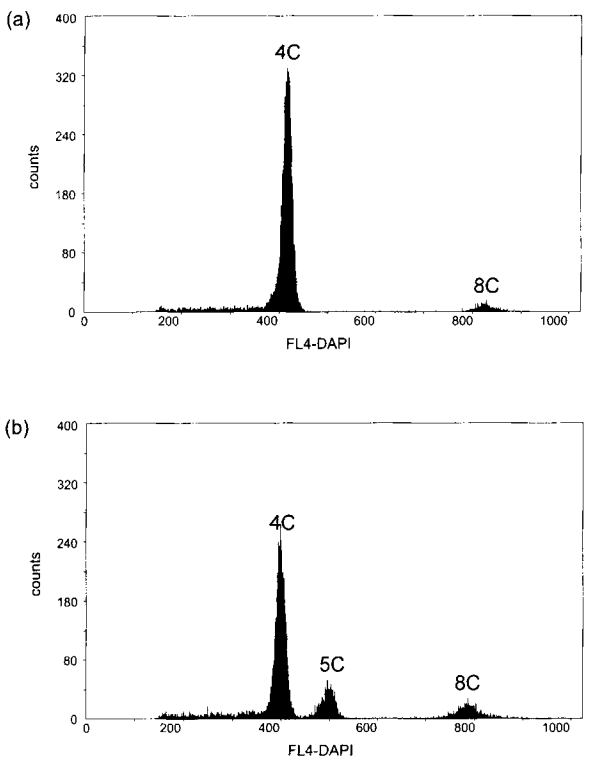

Fig. 6. Flow cytometric analysis of seed sample of tetraploid pollinated by the pollen of diploid (C-value represents amount of nuclear DNA in unreplicated haploid nucleus). (a) – pure tetraploid progeny formed by apomixis (4C embryo and 8C endosperm DNA peak); sample size 30 seeds, coefficient of variation for the peaks: 2.39, 2.70; (b) – pentaploid B_{III} hybrid (5C embryo DNA peak) among tetraploid apomictic progeny; sample size 30 seeds, coefficient of variation for the peaks: 2.53, 2.92, 2.88.

localities in Košice and its surroundings with the *Taraxacum* sect. *Ruderalia* diploids prevail (unpubl. data) and the insects visit particular anthodia many times, JENNISKENS et al. (1985)]. Seed set in these experiments corresponded approximately to the crosses between two diploids. It is interesting that FCSS did not reveal polyploid hybrids in the progeny of these crosses: the progeny was completely diploid or near-diploid (in the case where the aneuploid parent was used). The insect-pollinated plants gave the same results. The seeds of wild growing plants were mostly collected at the localities with a high proportion of polyploids, namely in disturbed habitats with low-density of plant cover (around new buildings, abandoned fields, etc.), so the diploids were supposed to receive a sufficient amount of the pollen of triploids from their proximity (together with the pollen of neibourghing diploids), however, no polyploid hybrids appeared in the progeny. In four of the FCSS of insect pollinated plants, a peak corresponding to embryos 2n=17 appeared besides that of 2n=16. This situation was similar to that of diploids pollinated with the mixture of the pollen of diploid and triploid, where one of the diploid parents was aneuploid (2n=17). On the basis of this analogy and of the present knowledge we can conclude that aneuploids in these progenies (in considerable amount here) were generated by aneuploid gametes of a diploid parent, not by the aneuploid (n=9) pollen of polyploid pollen parent.

It seems that there is some mechanism that prevents pollen of triploids from fertilizing ovules of diploids when there is pollen of another diploid sexual available. Thus the possibility of the rise of hybrids between diploid maternal plants and triploid pollen donors is much reduced in the wild.

Table 4. Results of crossing experiments with diploid maternal plants (in brackets) and triploid pollen donor. Estimation of particular ploidy levels in the progeny.

Analysis	Number of the seeds analyzed	diploids (%)	triploids (%)	tetraploids (%)
(t132) × t11	30	86.7	13.3	0
(t123) × t32	14	71.4	14.3	14.3
(127) × t31	22	81.8	9.1	9.1
(t1) × t80	3	100	0	0
(t1) × t65	4	100	0	0
(t131) × t97	30	80	20	0
(t131) × t113	30	83.3	16.7	0
(t82) × 181	9	33.3	66.7	0
(127) × t108	12	100	0	0
(t123) × t50	5	80	0	20
(t82) × t67	30	53.3	46.7	0
(t26) × t101	30	76.7	23.3	0
(t26) × 181	30	76.7	20	3.3
(t123) × t40	30	73.3	26.7	0

Table 5. Results of crossing experiments with diploid maternal plants (in brackets) and tetraploid pollen donor. Estimation of particular ploidy levels in the progeny.

Analysis	Number of the seeds analyzed	diploids (%)	triploids (%)	tetraploids (%)
(t122) × t92	30	10	86.7	3.3
(t125) × t92	21	9.5	90.5	0
(t1) × t85	5	80	20	0
(t131) × t92	30	53.3	43.4	3.3
(t123) × t91	30	6.7	93.3	0
(t131) × t91	30	33.3	63.4	3.3
(t136) × t85	4	0	100	0
(127) × t91	30	0	100	0
(t122) × t91	30	0	96.7	3.3
(t82) × t81	30	0	100	0
(t9) × t91	30	0	100	0

Diploid hybrids occur much less often than polyploid ones (MORITA et al. 1990a, TAS & VAN DIJK 1999), thus the diploid hybrid progeny is improbable, although not excluded.

There is one more question that remains open – what is the probability of hybrid formation in diploid mothers pollinated with a mixture of diploid and tetraploid pollen. I suppose that the same mechanism will function as for the mixtures of the pollen of diploids and triploids. However, this should be further studied.

Apomicts as pollen receivers. Pathway B – B_{III} hybrid formation

In most of the crossing experiments carried out by various *Taraxacum* researchers the triploids were studied only as pollen donors (e.g. RICHARDS 1970a, JENNISKENS et al. 1985, HUGHES & RICHARDS 1988, MORITA et al. 1990a,b). The role of polyploids as maternal plants and possible hybrid formation is often neglected. The apomicts were examined as

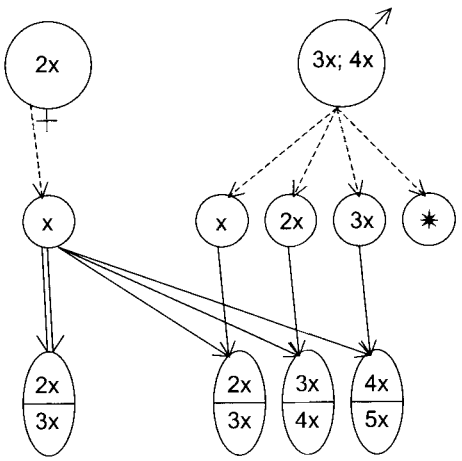

Fig. 7. Gene flow in *Taraxacum* sect. *Ruderalia*. Pathway A – apomicts as pollen donors. Big circle – ploidy of female or male parent; small circle – possible gametes (* – aneuploid gametes, that with high probability produce no vital progeny); oval – progeny, upper part stands for a ploidy level of embryo, lower one for ploidy level of endosperm. Dashed arrow – reduction; solid arrow – fertilization; double arrow – selfing.

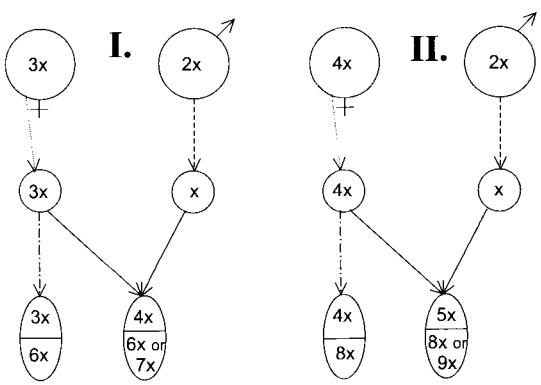

Fig. 8. Gene flow in *Taraxacum* sect. *Ruderalia*. Pathway B – B_{III} hybrid formation. I – Triploid maternal plant. II – Tetraploid maternal plant. Big circle – ploidy of female or male parent; small circle – possible gametes; oval – progeny, upper part stands for a ploidy level of embryo, lower one for ploidy level of endosperm. Dotted arrow – diplospory; dashed arrow – reduction; solid arrow – fertilization; dot-and-dashed arrow – parthenogenesis.

maternal plants only when facultative apomixis, i.e., the presence of reduced ovules in the anthodium and diploid B_{II} hybrid formation (fertilization of reduced egg cell, ASKER & JERLING 1992) was studied (e.g. RICHARDS 1970a,b, MAŁECKA 1973, JENNISKENS et al. 1985). B_{III} hybrids (i.e., those originating from fertilization of unreduced gametes), have been recorded rarely. MAŁECKA (1973) reported B_{III} hybrids in some species and the progeny of several crosses between various species within the section *Palustria*, and VAN DIJK et al. (1999) reported two experimental hybrid polyploids that exclusively produced the 2n+n progeny, i.e., the B_{III} hybrid type.

However, polyploid plants from natural localities are interesting from the point of view of B_{III} hybrid formation (Fig. 8). Present flow cytometric analyses showed B_{III} hybrids in the progeny of both triploid and tetraploid maternal plants. The B_{III} hybrids were formed more frequently in tetraploids – in 40% of the crosses the B_{III} hybrids were found in the progeny in quite high amounts (up to 50%). These B_{III} hybrids included mostly pentaploids, however, also hexaploid B_{III} hybrids were recorded (when the tetraploid was crossed with triploid). B_{III} hybrids were less frequent in triploids, however, not rare at all. The B_{III} hybrids coming from the progeny of triploid mothers were tetraploids. The formation of B_{III} hybrids gives an evidence for the lack of precocious embryony in at least some ovules. In the presence of cross-pollination they did not develop embryos partenogenetically, but sexually. The development of endosperm was autonomous. This fact corresponds well with the discovery of VAN BAARLEN

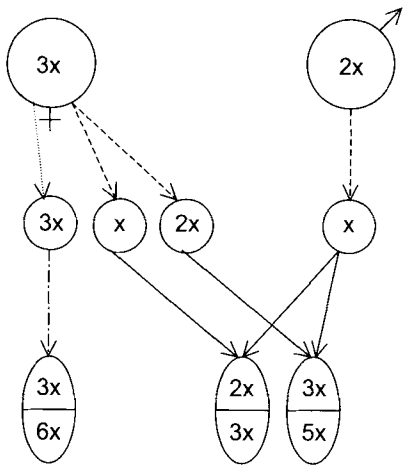

Fig. 9. Gene flow in *Taraxacum* sect. *Ruderalia*. Pathway C – facultative apomixis. Big circle – ploidy of female or male parent; small circle – possible gametes; oval – progeny, upper part stands for a ploidy level of embryo, lower one for ploidy level of endosperm. Dotted arrow – diplospory; dashed arrow – reduction; solid arrow – fertilization; dot-and-dashed arrow – parthenogenesis.

et al. (2002) who observed that megasporogenesis and gametogenesis proceeded asynchronously between florets within a single anthodium of natural apomicts. The endosperm developed autonomously, too.

In my studies, B_{III} hybrids are present both in the progeny of experimental crossings and insect pollinated apomicts in their natural localities, on the contrary to B_{II} hybrids between diploid maternal plants and apomictic pollen donors, which were never recorded in natural populations.

Apomicts as pollen receivers. Pathway C – facultative apomicts

Cytoembryological investigations in the genus *Taraxacum* proved that within one anthodium besides unreduced ovules some reduced ovules, which seem to behave sexually, can be formed (MAŁECKA 1973). RICHARDS (1970b) showed that triploid plants with almost regular pollen exhibited partial seed set in the absence of cross pollination. Moreover, they also gave some diploid offspring, being pollinated by a haploid pollen of diploid sexual. These triploid maternal plants were assumed facultatively apomictic, producing progeny in both sexual and apomictic way.

In *Taraxacum*, two groups of facultative apomicts can be distinguished, according to ploidy of progeny formed in crosses with a diploid sexual pollen donor (Fig. 9):

(i) The group in which the progeny produced in a sexual way is of a different ploidy level than that produced in an apomictic way. In this group, triploid maternal plants producing haploid ovules and tetraploid maternal plants producing haploid or diploid ovules, are included. The plants with subregular pollen, which are believed to be the most common facultative apomicts in *Taraxacum* (RICHARDS 1970b), are included in this group. These plants were, however, difficult to find. I found 11 triploids of this pollen type in the field, 8 of them survived the cultivation. 20 crosses of these plants with various diploids were carried out. The progeny consisted only of triploids in 19 of the 20 cases analyzed. In one case, flow-cytometric analysis showed triploid embryos and one little tetraploid peak. This peak represented embryo of B_{III} hybrid. Thus it can be concluded that flow-cytometric analyses revealed no hybrids originating from reduced egg cells of the mother plants with subregular pollen – none of these plants behaved as facultative apomict.

As far as tetraploids are concerned, ovule reduction to diploid level is expected (MAŁECKA, 1967, noted this in the sect. *Palustria*). However, in my collection of tetraploid mothers, no such behavior was found.

(ii) The group in which the progeny produced in a sexual way is of the same ploidy level as that produced in an apomictic way. Triploids producing diploid ovules and tetraploids producing triploid ovules are addressed here. Thus B_{II} hybrids are not distinguishable from the progeny formed by apomixis by chromosome counts. However, the results of FCSS would distinguish their endosperm: in the case of triploid hybrids their endosperm would be either tetraploid (if developed autonomously) or pentaploid (if arisen after fertilization), in the case of tetraploid hybrids the endosperm would be pentaploid (autonomous) or hexaploid (arisen after fertitlization). In my crossings of triploid mothers with diploid plants a tetraploid peak occurred in the FCSS in several cases and in the crosses of tetraploid mothers with diploids pentaploid peaks occurred even in 40% of the cases. However, these cannot be considered evidence of facultative apomixis. In about one half of these analyses tetraploid and pentaploid peaks were quite high (Fig. 6b) and thus clearly represented embryos of B_{III} hybrids (a similar situation for *Hypericum perforatum* was reported by MATZK 2001). In the rest of the cases, these peaks were smaller. Two of these analyses were carried at the time when only embryo peaks were detectable, thus the smaller peaks represent surely embryos of B_{III} hybrids. In the remaining cases, the situation is unequivocal. However, when the tetraploid or pentaploid peaks in the remaining analyses are considered to represent endosperm, there would be rather many endospermal nuclei (in ratio to embryo). Their size, however, suits one embryo per 30 plants analyzed. Thus the tetraploid and pentaploid peaks concerned seem to represent embryo of B_{III} hybrids, too.

Future prospects

The possibilities of gene flow between particular ploidy levels are quite clear from numerous experimental studies of several *Taraxacum* researchers. Based on these works, the main possibilities are seen in the formation of B_{II} hybrids between diploid sexuals and apomictic pollen donors and between facultative apomicts and diploids as pollen donors. B_{III} hybrids represent gene flow to higher ploidy levels and may represent intermediates to further polyploids. It is clear, that gene flow exists between particular ploidy levels in natural conditions, which was shown, e.g. by MENKEN et al. (1995) who revealed in their study that diploids and triploids share all major and most minor polymorphisms. MEIRMANS et al. (2003), however, studied the same populations as MENKEN et al. (1995) did and stated significant differences in allele frequences between certain subpopulations. They found congruence between spatial genetic patterns of diploids and triploids and ascribe this to gene flow between particular ploidy levels. The question what is gene flow like, what is its intensity in nature, remains open. Natural conditions differ substantially from the experimental ones – the plants receive a mixture of pollen of the plants growing around, i.e., mixture of diploid and triploid (or, as the case may be, tetraploid) pollen in mixed diploid-polyploid population. Even though I analyzed quite a good amount of samples from natural conditions and simulated natural situation I discovered no polyploid B_{II} hybrids. However, the study presented was aimed at the studies of possibilities of gene flow and the insight to its reality in natural conditions was considered preliminary. Thus, the situation in natural conditions requires further study. Especially the question of tetraploids as pollen donors in natural conditions has not been studied so far. Attention should also be paid to B_{II} hybrids from

diploid maternal plant – polyploid pollen donor crosses and B_{III} hybrids from polyploid maternal plant – diploid or polyploid pollen donor crosses. What is their vitality, fertility, and breeding behaviour? What are the potential diploid hybrids like, which have been distinguishable only by isosyme analysis in experimental condition so far, and what is their vitality and breeding behaviour? Special attention is to be paid to the progeny of diploid-tetraploid crosses. Another question to be answered in future is the question of facultative apomicts and their incidence not only among the plants with subregular pollen. The answer to these questions will bring more light to the gene flow in natural conditions.

Acknowledgement: I am much obliged to J. Suda (Department of Botany, Faculty of Science, Charles University in Prague, Czech Republic) for teaching me basics of flow-cytometric methods, J. Štěpánek (Department of Botany, Faculty of Science, Charles University in Prague, Czech Republic) for the determination of some plant material, J. Kirschner (Botanical Institute, Academy of Sciences of Czech Republic, Průhonice) and K. Marhold (Institute of Botany, Slovak Academy of Sciences, Bratislava) for language revision and two anonymous reviewers for their valuable comments. The research was supported by the Grant Agency for Science (VEGA), Bratislava, Slovakia (grant no. 1/9206/02).

REFERENCES

ASKER S.E. & JERLING L. (1992): *Apomixis in plants*. CRC Press, Boca Raton, Ann Arbor, London, Tokyo.

DOLL R. (1982): Grundriss der Evolution der Gattung *Taraxacum* ZINN. *Feddes Repert.* 93: 481–624.

FÜRNKRANTZ D. (1961): Cytogenetische Untersuchungen an *Taraxacum* im Raume von Wein. II. Hybriden zwichen *T. officinale* und *T. palustre*. *Oesterr. Bot. Z.* 108: 408–415.

HUGHES J. & RICHARDS A.J. (1988): The genetic structure of populations of sexual and asexual *Taraxacum* (dandelions). *Heredity* 60: 161–171.

JENNISKENS M.P.J., DEN NIJS J.C.M. & STERK A.A. (1985): Crossability and hybridization of taxa of *Taraxacum* section *Taraxacum* from central and western Europe. *Proc. Kon. Ned. Akad. Wetensch. C* 88: 297–338.

KIRSCHNER J. & ŠTĚPÁNEK J. (1996): Modes of speciation and evolution of the sections in *Taraxacum*. *Folia Geobot. Phytotax.* 31: 415–426.

KIRSCHNER J. & ŠTĚPÁNEK J. (1998): A monograph of *Taraxacum* sect. *Palustria*. Institute of Botany, Průhonice.

KIRSCHNER J. & ŠTĚPÁNEK J. (2004): New sections in *Taraxacum*. *Folia Geobot.* 39: 259–274.

MAŁECKA J. (1965): Embryological studies in *Taraxacum palustre*. *Acta Biol. Cracov., Ser. Bot.* 8: 223–235.

MAŁECKA J. (1967): Processes of intraspecific differentiation in the genus *Taraxacum*. *Genet. Polon.* 8: 185–188.

MAŁECKA J. (1973): Problems of the mode of reproduction in microspecies of *Taraxacum* section *Palustria* DAHLSTEDT. *Acta Biol. Cracov., Ser. Bot.* 16: 37–84.

MATZK F., MEISTER A. & SCHUBERT I. (2000): An efficient screen for reproductive pathways using mature seeds of monocots and dicots. *Pl. J.* 21: 97–108.

MATZK F., MEISTER A., BRUTOVSKÁ R. & SCHUBERT I. (2001): Reconstruction of reproductive diversity in *Hypericum perforatum* L. opens novel strategies to manage apomixis. *Pl. J.* 26: 275–282.

MENKEN S.B.J., SMIT E. & DEN NIJS J.C.M. (1995): Genetical population structure in plants: gene flow between diploid sexual and triploid asexual dandelions (*Taraxacum* section *Ruderalia*). *Evolution* 49: 1108–1118.

MEIRMANS P.G., VLOT E.C., DEN NIJS J.C.M. & MENKEN S.B.J. (2003): Spatial ecological and genetic structure of a mixed population of sexual diploid and apomicitic triploid dandelions. *J. Evol. Biol.* 16: 343–352.

MOGIE M. & FORD H. (1988): Sexual and asexual *Taraxacum* species. *Biol. J. Linn. Soc.* 35: 155–168.

MORITA T., MENKEN S.B.J. & STERK A.A. (1990a): Hybridization between European and Asian dandelions (*Taraxacum* section *Ruderalia* and section *Mongolica*). 1. Crossability and breakdown of self-incompatibility. *New Phytol.* 114: 519–529.

MORITA T., STERK A.A., DEN NIJS J.C.M. (1990b): The significance of agamospermous triploid pollen donors in the sexual relationship between diploids and triploids in *Taraxacum* (*Compositae*). *Pl. Spec. Biol.* 5: 167–176.

MURÍN A. (1960): Substitution of cellophane for glass covers to facilitate preparation of permanent squashes and smears. *Stain Technol.* 35: 351–353.

RICHARDS A.J. (1970a): Hybridization in *Taraxacum*. *New Phytol.* 69: 1103–1121.

RICHARDS A.J. (1970b): Eutriploid facultative agamospermy in *Taraxacum*. *New Phytol.* 69: 761–774.

RICHARDS A.J. (1973): The origin of *Taraxacum* agamospecies. *Bot. J. Linn. Soc.* 66: 189–211.

RICHARDS A.J. (1997): *Plant breeding systems*. Ed. 2. Chapman & Hall, London, etc..

SORENSEN T. (1958): Sexual chromosome aberants in triploid apomictic Taraxaca. *Bot. Tidsskr.* 54: 1–22.

STERK A.A. (1987): Aspects of the population biology of sexual dandelions in the Netherlands. In: HUISKES A.H.L., BLOOM C.W.P. M. & ROZEMA J. (eds.), *Vegetation between land and sea*, Junk Publishers, Dordrecht, pp. 284–291.

TAS I.C.Q. & VAN DIJK P.J. (1999): Crosses between sexual and apomictic dandelions (*Taraxacum*). I. The inheritance of apomixis. *Heredity* 83: 707–714.

TCHERMAK-WOESS E. (1949): Diploides *Taraxacum vulgare* in Wien und Niederösterrrieich. *Oesterr. Bot. Z.* 96: 56–63.

UHLEMANN I., KIRSCHNER J. & ŠTĚPÁNEK J. (2004): The genus *Taraxacum* (*Asteraceae*) in the southern Hemisphere. I. The section *Antarctica* HANDEL-MAZZETTI and notes on dandelions of Australasia. *Folia Geobot.* 39: 205–220.

VAN BAARLEN P., DE JONG J.H. & VAN DIJK P.J. (2002): Comparative cyto-embryological investigations of sexual and apomictic dandelions (*Taraxacum*) and their apomictic hybrids. *Sex. Pl. Reprod.* 15: 31–38.

VAN DIJK P.J., TAS I.C.Q., FALQUE M. & BAKX-SCHOTMAN T. (1999): Crosses between sexual and apomictic dandelions (*Taraxacum*). II. The breakdown of apomixis. *Heredity* 83: 715–721.

ZÁVESKÝ L., JAROLÍMOVÁ V. & ŠTĚPÁNEK J. (2005): Nuclear DNA content variation within the genus *Taraxacum* (*Asteraceae*). *Folia Geobot.* 40: 91–104.

Received 12 October 2004, first revision received 28 February 2005, second revision received and accepted 17 June 2005