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POLYPLOID PROGENY FROM CROSSES BETWEEN DIPLOID SEXUALS AND TETRAPLOID APOMICTIC POLLEN DONORS IN *TARAXACUM* SECT. *RUDERALIA*

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The paper reports a study of polyploid progeny of crosses between diploid sexual maternal plants and tetraploid pollen donor plants in the genus *Taraxacum* sect. *Ruderalia*. All polyploid progeny plants were triploids; no tetraploids were found. Two types of experiments were done with each plant: crossing of some capitula with diploid pollen donor, and isolation of other capitula. Flow cytometric seed screening, together with analysis of seed set, were used to determine the breeding system of particular hybrids. Of the 29 triploid hybrids studied, 7 plants were apomictic. Seventeen triploid hybrids produced progeny sexually, reduced ovules were fertilized, and seed set was low. Three plants produced (near)tetraploid progeny – B_{III} hybrids with autonomous endosperm. The remaining 2 triploid hybrids were nonapomicts, but their type could not be distinguished. Compared with the crosses with triploid pollen donors, the crosses of diploid with tetraploid pollen donors produced fewer apomictic progeny and more nonapomictic progeny with reduced, irregular chromosome numbers. However, the total number of developed seeds per capitulum was substantially higher in diploid × tetraploid crosses, and their impact in microevolutionary processes may be considerable. In both types of crosses, diplosporous plants lacking the capacity for parthenogenesis were produced, confirming the breakdown of apomixis into its elements.

Key words: *Taraxacum* sect. *Ruderalia*, Asteraceae, breeding system, flow cytometry, hybridization, apomixis, polyploid progeny.

INTRODUCTION

Taraxacum Wigg. is a widespread and common genus, found native to all continents except Antarctica (Richards, 1973). Taxonomically it is very complicated, with some 2000 microspecies described (Mogie and Ford, 1988), grouped to over 40 sections (Kirschner and Štěpánek, 1996), and new sections are still being described (Kirschner and Štěpánek, 2004; Uhlemann et al., 2004). This taxonomic complexity follows from the breeding system in the genus.

The section *Ruderalia* Kirschner, H. Øllg. et Štěpánek represents, like other *Taraxacum* sections, a polyploid series with the basic chromosome number x = 8. Diploids in the section *Ruderalia* are obligate sexuals, allogamous in natural conditions (Menken et al., 1995). Polyploids are apomicts. Three basic elements characterize apomictic megaspore and embryo development (van Dijk et al., 2003): avoidance of meiosis, parthenogenetic embryo development, and autonomous endosperm development. Male meiosis is disturbed, resulting in pollen grains of different sizes. Residual sexuality has been found in polyploid *Taraxacum*. Facultative agamospermy, that is, the presence of both reduced and unreduced ovules in one capitulum, was reported by Richards (1970b) and Małecka (1973). B_{III} hybrid formation (fertilization of an unreduced ovule) was found by Małecka (1973) and later by Mártonfiová (2006).

Intersectional crosses have been made in order to investigate the relationships between particular sections of *Taraxacum*, but often also to study breeding systems and crossability in this genus (e.g., Richards 1970a; Hughes and Richards 1988; Morita et al., 1990a). Intrasectional crosses usually have been made in order to investigate breeding systems, gene flow and microevolutionary processes in populations with co-occurrence of diploids and poly-

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ploids (Jenniskens et al., 1985; Sterk, 1987; Tas and van Dijk, 1999; van Dijk et al., 1999; de Kovel and de Jong, 2000; van Baarlen et al., 2002; Verduijn et al., 2004). Intrasectional crossing experiments have also been used to produce artificial populations for studying the genetics of breeding systems (Vijverberg et al., 2004).

The progeny of crosses between diploids and triploids in the section *Ruderalia* usually consist of diploids, triploids and tetraploids (Jenniskens et al., 1985; Sterk, 1987; Tas and van Dijk, 1999). These progeny were studied in detail in terms of breeding systems by van Dijk et al. (1999); in crosses of diploid sexuals with triploid pollen donors, they obtained 15 triploid hybrids and 7 tetraploid hybrids. All tetraploid progeny and five of the triploid hybrids were apomicts. The nonapomictic progeny were one of the following types (van Dijk et al., 1999, 2003):

- Type A hybrids were sexuals lacking all elements of apomixis.
- Type B hybrids were diplosporous plants with the capacity for parthenogenesis, lacking the capacity for autonomous endosperm formation; nuclear restitution was often incomplete.
- Type C hybrids were diplosporous plants lacking egg cell parthenogenesis, possessing autonomous endosperm formation.

Later, van Dijk and Bakx-Schotman (2004) discovered that diplospory is controlled by dominant allele D on a locus named DIP (diplosporous), and diplosporous plants have a simplex genotype, Ddd or Dddd.

Tetraploids seem very important from the point of view of gene flow and microevolutionary processes in the genus *Taraxacum* (Kirschner and Štěpánek, 1996; Verduijn et al., 2004; Mártonfiová, 2006). The latter studied gene flow between different ploidy levels and the breeding system in many crosses between diploids and tetraploids. The progeny of these crosses was used in the present study to address several questions: (i) What is their breeding system? (ii) Are there any important differences in progeny characteristics when compared with the progeny of the diploid × triploid crosses studied by van Dijk et al. (1999)? (iii) Does analysis of these progeny also confirm the breakdown of apomixis? (iv) What are the possibilities for this progeny to participate in microevolutionary processes – the formation of new triploid and tetraploid lineages?

MATERIALS AND METHODS

Numerous crosses between diploid sexual and polyploid (triploid, tetraploid) dandelions were made in order to study the breeding system and gene flow in *Taraxacum* sect. *Ruderalia* (Mártonfiová, 2006).

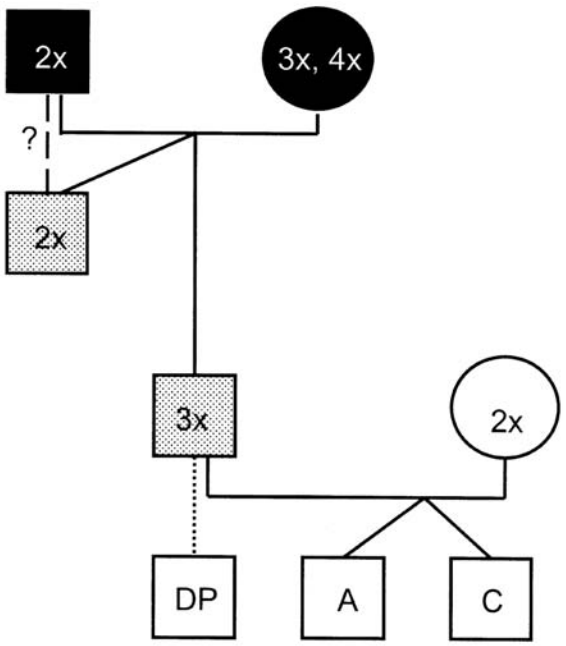

Fig. 1. Crossing scheme of the experiments leading to the progeny plants studied. Circle – pollen partner; dashed line – induced selfing; dotted line – apomixis; black – parental generation; dotted shading – first filial generation; DP – progeny of apomictic (diplosporous, parthenogenetic) hybrid; A – crossed progeny of type A plant (nondiplosporous, nonparthenogenetic hybrid), C – crossed progeny of type C plant (diplosporous, nonparthenogenetic hybrid).

Polyploid progeny (F_1, Fig. 1) from some of these crosses were used in the present study. The progeny plants studied were crossed with various diploid pollen donors (1–5 capitula) and isolated (1 or 2 capitula). The seeds obtained were studied by flow cytometric seed screening (FCSS, Matzk et al., 2000) technique in order to investigate their breeding system.

PLANT MATERIAL

The parent plants of the studied polyploid hybrids were collected in the city of Košice and vicinity. Detailed data on the material are given in Mártonfiová (2006).

The progeny (F_1) of the crosses between parent plants (P, Fig. 1) were analyzed by FCSS. On the basis of these results, several crosses were selected for further study of F_1 plants: the crosses that produced enough seeds (i.e., some seeds remained after FCSS) and showing some triploid or, as the case may be, tetraploid progeny; together, six crosses were selected. In one of the crosses the diploid mother was pollinated with a triploid pollen donor,

and the other crosses employed tetraploid pollen donors.

The seeds originating from the selected crosses were sown on wet filter paper in Petri dishes and grown until the first leaves appeared. Then the seedlings were transferred to small pots with soil and grown to maturity in an experimental garden. The chromosome numbers of the plants that survived in cultivation were counted and 29 polyploid hybrids were selected.

CROSSING EXPERIMENTS

The following spring, 1–5 capitula on each of these plants were pollinated with various diploid pollen donors and 1 or 2 capitula were isolated. Mature seeds were collected, the seeds were classified as developed or undeveloped according to Tas and van Dijk (1999), and seed set was determined.

FLOW CYTOMETRY

The developed seeds were analyzed by FCSS in order to reveal the reproduction mode of the maternal plants – the polyploid hybrids studied. According to the FCSS results the plants were classified in several types. A modification of van Dijk et al.'s (1999) classification was used. The following year, herbarium specimens of the plants studied were prepared and were deposited in KO.

KARYOLOGICAL TECHNIQUES

For chromosome number determination, root tip meristems of potted plants were used. They were pretreated in 0.1 or 0.05% aqueous solution of colchicine for ~90 min. The roots were fixed in acetic ethanol (ratio 1:3) for 2–24 h, washed in distilled water, macerated in 1N HCl at 60°C for 5 min and washed in distilled water. Then squashes were made using cellophane squares (Murín, 1960), stained in 10% solution of Giemsa stain stock solution in Sörensen phosphate buffer, washed, dried and observed in an oil drop.

CROSSING TECHNIQUES

The capitula were isolated in bud stage in small bags made of dense tissue impenetrable to pollen grains. Cross-pollination was carried by rubbing together two capitula at about the same stage of flowering, usually on three successive days, until one of them became swollen.

FCSS TECHNIQUES

For flow cytometry, 25 seeds per sample (or less if there was lower seed yield from the respective crossing) from one capitulum were used. The FCSS method followed Matzk et al. (2000). The analyses were made with a Becton Dickinson FACSCalibur flow cytometer with BD CellQuest Pro Software. Combined extraction and staining was used (buffer: 0.107 g $MgCl_2$ $6H_2O$, 0.5 g NaCl, 1.211 g Tris, 0.1 ml Triton × 100 in 200 ml aqueous solution, pH 7.0, supplemented with 50 μg propidium iodide and 50 μg RNAse). As the internal standard, older but viable seeds of a diploid for which no peak for endosperm was detectable were used (in older but still viable *Taraxacum* seeds, endosperm is not detectable by FCSS; Mártonfiová, 2006). In most cases the sample was analyzed both with and without the internal standard in order to check where the diploid peaks were superimposed. The breeding behavior of particular maternal plants was evaluated according to the embryo:endosperm ploidy ratio (Matzk et al., 2000). In *Taraxacum*, this ratio is 2:3 in sexually arisen progeny (1C ovule + 1C sperm cell for embryo and 1C + 1C polar bodies + 1C sperm cell for endosperm; C-value represents amount of nuclear DNA in unreplicated haploid nucleus) and 3:6 (3C unreduced ovule and 3C + 3C polar bodies in autonomous endosperm) for the triploid progeny arisen by apomixis; the ratio is similarly modified for other ploidy levels involved in sexual or apomictic reproduction. In facultative apomicts (both apomictic and sexual progeny from one capitulum), mixing of peaks for both reproduction modes is expected (some may be superimposed). Since in *Taraxacum* the number of endosperm cells is much lower than that of embryo cells, the peaks for embryo and endosperm can be clearly distinguished. The ploidy levels of the embryo and endosperm can also be estimated.

RESULTS

Karyological studies (Tab. 1) showed that the progeny from the selected six crosses (one crossing of diploid mother with triploid pollen donor and five crossings of diploid mother with tetraploid pollen donor) consisted only of diploids and triploids; no tetraploid progeny were found.

SEED DEVELOPMENT

Seed development was highly variable in the triploid hybrids studied (see Table 2). After isolation, some plants developed seeds (in 10 of the hybrids studied) and the others produced no or only undeveloped seeds. After cross-pollination with diploids all the triploid hybrid plants produced some developed seeds.

TABLE 1. Chromosome numbers of the progeny of selected crosses between diploid maternal plants and polyploid pollen donors

Crossing number	Pollen parent	Diploid progeny plant	Triploid progeny plant
33	triploid	33/1, 33/2, 33/4, 33/5, 33/6, 33/9, 33/10, 33/13	33/7, 33/14
34	tetraploid		34/6, 34/11, 34/12, 34/15, 34/16, 34/21, 34/23
48	tetraploid	48/7, 48/12,	48/8, 48/14, 48/15
53	tetraploid		53/1, 53/4, 53/5, 53/6, 53/20
59	tetraploid		59/1, 59/4, 59/6, 59/7, 59/13, 59/15, 59/17, 59/19
76	tetraploid		76/4, 76/5, 76/7, 76/8, 76/9, 76/10, 76/11, 76/15

CLASSIFICATION OF THE TRIPLOID HYBRIDS

From each capitulum that produced some seeds, a seed sample was taken and subjected to FCSS to obtain information on progeny type. On the basis of this information, together with the data on seed development, the following hybrid types were distinguished:

Apomictic hybrids (7 plants)

The progeny of this type were predominantly (near)triploid formed by apomixis [the endosperm was (near)hexaploid]; some (near)tetraploid progeny also formed (found in 2 plants, 3 capitula), with endosperm always (near)hexaploid (Fig. 2). Seed set in these plants was high (Tab. 2), isolated capitula produced seeds by apomixis in 5 plants, and in 2 plants they were damaged due to manipulation in the course of crossing experiments.

Nonapomictic hybrids

Type A (17 plants) – chromosome numbers in type A progeny are from the interval 2n = 16 to 2n = 31, many capitula produced progeny only between 2n = 16 and 2n = 24 (23 of 41 capitula studied); in the majority of cases, the progeny did not exceed hypertriploid chromosome number. Type A plants produced progeny sexually, reduced ovules were fertilized, FCSS detected embryos ranging between 2n = 2x and 2n = 4x (x = 8, basic chromosome number), and the endosperm corresponded to sexual development (Fig. 3). In two cases (progeny with higher than hypertriploid chromosome number) FCSS detected autonomous endosperm. Seed set in type A plants was low (Tab. 2), isolated capitula produced either no seed (in most cases) or poorly developed embryos, and no peaks for embryo or endosperm appeared in FCSS (3 cases).

Type C (3 plants) – the progeny of type C plants was (near)tetraploid, and endosperm was (near) hexaploid (Fig. 4). This indicates fertilization of unreduced ovules (BIII hybrid formation) and

TABLE 2. Seed set after cross-pollination with diploid and reproduction mode of polyploid hybrids coming from diploid × tetraploid crosses of *Taraxacum* sect. *Ruderalia*. (*undeveloped seeds could not be counted). For definition of the A and C types, see Introduction

Plant no.	No. of capitula studied	No. of developed seeds per capitulum	% of developed seeds
Apomicts			
33/7	5	143.2	93.5
48/8	2	118.5	78.5
53/1	2	110.5	81.4
53/4	5	176.2	85.8
76/7	3	166.0	91.9
76/9	3	151.0	84.7
59/17	2	63.0	*
Nonapomicts Type A			
34/3	4	33.8	23.0
34/6	2	97.0	75.5
34/11	2	85.5	62.6
34/12	4	55.3	47.7
34/16	3	22.0	22.6
48/14	1	43.0	40.9
48/15	2	40.0	26.9
53/6	3	62.3	36.0
59/1	3	34.7	25.8
59/4	2	3.0	*
59/6	4	42.5	29.2
59/7	5	9.4	7.0
59/15	3	22.7	17.6
76/4	2	32.5	25.8
76/5	5	18.2	15.0
76/8	2	29.5	23.1
76/11	1	12.0	11.3
Nonapomicts Type C			
33/14	2	45.0	43.0
53/5	4	79.0	48.8
59/19	2	136.5	71.8

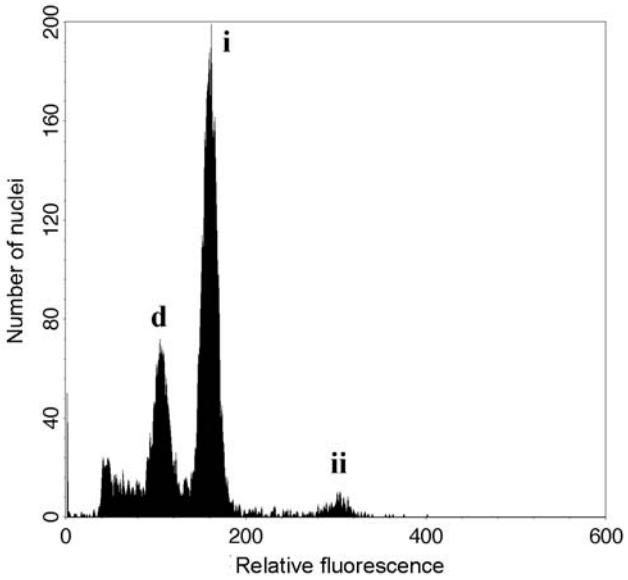

Fig. 2. Flow cytometric analysis of seed sample of apomictic triploid hybrid pollinated by diploid (C-value represents amount of nuclear DNA in unreplicated haploid nucleus); i – 3C embryo DNA peak; ii – 6C endosperm DNA peak; d – 2C embryo DNA peak of control plant.

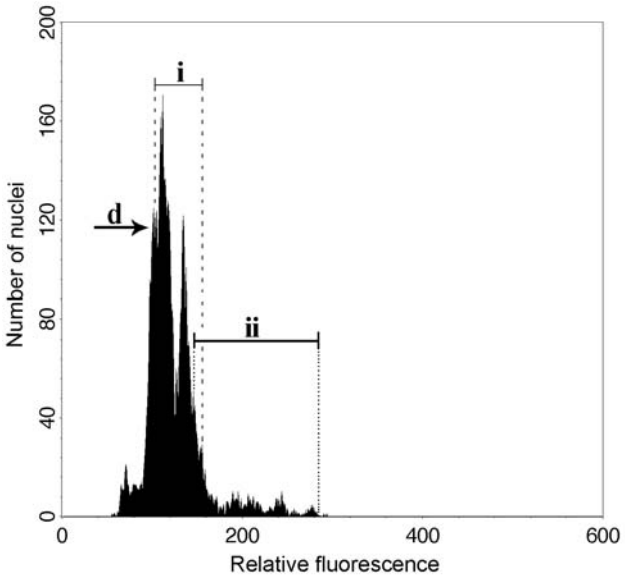

Fig. 3. Flow cytometric analysis of seed sample of non-apomictic triploid hybrid type A pollinated by diploid (C-value represents amount of nuclear DNA in unreplicated haploid nucleus); the peaks are the result of superimposing sexually developed embryo DNA peaks (i), corresponding endosperm DNA peaks (ii) and diploid control embryo peak (d).

autonomous endosperm development. Seed set in this plant type was intermediate between type A and apomicts (Tab. 2). After isolation, seed production was very low (maximum 2 developed seeds per capitulum), and in 2 cases seeds were formed by autogamy (one peak for aneuploid embryo and a peak for autonomous endosperm).

One of the plants studied (34/15) produced pentaploid embryos and octoploid endosperm. The remaining plant (34/12) was nonapomictic, but only an isolated capitulum developed (the crossed one was undeveloped, probably due to trauma caused by isolation and manipulation), and thus no conclusions on the hybrid type were drawn. The majority of plants in our study came from crosses of diploid sexuals with tetraploid pollen donors. Only two of the plants came from crossing between diploid sexual and triploid pollen donors. One of these two was apomictic, and the other was a type C plant.

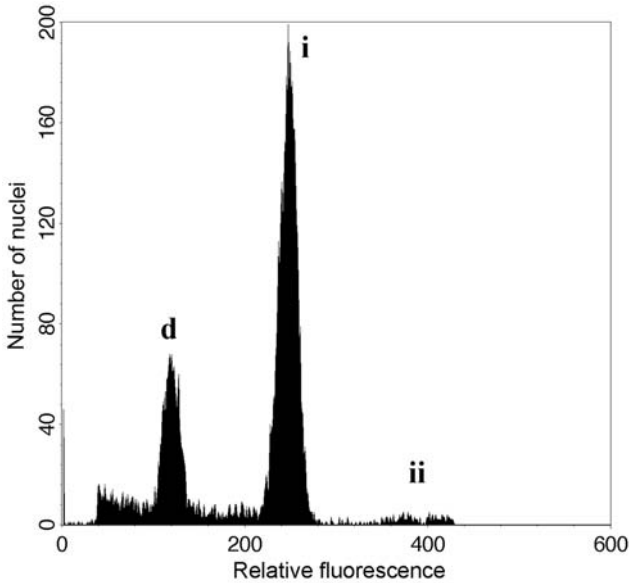

Fig. 4. Flow cytometric analysis of seed sample of non-apomictic triploid hybrid type C pollinated by diploid (C-value represents amount of nuclear DNA in unreplicated haploid nucleus); i – 4C B_{III} hybrid embryo DNA peak; ii – 6C autonomous endosperm DNA peak; d – 2C embryo DNA peak of control plant.

DISCUSSION

BREEDING SYSTEM OF POLYPLOID HYBRIDS

Apomictic hybrids

The majority of progeny of apomictic hybrids were (near)triploid, formed by apomixis. The tetraploid progeny of triploid apomictic hybrids studied in this paper are presumed to be B_{III} hybrids (Mártonfiová, 2006). In both cases, the endosperm was autonomous.

Nonapomictic hybrids

Type A plants (nondiplosporous, nonparthenogenetic hybrids). The type A crossed progeny ranged between 2n = 16 and 2n = 31, that is, the chromosome number of ovules (the result of distraction of 8 paternal chromosomes) ranged between n = 8 and n = 23. This means that female meiosis occurred; the type A plants lacked diplospory. These plants lacked the ability to develop parthenogenetically – the isolated capitula usually produced no progeny. In a few cases, poorly developed seeds were produced after isolation, and showed no peaks in flow cytometry.

Type B plants (diplosporous hybrids, facultatively parthenogenetic, without autonomous endosperm formation). Type B plants are nonapomictic hybrids – diplosporous plants with low penetrance of diplospory, producing, after cross-pollination, some mixture of (near)triploid and (near)tetraploid progeny (with a considerable share of aneuploid progeny), lacking the capacity for autonomous endosperm formation (van Dijk et al., 2003). Type B plants were not found in the progeny of diploid × tetraploid crosses.

Type C plants (diplosporous, nonparthenogenetic hybrids with autonomous endosperm formation). Type C plants produced B_{III} hybrids when cross-pollinated. The autogamous production of some seeds after isolation confirms the loss of parthenogenetic development capacity in this type of plant.

DIFFERENCES BETWEEN THE PROGENY OF TRIPLOID AND TETRAPLOID POLLEN DONORS

The progeny of crosses between diploid sexuals and triploid pollen donors was studied in detail by Tas and van Dijk (1999) and van Dijk et al. (1999). Our studies discovered similar types of hybrid polyploid plants coming from diploid × tetraploid crosses as given by van Dijk et al. (1999). This was expected. On the basis of previous work (Mártonfiová, 2006) we supposed that tetraploids in natural populations are usually B_{III} hybrids originating from an unreduced ovule of a triploid fertilized by haploid pollen of a diploid. Since such a tetraploid has three chromosome sets (complete genome) of a triploid, the same types of hybrid plants are expected among the progeny of a tetraploid pollen donor as in a triploid one. However, some differences are obvious.

Both types of crosses produced some apomictic and nonapomictic triploid progeny. In diploid triploid crosses the ratio was 1:2 (Tas and van Dijk, 1999); in our studies of diploid × tetraploid crosses this ratio was ~1:3. This result was expected theoretically. Pollen parents, diplosporous triploids and diplosporous tetraploids have Ddd and Dddd genotypes, respectively (D is the dominant allele for diplospory, van Dijk and Bakx-Schotman, 2004); the quotient of diploid gametes transferring diplospory and diploid gametes not transferring diplospory is 2:1 in the former and 1:1 in the latter. More diplosporous plants (apomicts + type C + type B nonapomicts) are thus expected theoretically in the progeny of diploid × triploid more than in diploid × tetraploid crosses.

In the progeny of diploid × tetraploid crosses studied by Mártonfiová (2006), tetraploids were quite rare, so it is not surprising that no tetraploid hybrids were found by chromosome counting in the present study. Tetraploids are more frequent in diploid × triploid crosses (Mártonfiová, 2006); this conclusion agrees with the finding that 7 tetraploid apomictic hybrids were present among 22 polyploid hybrids in a study of diploid × triploid crosses (Tas and van Dijk, 1999).

Nonapomictic triploids of type A coming from diploid × tetraploid crosses showed reduction in ovules ranging between n = 8 and n = 23, while in diploid × triploid crosses studied by van Dijk et al. (1999) the reduction was between n = 8 and n = 16; in that paper, however, the sample size was small, with only two type A hybrids. In our type A hybrids, many capitula produced progeny ranging between 2n = 16 and 2n = 24 only, and in the others (often coming from the same hybrid) the progeny ranged between 2n = 16 and 2n = 31.

There are evident differences in the frequency of type A plants. Among the progeny of diploid × tetraploid crosses, type A plants were the most frequent – 17 of the 27 plants studied. However, van Dijk et al. (1999) gives only 2 or 3 type A plants among 22 polyploid progeny of diploid × triploid crosses. Differences in the frequency of nondiplosporous hybrids (type A plants) in the triploid progeny of diploid-triploid crosses and diploid-tetraploid crosses were, as stated above, expected, but were not expected to be so striking.

In the study of van Dijk et al. (1999), type B was the most frequent one (4 of 10 plants) among the nonapomictic polyploid hybrids, but type B plants were totally absent from the progeny of diploid tetraploid crosses. All of the plants that produced (near)triploids or some mixture of (near)triploids and (near)tetraploids were apomicts. FCSS showed apomictic embryo development for triploid embryos, and the tetraploids were very probably B_{III} hybrids (i.e., hybrids originating from fertilization of unreduced egg cells; Asker and Jerling, 1992). No evidence of pseudogamy was found in the FCSS results.

The reason type B plants were not found among the progeny of diploid × tetraploid crosses remains unknown. As stated earlier here, the hybrid types found in the progeny of diploid × triploid crosses

are also expected in the progeny of diploid × tetraploid crosses. For some unknown (genetic?) reason, type B hybrids either are totally absent from the progeny of diploid × tetraploid crosses or else are quite rare and were not registered among the 27 progeny plants. This finding does not contradict multigenic control of apomixis; it only underlines that this phenomenon is a very complex one.

BREAKDOWN OF APOMIXIS

The presence of type C plants also among the progeny of diploid × tetraploid crosses confirms the breakdown of apomixis and supports the multigenic control of apomixis as described by van Dijk et al. (1999). Multigenic control of apomixis has recently been described in another diplosporous parthenogenetic genus – *Erigeron* (*Asteraceae*) – by Noyes (2005, 2006).

PARTICIPATION IN MICROEVOLUTIONARY PROCESSES

In relation to microevolutionary processes we can conclude the following: (1) Both triploid and tetraploid pollen donors have the capacity to participate in the formation of new triploid apomicts and, presumably, new triploid apomictic lineages. The diploid mothers produced seeds much more easily with tetraploid pollen donors than with triploid pollen donors (Mártonfiová, 2006), so the contribution of tetraploid pollen donors to the formation of new apomicts may be conspicuously higher even though the quotient of apomicts is higher in the progeny of diploid × triploid crosses. (2) Type C plants can be suggested to be mediators to new apomictic tetraploids. (3) Type A plants produce progeny which probably are of very low vitality due to incompleteness of chromosome sets, and their microevolutionary impact as maternal plants is expected to be low. This is in accordance with the situation in natural localities – aneuploids are quite rarely reported, and these reports refer only to plants lacking one or two chromosomes at maximum, or having one surplus chromosome (Sørensen, 1958; Mártonfiová, 2006). (4) The question of the microevolutionary importance of the hybrids as pollen donors, as well as that of diploid hybrids coming from crosses of diploids with tetraploid pollen donors, remains open and requires further study.

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REFERENCES

Asker SE, and Jerling L. 1992. *Apomixis in plants*. CRC Press, Boca Raton, Ann Arbor, London, Tokyo.

de Kovel CGF, and de Jong G. 2000. Selection on apomictic lineages of *Taraxacum* at establishment in a mixed sexual-apomictic population. *Journal of Evolutionary Biology* 13: 561–568.

Hughes J, and Richards AJ. 1988. The genetic structure of populations of sexual and asexual *Taraxacum* (dandelions). *Heredity* 60: 161–171.

Jenniskens MPJ, den Nijs JCM, and Sterk AA. 1985. Crossability and hybridization of taxa of *Taraxacum* section *Taraxacum* from central and western Europe. *Proceedings of the Koninklijke Nederlandse Akademie van Wetenschappen: Series C: Biological and Medical Sciences* 88: 297–338.

Kirschner J, and Štěpánek J. 1996. Modes of speciation and evolution of the sections in *Taraxacum*. *Folia Geobotanica et Phytotaxonomica* 31: 415–426.

Kirschner J, and Štěpánek J. 2004. New sections in *Taraxacum*. *Folia Geobotanica* 39: 259–274.

Małecka J. 1973. Problems of the mode of reproduction in microspecies of *Taraxacum* section Palustria Dahlstedt. *Acta Biologica Cracoviensia Series Botanica* 16: 37–84.

Mártonfiová L. 2006. Possible pathways of the gene flow in the genus *Taraxacum* section *Ruderalia*. *Folia Geobotanica* 41: 183–201.

Matzk F, Meister A, and Schubert I. 2000. An efficient screen for reproductive pathways using mature seeds of monocots and dicots. *Plant Journal* 21: 97–108.

Menken SBJ, Smit E, and den Nijs JCM. 1995. Genetical population structure in plants: gene flow between diploid sexual and triploid asexual dandelions (*Taraxacum* section *Ruderalia*). *Evolution* 49: 1108–1118.

Mogie M, and Ford H. 1988. Sexual and asexual *Taraxacum* species. *Biological Journal of Linnean Society* 35: 155–168.

Morita T, Menken SBJ, and Sterk A.A. 1990. Hybridization between European and Asian dandelions (*Taraxacum* section *Ruderalia* and section *Mongolica*). 1. Crossability and breakdown of self-incompatibility. *New Phytologist* 114: 519–529.

Murín A. 1960. Substitution of cellophane for glass covers to facilitate preparation of permanent squashes and smears. *Stain Technology* 35: 351–353.

Noyes RD. 2005. Inheritance of Apomeiosis (diplospory) in fleabanes (*Erigeron, Asteraceae*). *Heredity* 94: 193–198.

Noyes RD. 2006. Apomixis via recombination of genome regions for apomeiosis (diplospory) and parthenogenesis in *Erigeron* (daisy fleabane, *Asteraceae*). *Sexual Plant Reproduction* 19: 7–18.

Richards AJ. 1970a. Hybridization in *Taraxacum*. *New Phytologist* 69: 1103–1121.

Richards AJ. 1970b. Eutriploid facultative agamospermy in *Taraxacum*. *New Phytologist* 69: 761–774.

Richards AJ. 1973. The origin of *Taraxacum* agamospecies. *Botanical Journal of Linnean Society* 66: 189–211.

Sørensen T. 1958. Sexual chromosome aberrants in triploid apomictic Taraxaca. *Svensk Botanisk Tidskrift*

Utgifven af Svenska Botaniska Foreningen, Stockholm 54: 1–22.

STERK AA. 1987. Aspects of the population biology of sexual dandelions in the Netherlands. In: Huiskes AHL, Bloom CWPM, and Rozema J [eds.], *Vegetation between land and sea*, 284–291. Junk Publishers, Dordrecht.

TAS ICQ, and VAN DIJK PJ. 1999. Crosses between sexual and apomictic dandelions (*Taraxacum*). I. The inheritance of apomixis. *Heredity* 83: 707–714.

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

VAN BAARLEN P, DE JONG JH, and VAN DIJK PJ. 2002. Comparative cyto-embryological investigations of sexual and apomictic dandelions (*Taraxacum*) and their apomictic hybrids. *Sexual Plant Reproduction* 15: 31–38.

VAN DIJK PJ, and BAKX-SCHOTMAN JMT. 2004. Formation of unreduced megaspores (diplospory) in apomictic dandelions (*Taraxacum officinale* s.l.) is controlled by a sex-specific dominant locus. *Genetics* 166: 483–492.

VAN DIJK PJ, TAS ICQ, FALQUE M, and BAKX-SCHOTMAN JMT. 1999. Crosses between sexual and apomictic dandelions (*Taraxacum*). II. The breakdown of apomixis. *Heredity* 83: 715–721.

VAN DIJK PJ, VAN BAARLEN P, and DE JONG JH. 2003. The occurrence of phenotypically complementary apomixis-recombinants in crosses between sexual and apomictic dandelions (*Taraxacum* officinale). *Sexual Plant Reproduction* 16: 71–76.

VERDUIJN MH, VAN DIJK PJ, and VAN DAMME JMM. 2004. The role of tetraploids in the sexual – asexual cycle in dandelions (*Taraxacum*). *Heredity* 93: 390–398.

VIJVERBERG K, VAN DER HULST RGM, LINDHOUT P, and VAN DIJK P. 2004. A genetic linkage map of the diplosporous chromosomal region in *Taraxacum officinale* (common dandelion; *Asteraceae*). *Theoretical and Applied Genetics* 108: 725–732.