

Crosses between sexual and apomictic dandelions (*Taraxacum*). I. The inheritance of apomixis

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Some dandelions, *Taraxacum*, are diplosporous gametophytic apomicts. Crosses between closely related diploid sexuals and triploid apomicts were made to study the inheritance of apomixis. Seed-set was less than one-third of that in diploid \times diploid crosses, probably because of the inviability of aneuploid pollen or zygotes. Almost 90% of the viable offspring were diploid and the result of selfing, as was shown by a discriminating allozyme marker. Aneuploid outcross pollen had a mentor effect on self-pollen, causing a breakdown of the sporophytic self-incompatibility system. A similar phenomenon has been reported before in wide crosses. Of the 26 allozyme-confirmed hybrids, four were diploids, 15 were triploids and seven were tetraploids. Diploid hybrids were significantly less frequent than triploid hybrids, suggesting either low fitness of haploid pollen or more numerous formation of diploid pollen. Emasculation and bagging of flowers indicated apomictic seed-set in none of the diploid, in one-third of the triploid and in all of the tetraploid hybrids. All apomictic hybrids showed partial seed-set, but additional cross-pollination did not increase seed-set. Cytological analysis of the F_2 progeny confirmed that partial apomixis was caused by semisterility and not by residual sexuality (facultative apomixis). The difference in segregation for apomixis between triploid and tetraploid hybrids may be because the triploids originated from partially reduced diploid pollen grains, whereas the tetraploids originated from unreduced triploid pollen grains.

Keywords: $2n$ -gametes, apomixis, mentor effect, parthenogenesis, self-incompatibility, *Taraxacum*.

Introduction

Apomixis is asexual seed production in plants (Nogler, 1984; Asker & Jerling, 1992; Koltunow, 1993). Although apomixis is an uncommon breeding system in angiosperms, it is of considerable interest in the study of the evolution and maintenance of sexual reproduction (Bell, 1982; Stearns, 1987) and for its potential in crop improvement (Koltunow *et al.*, 1995; Vielle Calzada *et al.*, 1996; Van Baarlen *et al.*, 1999). Apomicts circumvent the sexual processes of meiotic reduction and gamete fusion. Instead, apomictic offspring are the result of parthenogenetic development of unreduced egg cells. In this paper we report on the inheritance of apomixis in experimental crosses between sexual and apomictic dandelions (*Taraxacum*).

Gametophytic apomicts form unreduced megagametophytes or embryo sacs to give rise to a partheno-

genetic embryo (Nogler, 1984). Nearly all gametophytic apomicts are polyploid (Asker & Jerling, 1992; Mogie, 1992). This is also the case in *Taraxacum*, where sexuals are diploids ($2n = 2x = 16$), and apomicts are commonly triploids ($2n = 3x = 24$) (Richards, 1973). In apomictic *Taraxacum*, unreduced embryo sacs are derived from unreduced megaspores, which are produced by a first meiotic division restitution (Juel, 1906; Gustafsson, 1946). After the second meiotic division two megaspores are formed (diplospory), instead of four as with normal meiosis. Diplospory is less common among apomictic angiosperms than apospory, the other main type of gametophytic apomixis (Nogler, 1984). In apospory an unreduced somatic nucellar cell replaces the meiotic megaspore and forms an unreduced embryo sac.

During the last two decades, a number of studies have dealt with the inheritance of aposporous apomixis (e.g. *Ranunculus auricomus*, Nogler, 1984; *Panicum maximum*, Savidan, 1980; *Hieracium pilosella*, Gadella, 1987; *Pennisetum squamulatum*, Ozias-Akins *et al.*,

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1998). In all these studies there is evidence for single-locus dominant control of aposporous apomixis. In contrast, much less is known about the genetic control of diplosporous apomixis (Nogler, 1984; Asker & Jerling, 1992). According to Nogler (1984), 'It is an open question whether the different forms of diplospory are, from a genetic point of view, fundamentally different from apospory or whether there is a common basis.'

Studies on crosses between diploid sexual and triploid apomicts in *Taraxacum* have been reviewed by Morita *et al.* (1990b). Typically the majority of the offspring from such crosses was (near-) diploid, whereas low frequencies of polyploid offspring were obtained. However, the hybrid origin of diploid offspring in these crosses has been questioned by Morita *et al.* (1990b) because Morita *et al.* (1990a) showed, by using diagnostic allozyme markers, that all diploids in crosses between diploid sexuals and triploid apomicts had arisen by selfing. As the diploid mother plants were self-incompatible, Morita *et al.* (1990a) suggested that nonfunctional aneuploid pollen from the triploid pollen donors caused a weakening of the self-incompatibility system. Such 'mentor effects' are well known from heterospecific or dead pollen (De Nettancourt, 1977; Richards, 1986). The mentor effect in *Taraxacum* was, however, observed in wide crosses (between Japanese plants from the section *Mongolica* and European plants from the section *Ruderalia*) and it was unclear whether the same phenomenon also occurs in narrow crosses.

Apomictic *Taraxacum* are nearly always obligate apomicts, although facultative apomicts, partially reproducing sexually, have also been reported (Malecka, 1967; Richards, 1970a). Apomixis in *Taraxacum* is autonomous, implying that no pollen is necessary for the development of endosperm as in pseudogamous apomicts. However, in cyto-embryological studies in the section *Palustris*, Malecka (1973) observed cases of parthenogenetic embryo development with sexual endosperm (pseudogamy). Little is known about the occurrence of facultative apomixis in crosses between sexuals and apomicts.

In order to study the inheritance of apomixis in *Taraxacum* in narrow crosses, diploid sexuals and triploid apomicts from the section *Ruderalia* in the Netherlands were crossed and the reproductive mode of the offspring was investigated. We were specifically interested in the following questions. Does breakdown of self-incompatibility (SI) by mentor pollen occur in narrow crosses? What are the ploidy levels of the hybrids? And what is the relationship between reproductive mode and ploidy level?

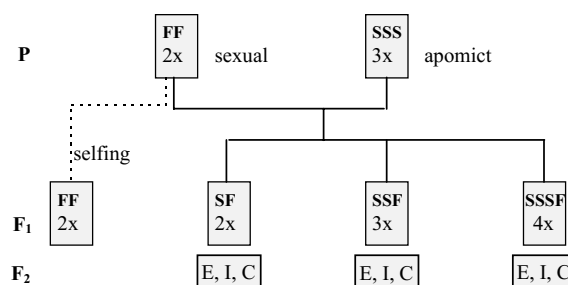


Fig. 1 Scheme of the diploid sexual \times triploid apomict crosses in *Taraxacum* and the breeding system tests of the F₁ hybrids. The ploidy levels and the genotypes for *6-pgd-2* (Slow, Fast) are indicated. The dashed line indicates the selfed offspring of the diploid seed parent. The flowers of F₁ plants were emasculated (E), isolated (I) and cross-pollinated with diploid pollen donors (C).

Materials and methods

Crosses

Crosses were made using sexual diploids as seed parents and apomictic triploids as pollen parents. The crossing scheme is shown in Fig. 1. All the plants belonged to the section *Ruderalia* and originated from populations in the centre of the Netherlands: sexuals from Wageningen (L 2x plants), apomicts from nearby populations (Slijk-Ewijk, SE 3x; Renkum, TE 3x; and Heteren, CT 3x).

None of the eight diploid sexual mother plants set seed spontaneously. However, to be able to discriminate between selfed and hybrid diploid offspring in crosses, plants were crossed that were homozygous for a different allele of the 6-phosphogluconate dehydrogenase-2 (*6-pgd-2*) locus. Mendelian codominant inheritance for this allozyme locus has been demonstrated in *Taraxacum* (Menken *et al.*, 1989). Sample preparation and vertical polyacrylamide electrophoresis of 6-PGD followed the protocols of Menken *et al.* (1989). Eleven $FF \times SSS$ crosses could be made (Table 1). Offspring only carrying *F* allozymes would result from selfing, whereas offspring carrying both the *S* and *F* alleles would result from real crossing. Based on the relative intensities of the dimeric *ss*, *ff* and *sf* 6-PGD-2-bands on the zymograms, the parental gene dosages might be estimated and the ploidy levels of the uniting parental gametes could be inferred. Assuming no gene dosage compensation, the relative intensities for the *ss*, *sf* and *ff* bands are 1:2:1 for *SF*, 4:4:1 for *SSF* and 9:6:1 for *SSSF*.

Crosses were made in the greenhouse in April 1995 by thoroughly rubbing the flowers together on two consecutive days. Seeds were collected two weeks later

Table 1 Crosses between diploid sexual and triploid apomictic *Taraxacum* that were homozygous for different alleles of the *6-pgd-2* locus. The diploid seed parents were all *FF*, the triploid pollen parents were all *SSS*. The mean seed-set of all crosses is indicated with its standard error

Cross	2x seed parent	3x pollen parent	Seed-set	Germination rate	No. seeds tested
1	L 2x-8-6	CT 3x-1	0.31	0.93	15
2	L 2x-17-6	SE 3x-6	0.29	0.71	45
3	L 2x-6-1	SE 3x-6	0.23	0.93	94
4	L 2x-17-18	SE 3x-4	0.15	0.67	15
5	L 2x-17-17	CT 3x-1	0.05	0.50	12
6	L 2x-17-18	SE 3x-6	0.09	0.67	55
7	L 2x-7-0	SE 3x-3	0.02	0.33	3
8	L 2x-8-6	TE 3x-2	0.32	0.83	60
9	L 2x-17-24	SE 3x-3	0.28	0.53	15
10	L 2x-17-5	TE 3x-2	0.24	0.73	15
11	L 2x-6-1	SE 3x-4	0.22	0.93	15
Mean \pm SE			0.22 \pm 0.03	0.74 \pm 0.05	Σ = 344

(in fact, in botanical terms the seed of *Taraxacum* is an achene: a single-seeded fruit). Seed-set was determined as the percentage of developed seeds among all seeds within a seed head (capitulum). Seeds were classified as developed or undeveloped, according to the descriptions given by Richards (1970a,b). Developed seeds are plump and well-pigmented brown or reddish, whereas undeveloped seeds are narrow, papery and unpigmented. In developed seeds the rostrum or beak (the part between the seed proper and the pappus) is elongated, causing the seed head to expand during ripening. Seed heads without developing seeds remain contracted.

A total of 344 F_1 seeds were put to germinate in Petri dishes on wet filter paper (21°C and 16 h light:15°C and 8 h dark). After germination and potting, 245 offspring plants could be analysed cytologically and electrophoretically.

Breeding system of the F_1 progenies

Hybrids were stored for vernalization for 2.5 months in a cold room at 4°C. The plants were then repotted and transferred to an insect-free, warm greenhouse (21°C and 16 h day:18°C and 8 h night).

To test whether hybrids were able to produce seed-set apomictically, two flowers per plant were emasculated (Raunkiaer, 1903; Richards, 1986). When still closed, the upper part of the bud was cut off with a sharp razor blade, removing the anthers and stigmas. This makes fertilization and sexual seed development impossible. Because endosperm development in apomictic *Taraxacum* is autonomous, apomicts are able to produce seeds, although seed-set may be reduced by damaging

effects. To quantify apomictic seed-set more precisely, seed-set was determined on flowers on which cross-pollination was prevented by pollen-proof paper bags (isolation treatment). To investigate whether cross-pollination could increase seed-set in apomictic hybrids, plants were cross-pollinated with unrelated diploid plants originating from the Wageningen population (pollination treatment).

Germination capacity of seeds produced by the F_1 hybrids after isolation and pollination treatments was tested by germinating 50 seeds (or all the seeds if fewer were available) per treatment. Viable seed-set was calculated as the product of seed-set and germination proportion. Seeds that did not germinate after 2.5 weeks were dissected and inspected under a dissecting microscope to see whether embryos were present. Ploidy levels of F_2 offspring produced after both treatments were determined by flow cytometry.

Cytological analyses

Chromosome numbers were counted in mitotic root-tip cells that were pretreated with 2 mM 8-hydroxyquinoline for 5 h at 5°C, squashed and stained with lacto-aceto-orcein. The ploidy levels of the offspring of hybrids were determined by measuring the nuclear DNA amount with a UV flow cytometer (PARTEC Ploidy Analyser, PARTEC GmbH, Münster, Germany), using a modified protocol of Ulrich & Ulrich (1991). Fresh leaf (1 cm²) was chopped with a sharp razor blade in 1 mL nuclei extraction buffer (0.1 M citric acid containing 0.5% Tween 20). After filtration through a 50 μ m filter (CellTrics, PARTEC), 1 mL of DAPI fluorescence solution (0.4 M sodium hydrogen phosphate, 0.2 M NaCl

and 5 mg L⁻¹ DAPI (4',6-diamidino-2-phenylindole)) was added and samples were measured directly.

Mixtures of leaf extracts of plants in which 23 and 24 chromosomes had been counted produced two well-separated fluorescence peaks with this protocol. To determine the chromosome number of an unknown plant, a known diploid (or triploid) plant was used as an internal reference and the relative peak positions were calculated. For triploids and tetraploids this ratio to the known diploid is 1.50 and 2.00, respectively. A step of 0.0625 was interpreted as a change of one in the chromosome number, compared to the internal standard. A test-series including diploids, hypotriploids, triploids, hypotetraploids and tetraploids gave a highly significant correlation between counted chromosome number and flow cytometric inferred chromosome number ($r=0.985$; $P < 0.001$; $N=24$) (P. van Dijk & H. de Jong, unpubl. data).

Results

The progeny types and their origin

The proportion of developed seeds in the diploid \times triploid crosses was low. Mean seed-set per cross ranged from 0.02 to 0.32 with an average of 0.22 (Table 1). The mean germination rate of the seeds in the crosses was 0.74.

The results of the electrophoretic analysis of the 11 $FF \times SSS$ crosses are given in Table 2. Figure 2 shows a typical zymogram. The large majority (89%) of the 245 offspring was homozygous for the maternal F allele. Cytological analysis of a subsample of the FF homozygotes revealed only diploids. Therefore these diploid offspring plants must be from selfing in the

Table 2 *6-pgd-2* progeny analysis of the 11 diploid $FF \times$ triploid SSS crosses in *Taraxacum*. The selfing rate is the proportion of FF homozygotes. The mean and standard error of the selfing rate is given for all crosses, except cross 7

Cross	No. seedlings analysed	FF	SF	SSF	$SSSF$	Selfing rate
1	12	9	—	—	3	0.75
2	22	17	1	3	1	0.78
3	84	75	2	7	—	0.89
4	7	6	1	—	—	0.86
5	6	4	—	—	2	0.67
6	36	31	—	5	—	0.86
7	1	0	—	—	1	0.00
8	48	48	—	—	—	1.00
9	7	7	—	—	—	1.00
10	9	9	—	—	—	1.00
11	13	13	—	—	—	1.00
Total	245	219	4	15	7	Mean: 0.88 \pm 0.03

crosses. High selfing rates occurred in all crosses, except in cross 7, in which seed-set was very low. In the other 10 crosses, there were no significant correlations between seed-set, germination rate and selfing rate.

Twenty-six (11%) of the F_1 plants carried maternal F and paternal S alleles and therefore were true F_1 hybrids. Chromosome counts revealed that four plants were diploid ($2n=2x=16$), 15 were triploid ($2n=3x=24$) and seven were tetraploid ($2n=4x=32$). Aneuploid hybrids were absent.

In the diploid hybrids the S and F alleles were equally strongly expressed, with the exception of H3-3. In this plant the paternal S allele was more weakly expressed than the maternal F allele. In the triploid hybrids the

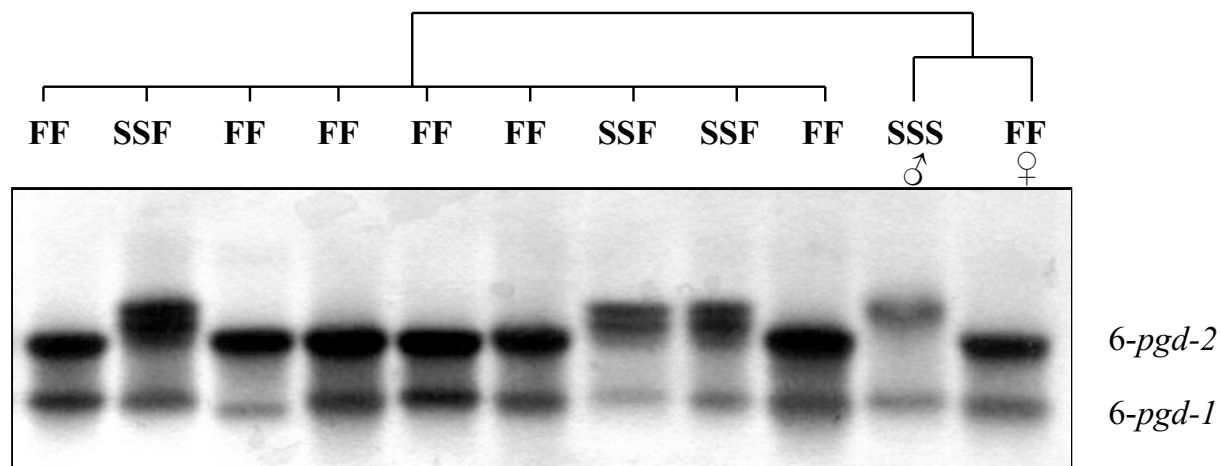


Fig. 2 An example of *6-pgd-2* progeny analysis in *Taraxacum*. The triploid SSS father and the diploid FF mother are in lanes 10 and 11, respectively. Lanes 2, 7 and 8 contain true hybrids ($3x$, SSF), the other lanes contain self offspring ($2x$, FF).

Table 3 Seed production of the *Taraxacum* hybrids that were apomictic as indicated by seed-set after emasculation. Two treatments were used: isolation and cross-pollination. Viable seed-set is the product of seed-set and germination rate

Hybrid	Ploidy	Isolation			Pollination		
		Seed-set	Germination rate	Viable seed-set	Seed-set	Germination rate	Viable seed-set
2-5	3x	0.85	0.68	0.58	0.90	0.60	0.54
3-2	3x	0.89	0.80	0.71	0.92	0.76	0.70
6-1	3x	0.69	0.84	0.58	0.72	0.72	0.52
6-2	3x	0.80	0.88	0.70	0.85	0.84	0.71
6-5	3x	0.89	0.76	0.68	0.96	0.68	0.65
1-1	4x	0.94	0.72	0.68	0.83	0.82	0.68
1-2	4x	0.35	0.60	0.21	0.35	0.72	0.25
1-3	4x	0.91	0.78	0.71	0.84	0.78	0.66
2-2	4x	0.12	0.72	0.09	0.19	0.68	0.13
5-1	4x	0.72	0.90	0.65	—	—	—
5-2	4x	0.80	0.86	0.69	0.85	0.78	0.66
7-1	4x	0.74	0.82	0.61	0.75	0.78	0.59

relative intensities of the *ss*, *sf* and *ff* bands were in close agreement with the expected 4:4:1 ratio for the *SSF* genotype. In the tetraploid hybrids the relative intensities of the *ss*, *sf* and *ff* bands were in agreement with the expected 9:6:1 ratio for the *SSSF* genotype.

The genotypic constitution of the hybrids implies that all egg cells from the diploid mother were reduced, haploid, as would be expected in a sexual diploid. The triploid fathers, however, produced reduced haploid ($n = x$) and partially reduced diploid pollen grains ($n = 2x$), as well as unreduced triploid pollen grains ($2n = 3x$). There were clear differences between the triploid pollen donors in hybrid production (Table 2). No hybrids were detected in crosses 8 and 10 where TE 3x-2 was used as pollen donor. In contrast, fathers SE 3x-6 and CT 3x-1 frequently produced hybrids, the former mainly triploids (crosses 2, 3 and 6), the latter only tetraploids (crosses 1 and 5). SE 3x-6 produced significantly fewer diploid hybrids than triploid hybrids (three and 15, respectively, $\chi^2_1 = 8.00$, $P < 0.01$).

As a consequence of the low number of hybrids obtained per cross, it was not possible to analyse the segregation for apomixis in individual crosses. Therefore in the following sections on the breeding systems of the hybrids, all crosses are taken together and attention is focused on the relationship between ploidy levels and mode of reproduction.

The reproductive mode of the hybrids

All 26 hybrids flowered after vernalization. After emasculation, all seven tetraploids and five of the 15 triploids (H2-5, H3-2, H6-1, H6-2 and H6-5) spontaneously set

seeds. These 12 plants were thus capable of apomictic reproduction, because sexual seed-set is impossible after style removal. In the diploids and the other 10 triploids seed heads did not expand after emasculation and no seeds developed. Hence these hybrids were apparently not capable of apomictic reproduction.

In agreement with the results from the emasculation treatment, there was no spontaneous seed-set in the diploid hybrids on bagged flowers. Spontaneous seed-set on bagged flowers was highly variable in the triploid hybrids. In four of the 15 triploids no seed-set was observed. Six triploid hybrids, which did not produce seeds after emasculation, produced some seeds on bagged flowers. However, these seeds did not germinate and turned out to be completely empty.

Viable seeds on bagged flowers were only produced in the triploid and tetraploid hybrids that set seeds after emasculation. Seed-set and germination results are given in Table 3. Viable seed-set in triploids ranged from 0.58 to 0.71 and in tetraploids from 0.09 to 0.71. In most cases, seeds that did not germinate contained small embryos, but occasionally such seeds were empty. There were no significant differences between triploids and tetraploids in apomictic seed-set, germination rate or viable seed-set (*t*-test on arcsin-transformed data).

Flow cytometry indicated that the spontaneously produced offspring by the five triploid hybrids were (near-) triploid. Two of the 54 F₂ plants analysed were hypotriploid ($2n = 23$) (3.7%), the rest eutriploid ($2n = 24$). Absence of genome segregation strongly supports the apomictic origin of these F₂ plants.

Additional pollination with diploid pollen donors did not increase seed-set in the apomictic triploids (paired *t*-test, $t_4 = -2.17$, $P = 0.10$), nor in the apomictic tetraploids ($t_5 = -0.49$, $P = 0.65$) (Table 3). The progeny of the five apomictic triploids after cross-pollination were almost entirely triploid (64 out of 69). Four F_2 plants (5.9%) were hypotriploid ($2n = 23$). One F_2 plant in the cross progeny was tetraploid (1.4%), the only product of fertilization that was found in the sample.

Discussion

Seed-set in the diploid \times triploid crosses was low, on average 22%. This can be partly explained by the fact that not all the florets are receptive at the moment of crossing (Richards, 1970b). The crossing technique used here generally yields 50–75% seed-set when two diploid sexuals are crossed. Thus seed-set in the diploid \times triploid crosses is about one-third of that of crosses at the diploid level. Percentage germination of the seeds produced in the diploid \times triploid crosses was reduced. The mean germination percentage was 74%, whereas the germination percentage of normal diploid sexual and triploid apomictic seeds is always higher than 90%. This may be caused by inbreeding depression in selfed offspring (see below).

Pollen from triploid pollen donors showed a large variation in size, reflecting a high level of aneuploidy caused by a disturbed and irregular microsporogenesis (Gustafsson, 1946; Battaglia, 1948). Low seed-set in diploid \times triploid crosses is probably caused by low fertilization capacity of aneuploid pollen, or early abortion of aneuploid zygotes.

The breakdown of self-incompatibility

Allozyme analysis showed that 89% of the viable progeny were from selfing. The diploid mother plants used in the crosses did not produce seeds when cross-pollination was prevented. *Taraxacum* species are generally self-incompatible (SI; Richards, 1973), caused by a sporophytic SI system (Okabe, 1956). Breakdown of SI in interploidy crosses in *Taraxacum* has been reported before by Morita *et al.* (1990a). These authors found 87% selfing in wide crosses between sexual diploids from the section *Mongolica* from Japan and apomictic triploids from the section *Ruderalia* from France.

The induction of self-compatibility in SI pollen in mixtures with foreign pollen has been described as 'mentor effects' (De Nettancourt, 1977; Richards, 1986). Heterospecific pollen may cause such mentor effects elsewhere in the Asteraceae (e.g. *Helianthus* species; Desrochers & Rieseberg, 1998). Morita *et al.* (1990a)

assumed that the mentor effects in *Taraxacum* were caused by high loads of (semi-) sterile aneuploid pollen produced by the triploid pollen donors. However, because heterospecific pollen may also cause mentor effects, their results could also have resulted from the wide crosses made. Our study shows that the same phenomenon also occurs in narrow crosses.

Morita *et al.* (1990b) argued that allozyme segregation data from Hughes & Richards (1988) concerning crosses between sexual diploids and apomictic triploids originating from France, could be better explained by at least partial selfing. In the absence of informative markers, diploid progenies of diploid \times triploid crosses should be interpreted with care. Müller (1972) reported 96.7% nonapomictic diploid offspring in crosses between sexual diploids and apomictic triploids in the section *Ruderalia* (formerly *Vulgaria*). If diploids in reality arose through selfing, absence of apomixis in these plants is meaningless for the study of the inheritance of apomixis.

It is noteworthy that although most of the viable seeds resulted from mentor-induced selfing, seed-sets were nevertheless poor. There may be several explanations for this. It may be that most self-pollen is outcompeted by cross-pollen, but that aneuploid embryos abort. It may also be that the SI system is still working quite strongly and that only few self-grains escape the SI system, possibly only on the stigmas with very high mentor-pollen loads. Alternatively, low seed-set may be caused by early abortion of selfed seeds as a consequence of inbreeding depression in this normally out-crossed species.

The hybrids and their reproductive mode

The apomictic pollen donors showed clear differences in hybrid production, both in numbers produced and their ploidy levels. These differences probably reflect variations in the course of pollen meiosis of these triploid plants.

The primary hybrids obtained in this study were all euploid, suggesting either inviability of aneuploid pollen and zygotes, or strong selection against these types. Aneuploids were also absent in diploid \times triploid crosses made by Müller (1972). In contrast, Richards (1970a,b) found hyperdiploids ($2n = 17, 18$ and 19) to be common and hypotriploids ($2n = 20, 21, 22$ and 23) to occur in such crosses. When aneuploids were obtained in *Taraxacum*, they were weak and had a low survival (Sørensen, 1958; Richards, 1970a,b).

Only four diploid hybrids were obtained in the present study, compared to 15 triploid hybrids. Morita *et al.* (1990a) reported 13 triploid allozyme hybrids and no diploid hybrids at all. Both studies suggest that

haploid pollen grains yield fewer offspring than diploid pollen grains. The lack of fertilization success could be caused by a lower fitness of haploid pollen. Deleterious mutations can be sheltered in a heterozygous condition in diploid pollen, but will be fully expressed in haploid pollen. Population genetics theories predict that asexual organisms accumulate deleterious mutations, because these cannot be purged by natural selection (Muller, 1964; Kondrashov, 1982). Alternatively, pollen meiosis in triploids could be biased towards diploid pollen grains. Secondary chromosome associations, which may bring about common movement and segregation of chromosomes, have been observed during male meiosis in triploid *Taraxacum* by Gustafsson (1935) and Richards (1970a). In addition, although haploid pollen has to be perfectly balanced, diploid pollen may include several classes of pollen grains that are not perfectly balanced.

Apomixis was absent in the four diploid hybrids obtained in this study. This is consistent with the absence of apomixis in natural diploids. Nogler (1982) suggested, based on his work in *Ranunculus auricomus*, that apomixis genes cannot be transmitted by haploid gametes, because of recessive lethality. Diploid apomicts could, however, be obtained in *Ranunculus auricomus* through di-haploidy avoiding a haploid state. Referring to the many nonapomictic diploids obtained by Müller (1972) in *Taraxacum*, Nogler (1982) speculated that a similar phenomenon could also be the case in *Taraxacum*. However, as indicated above, Müller's diploids could well have arisen from selfing. Unfortunately, the present sample of four diploid hybrids is too small to conclude that apomixis is incompatible with zygotic diploidy in *Taraxacum*.

Apomictic seed-set was restricted to polyploid hybrids. However, seed-set was variable and consistently lower than that of the natural apomictic fathers. The high percentage of undeveloped seeds could be because a proportion of the egg cells need fertilization for embryo development or that some central cells need fertilization for endosperm development (pseudogamy). However, additional pollinations did not increase seed-set. Only one fertilized unreduced egg cell was found (a $2n + n$ hybrid according to the terminology of Harlan & DeWet, 1975). Thus partial apomictic seed-set resulted from semisterility rather than from facultative apomixis.

The germination rate of the apomictic hybrids was also lower than that of the triploid fathers. Cross-sectioning showed that this was generally because of poorly developed, small embryos. Whereas in sexual *Taraxacum*, the endosperm has an important function in nourishing the developing embryo, in apomictic *Taraxacum*, this nutritive function is largely taken over by nucellar tissues (Cooper & Brink, 1949). Variation in development and functioning of these nucellar tissues

may be responsible for variation in development of apomictic embryos and viable apomictic seed-set between hybrids.

The triploid hybrids segregated for apomixis; one-third reproduced apomictically, two-thirds did not. In contrast, all tetraploid hybrids were capable of apomictic reproduction. In an accompanying paper, it is shown that apomixis in dandelions is likely to be controlled by more than one locus (Van Dijk *et al.*, 1999). Levels of gene segregation and recombination are higher in reduced than in unreduced gametes (Mendiburu & Peloquin, 1979; Ramanna, 1979). The differences in segregation for apomixis observed in the triploid and tetraploid hybrids in the present study may be related to a greater breakdown of the paternal apomictic genotype in the reduced pollen grains that gave rise to the triploid hybrids than in the unreduced triploid pollen grains that gave rise to the tetraploid hybrids.

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