Theor Appl Genet (1991) 82:645–656

THEORETICAL AND APPLIED GENETICS

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Formation of first division restitution (FDR) 2n-megaspores through pseudohomotypic division in *ds-1* (desynapsis) mutants of diploid potato: routine production of tetraploid progeny from 2xFDR × 2xFDR crosses

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Received January 15, 1991; Accepted February 20, 1991
Communicated by H.F. Linskens

Summary. The level and mode of 2n megaspore formation was studied in full-sib diploid potato clones with either normal or desynaptic (*ds-1ds-1*) meiosis. Cytological analysis revealed that functional 2n megaspores produced by normal and desynaptic clones originate exclusively from 'second division restitution (SDR)' and 'first division restitution (FDR)', respectively. SDR 2n megaspores resulted from the omission of the second meiotic division following chromosome doubling after anaphase I, whereas FDR 2n megaspores resulted from a direct equational division of univalent chromosomes at anaphase I (pseudohomotypic division). Comparative data strongly indicated that the observed mechanisms of SDR and FDR 2n megaspore formation are extremes of a continuum that is being brought about by common genes for precocious chromosome division. Depending on the relative timing of cell cycle and chromosome division, this precocious chromosome division may impose postreductional (SDR) or prereductional (FDR) 'restitution' of the sporophytic chromosome number under normal synaptic and desynaptic conditions, respectively. The observed frequencies of 2n megaspores closely correlated with seed set, following pollination by tetraploid varieties and by desynaptic diploid clones with exclusive FDR 2n pollen formation. Up to 54.0 and 21.5 seeds/fruit were obtained from normal synaptic (SDR) and desynaptic (FDR) progeny, respectively. The high frequency of segregants with either SDR or FDR 2n megaspore formation (78.0 and 45.2%, respectively) supports the hypothesis that sexual polyploidization is the driving force behind the origin and evolution of polyploid *Solanum* species. The present identification of diploid potato clones with consistent FDR 2n megaspore formation extends the opportunities for direct transfer of enhanced diploid germ plasm to tetraploids, and particularly advocates the feasibility of 2x(*ds-1*; FDR) × 2x(*ds-1*; FDR) breeding schemes in cultivar development and the production of relatively vigorous and uniform true potato seed (TPS) varieties. Its potential value and limitations for breeding and the experimental induction of diplosporic apomixis are discussed.

Key words: 2n Megaspores – Desynapsis – Pseudohomotypic division – Sexual polyploidization – apomixis

Introduction

Sexual polyploidization via numerically unreduced or 2n gametes has been identified as being the driving force behind the origin and evolution of polyploid plant species (Harlan and de Wet 1975). As for potato, the frequent occurrence of 2n gamete formation in many diploid species has substantiated their evolutionary significance in the origin of the polyploid complexes found in some taxonomic series of the tuber-bearing *Solanums* (Den Nijs and Peloquin 1977). More important, 2n gametes also enable the adoption of relatively efficient breeding schemes, which basically consist of direct transfer of enhanced diploid germ plasm to tetraploids through unilateral (4x × 2x crosses) and bilateral (2x × 2x crosses) sexual polyploidization, for both cultivar development and the production of TPS varieties (Mendiburu et al. 1974; Peloquin 1982; Hermsen 1984a).

Unreduced gametes may result from a number of different meiotic 'abnormalities'. According to the genet-

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ic consequences, however, only two distinct types, first division restitution (FDR) and second division restitution (SDR), are distinguished. Where FDR 2n gametes essentially derive from an equational division of the entire chromosome complement and thus include 'non-sister' chromatids, SDR 2n gametes result from chromosome doubling following reductional chromosome division and comprise 'sister chromatids'. For breeding schemes employing sexual polyploidization, the actual mode of 2n gamete formation in the diploid parental clones is of practical significance. FDR 2n gametes are by far superior in preserving parental heterozygosity, and thus significantly contribute to both vigour and homogeneity of tetraploid progeny recovered by sexual polyploidization (Mendiburu et al. 1974; Peloquin 1982; Hermsen 1984 b). In addition, mutant synaptic genes are known to cause the exclusive occurrence of functional FDR 2n gametes (Ramanna 1983; Jongedijk 1985) and, owing to reduced gene recombination, to particularly increase the ability of FDR 2n gametes to maintain the genetic constitution of parental clones, including complex types of favorable epistasis, with a minimum amount of reassortment (Douches and Quiros 1988 a; Jongedijk et al. 1991). Maximum possible performance and uniformity might thus be attained in tetraploid progeny from 2xFDR × 2xFDR crosses when genetic recombination is largely lacking in both parental clones. As to TPS technology, nearly complete uniformity might also be achieved by diplosporic apomixis. The latter could possibly be obtained by combining genes for asynapsis/desynapsis, FDR 2n megaspore formation, and pseudogamous seed development (Hermsen 1980; Hermsen et al. 1985; Jongedijk 1985).

For the efficient use of 2n gametes in breeding, knowledge of the cytological mechanisms of SDR and FDR and their inheritance is indispensable. Whereas the mechanisms and genetic control of SDR and FDR 2n pollen formation have been well established (Ramanna 1974, 1979, 1983; Mok and Peloquin 1975; Veilleux et al. 1982), cytological data concerning 2n megaspore formation are relatively scarce. So far, genetic and cytological studies have indicated the predominant occurrence of SDR with normal synapsis and revealed that the prevailing mechanism of SDR 2n gamete formation consists of 'omission of the second meiotic division' (Stelly and Peloquin 1986 a, b; Werner and Peloquin 1987, 1991; Douches and Quiros 1988 b). Similar mechanisms of SDR 2n megaspore formation have been reported for *Datura*, maize, and barley (Satina and Blakeslee 1935; Rhoades and Dempsey 1966; Finch and Bennett 1979). According to Werner and Peloquin (1987, 1991) SDR 2n megaspores may also be formed through the failure of cytokinesis after the second meiotic division and subsequent fusion of daughter nuclei prior to embryo sac development, or by nuclear restitution following irregular second meiotic divisions. The former mechansim has previously been noted to cause SDR 2n megaspore formation in alfalfa (Pfeiffer and Bingham 1983). As expected with mutant synaptic conditions being required for consistent FDR 2n megaspore formation (Jongedijk 1985), the latter has only been inferred to occur in some of the synaptic mutants tested so far. Although cytological studies of female meiosis in these mutants revealed occasional nuclear restitution following typically desynaptic metaphases (Iwanaga and Peloquin 1979, Werner and Peloquin 1987, 1991), conclusive evidence for FDR 2n megaspore formation by the formation of restitution nuclei including all chromosomes and their subsequent division in the second meiotic division has not yet been provided.

In this paper, consistent FDR 2n megaspore formation in desynaptic mutants through direct equational division of univalent chromosomes and subsequent omission of the second meiotic division (pseudohomotypic division) is reported. In addition, comparative data on SDR and FDR 2n megaspore formation are provided which suggest that both are caused by common genes for precocious chromosome division.

Materials and methods

Plant material

The formation of 2n megaspores was studied in the diploid parental clones USW5295-7 (coded B), USW 5337-3 (coded C), USW 7589-2 (coded D), 77-2102-37 (coded E), and derived F_1 hybrids (two letter codes). Detailed information on the origin and pedigree of the parental clones has been summarized earlier (Jongedijk and Ramanna 1988). The F_1 hybrids included both normal (*Ds-1.*) and desynaptic (*ds-1 ds-1*) segregants (Jongedijk and Ramanna 1988, 1989). Levels of 2n megaspore formation were estimated on the basis of seed set, following pollination by the tetraploid potato cultivars Gineke, Libertas, Chippewa, and Katahdin (2x × 4x testcrosses) and by diploid desynaptic clones with exclusive FDR 2n pollen formation [2x × 2x(*ds-1*; FDR) testcrosses] and with high seed set in 4x × 2x crosses (Table 1). At least 50 flowers of each clone were pollinated with four to six different male parents. To exclude selfing, flowers were emasculated well before anthesis. Mature fruits were collected 6–8 weeks after pollination. To obtain a value measuring the average seed set on a particular clone, the data concerning different

Table 1. Percentage of stainable and 2n pollen in diploid (2x) desynaptic pollen parents, and seed set following pollination of tetraploid (4x) seed parents

Pollen	% Stainable pollen[a]	% 2n pollen[a]	Seeds/fruit from 4x × 2x crosses
CE-10	69.7	96.7	128.77
CE-101	69.1	94.8	97.73
BE-62	45.9	93.6	80.97
BE-67	63.1	96.2	65.72

[a] Data from Ramanna (1983)

pollen parents were pooled. Relevant data concerning the parental genotypes at the *Ds-1/ds-1* locus and parental '2x × 4x crossability' are summarized in Table 2.

Cytological analyses

Ploidy distributions in testcross progenies were checked by establishing the mean number of chloroplasts in stomatal guard cells (Frandsen 1968) or, in cases of doubt, by chromosome counts in root-tip meristems. For large-scale screening and detailed observations of megasporogenesis in young ovaries, a routine methyl salicylate clearing technique (Jongedijk 1987a) and an enzyme squash technique (Jongedijk 1987b) were used, respectively. Frequencies of 2n megaspores were estimated in random samples of about 500 ovules from three to four different ovaries with predominantly sporad stages. Frequencies of desynaptic and (partially) pseudohomotypic metaphase stages in *ds-1* mutants were estimated in random samples of about 150 megaspore mother cells from two to three different ovaries. All photographs were taken with a Zeiss Photomicroscope II, using a Zeiss Planapochromatic 63 PH3H/1.4 oil immersion objective on Kodak technical Pan Film 2415.

Table 2. Average seed set on diploid (2x) normal synaptic (*Ds-1ds-1*) parental clones from 2x × 4x and 2x × 2x (*ds-1*; FDR) testcrosses

Parental clone (code)	Genotype	Average seed set[a]			No. poll.
		f/p	s/f	s/p	
USW 5295-7 (B)	*Ds-1ds-1*	0.75	12.29	9.22	69
USW 5337-3 (C)	*Ds-1ds-1*	0.17	1.13	0.19	84
USW 7589-2 (D)	*Ds-1ds-1*	0.54	25.33	13.68	144
77-2102-37 (E)	*Ds-1ds-1*	0.71	1.81	1.28	160

[a] f = fruits, s = seeds, p = pollination

Results

Seed set in 2x × 4x and 2x × 2x(ds-1ds-1/FDR) testcrosses

In the absence of premeiotic and postmeiotic doubling, 2n megaspores from normal synaptic potato clones are expected to arise through SDR, whereas consistent FDR 2n megaspore formation requires mutant synaptic conditons (Jongedijk 1985). In the latter case, both SDR and 'reduced' megaspores may be formed, but these abort due to chromosome imbalance (Ramanna 1983; Jongedijk 1985). Because of the nearly complete 'triploid block' (Marks 1966) and the near absence of premeiotic and postmeiotic doubling in potato (Stelly and Peloquin 1986a; Werner and Peloquin 1987, 1991), the average seed set in 2x × 4x and 2x × 2x(*ds-1*; FDR) testcrosses may therefore be assumed to give rise to tetraploid offspring and to be a measure of the seed parents' ability to produce either SDR or FDR 2n megaspores in case of normal synapsis and desynapsis, respectively.

Seed set following 2x × 4x and 2x × 2x(*ds-1*;FDR) testcrosses was estimated for 123 normal synaptic (*Ds-1.*) and 126 desynaptic (*ds-1ds-1*) diploid F_1 hybrids from nine different, partly reciprocal crosses (Table 3). Data about seed set on normal synaptic segregants as well as on desynaptic segregants from reciprocal crosses were pooled. Among reciprocal hybrids within each category, only very few significant differences were observed [$P(\chi^2$ homogeneity$) \geq 0.01$]. The populations differed substantially in the percentage of diploid hybrids with seed set. In addition, this frequency was generally higher among

Table 3. Mean number of fruits/pollination (f/p), seeds/fruit (s/f), and seeds/pollination (s/p) obtained from 2x × 4x and 2x × 2x (*ds-1*; FDR) testcrosses and their relation in normal synaptic (*Ds-1.*) and desynaptic (*ds-1ds-1*) diploid progenies from five sets of (reciprocal) crosses

Hybrids (2n = 2x = 24)			No. poll.	Hybrids with seed set									
Parentage[a]	*Ds-1/ds-1*	No.		% clones	f/p		s/f		s/p		Correlation coefficient		
					Mean	Range	Mean	Range	Mean	Range	r(f/p, s/f)	r(f/p, s/p)	r(s/f, s/p)
BC/CB	*Ds-1.*	22	2186	54.5	0.25	0.01–0.82	2.46	1.00– 6.13	0.84	0.01– 5.04	0.66*	0.89*	0.83*
	ds-1ds-1	30	2098	56.7	0.15	0.02–0.49	6.47	1.00–21.53	1.29	0.02– 6.50	0.38	0.78*	0.78*
BE/EB	*Ds-1.*	17	1285	76.5	0.26	0.01–0.78	14.47	2.38–36.86	4.86	0.08–28.67	0.46	0.87*	0.77*
	ds-1ds-1	33	2212	39.4	0.11	0.01–0.50	4.43	1.00–13.78	0.75	0.02– 3.35	0.30	0.70*	0.83*
CE/EC	*Ds-1.*	42	2116	80.9	0.38	0.01–0.90	4.42	1.00–25.64	1.79	0.03–11.97	0.08	0.51*	0.82*
	ds-1ds-1[b]	19	2108	47.4	0.15	0.01–0.44	4.88	1.50–10.85	0.96	0.02– 3.78	0.49	0.83*	0.81*
DE/ED	*Ds-1.*	29	2249	86.2	0.57	0.06–1.00	15.92	1.17–54.00	9.08	0.29–54.00	0.27	0.54*	0.91*
	ds-1ds-1	34	2576	44.1	0.20	0.01–0.60	3.33	1.00–12.53	0.99	0.01– 5.52	0.50	0.76*	0.86*
DB	*Ds-1*	13	678	92.3	0.56	0.08–0.94	9.89	1.00–36.67	6.93	0.13–27.50	0.53	0.62*	0.98*
	ds-1ds-1	10	527	30.0	0.50	0.03–0.83	2.71	0.03– 6.00	1.92	1.00– 5.00	0.79	0.82	0.99
Total	*Ds-1.*	123	8514	78.0	0.42	0.01–1.00	9.21	1.00–54.00	4.63	0.01–54.00	0.30*	0.58*	0.86*
	ds-1ds-1	126	9521	45.2	0.17	0.01–0.83	4.73	1.00–21.53	1.14	0.01– 6.50	0.27*	0.77*	0.75*

[a] For explanation of codes, see section 'Plant material'
[b] *ds-1ds-1* hybrids from CE only; EC does not segregate (Jongedijk and Ramanna 1988)
* Significant at the 5% level

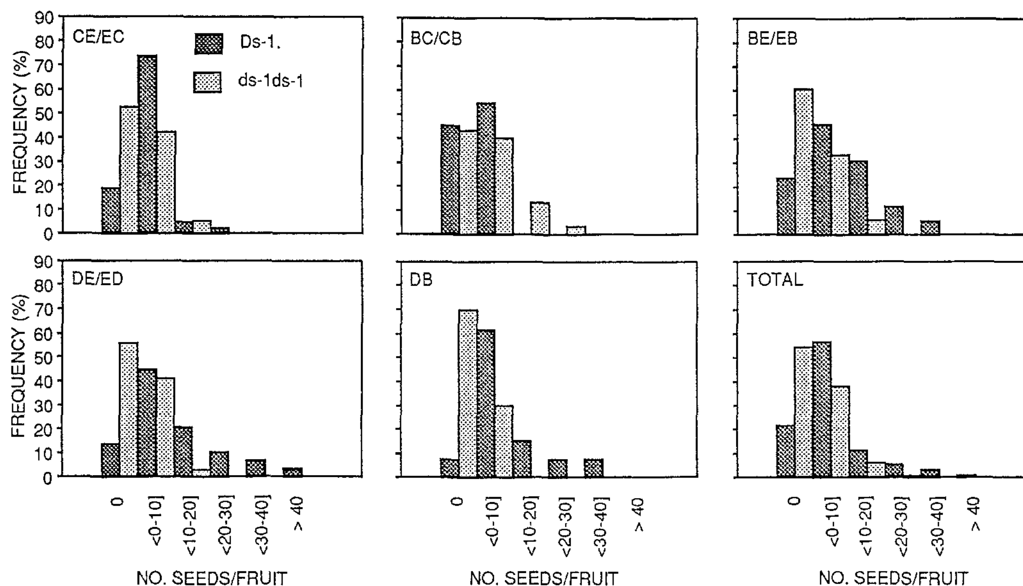


Fig. 1. Average numbers of seeds/fruit following testcrossing of normal synaptic (*Ds-1.*) and desynaptic (*ds-1ds-1*) diploid potato clones

the normal synaptic segregants than among the desynaptic segregants. On the whole, 78.0% of the normal synaptic progeny and 45.2% of the desynaptic mutants tested formed SDR and FDR 2n megaspores, respectively (Table 3). Both between and within different populations, the average numbers of fruits/pollination (f/p), seeds/fruit (s/f) and seeds/pollination (s/p) produced by normal synaptic and desynaptic segregants varied considerably, the averages and ranges usually being smaller among *ds-1* mutants (Table 3). Significant positive correlations were observed between the numbers of seeds/pollination, on the one hand, and the numbers of fruits/pollination and seeds/fruit, on the other, in all (sub)populations [correlation coefficients: r(f/p, s/p) = 0.51–0.89 and r(s/f, s/p) = 0.77–0.99]. However, the number of fruits/pollination generally did not significantly correlate with the number of seeds/fruit (Table 3). Similar amounts of seeds/pollination thus may result from relatively high berry set but moderate numbers of seeds/fruit, and from moderate berry set but relatively high numbers of seeds/fruit.

Normal synaptic F_1 hybrids with numbers of seeds/fruit exceeding that of the highest parental clone were observed in all crosses but BC/CB. As to the desynaptic seed parents, such F_1 hybrids were observed among BC/CB and CE/EC progeny only (Fig. 1). Following testcrossing, up to 54.0 and 21.5 seeds/fruit were obtained from normal synaptic and desynaptic clones, respectively. Seed parents with medium-high numbers of seeds/fruit generally produced sufficient berries to allow for routine production of extensive testcross progeny. The average number of seeds/pollination produced by normal synaptic plants with <0–10], <10–20], <20–30], >30–40], and >40 seeds/fruit amounted to 1.4, 6.5, 13.0, 27.6, and 54.0, respectively, whereas *ds-1* mutants representing the lower three classes produced on the average 0.6, 3.2, and 6.5 seeds/pollination.

Ploidy levels of testcross progeny

In order to make sure that seed set following the 2x × 4x and 2x × 2x(*ds-1*;FDR) testcrosses does accurately measure the seed parents' ability to produce 2n megaspores, ploidy levels of testcross progenies that involved normal synaptic and desynaptic seed parents with low-high seed set were checked. For comparison, ploidy levels among progeny derived from additional 4x × 2x and 2x × 2x (*Ds-1.*) crosses were established. Normal synaptic pollinators used in these latter crosses included the parental clones USW5295-7 (B), USW5337-3 (C), 77-2102-37 (E), and BE-44, which produce substantial amounts of predominantly FDR 2n pollen (Mok and Peloquin 1975; Jacobsen 1978; Jongedijk et al. 1991).

As expected with a nearly complete triploid block, the frequency of tetraploids among progeny derived from either 2x–4x crosses or 2x–2x crosses involving at least one desynaptic parent was extremely high (Table 4). However, due to the formation of reduced gametes in normal synaptic parents, the frequency of tetraploid progeny from 2x(*Ds-1.*) × 2x(*Ds-1.*) crosses was relatively low: even in crosses between normal synaptic parental clones with relatively high levels of 2n gamete formation only up to 35.1% tetraploids were observed (Table 4). It may thus be concluded that desynapsis conferred by the *ds-1* gene acts as an effective sieve against the formation of functional reduced (and SDR) gametes. Not surprisingly, in that case, the highest frequencies of tetraploids (99.6–99.9%) were observed in progenies involving only desynaptic diploid parents. The rare occurrence of

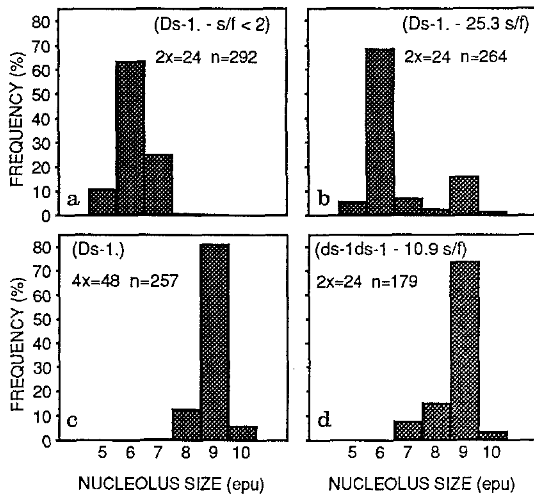


Fig. 2A–D. Average nucleolus sizes in uni- to tetranucleate embryo sacs from normal synaptic (*Ds-1.*) diploid potato clones with low (**A**) and high (**B**) levels of SDR, a normal synaptic tetraploid potato variety (**C**), and a desynaptic (*ds-1ds-1*) diploid potato clone with FDR (**D**). 1 epu (eye piece unit) ≈0.5 μm. n = no. embryo sacs

Table 4. Ploidy levels of progeny from 2x × 4x and 2x × 2x (*ds-1*; FDR) testcrosses and, for comparison, those of progeny from 4x × 2x and 2x × 2x (*Ds-1*) crosses

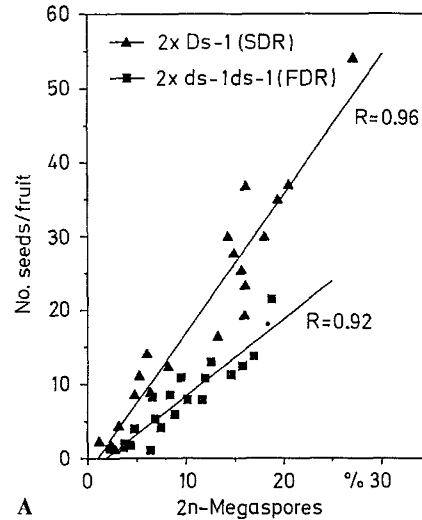
Mating type	No. crosses	No. progeny	Ploidy level (%)		
			2x	3x	4x
2x × 4x:					
2x *Ds-1* × 4x *tbr*	22	1385	0.9	0.4	98.7
2x *ds-1* × 4x *tbr*	17	1784	0.4	0.0	99.6
4x × 2x:					
4x *tbr* × 2x *Ds-1*	10	1227	0.8	0.2	99.0
4x *tbr* × 2x *ds-1*	11	1119	0.1	0.0	99.9
2x × 2x (*ds-1*; FDR):					
2x *Ds-1* × 2x *ds-1*	19	1900	1.0	0.2	98.8
2x *ds-1* × 2x *ds-1*	22	1624	0.2	0.1	99.7
2x × 2x (*Ds-1*):					
2x *Ds-1* × 2x *Ds-1*	36	5009	85.7	0.1	14.2[a]
2x *ds-1* × 2x *Ds-1*	21	1465	1.4	0.1	98.5

[a] Range (depending on cross combination): 0.0–35.1%

diploid (and triploid) progeny from 2x–2x crosses involving at least one desynaptic parent (Table 4) might be explained by the occasional formation of largely balanced, reduced gametes in *ds-1* mutants. The diploid progeny from 2x–4x crosses might result from pseudogamous parthenogenetic development of (un)reduced diploid egg cells.

Mechanisms of SDR and FDR 2n gamete formation

Female meiosis was studied in detail in 20 normal synaptic plants and 20 desynaptic mutants with low, medium



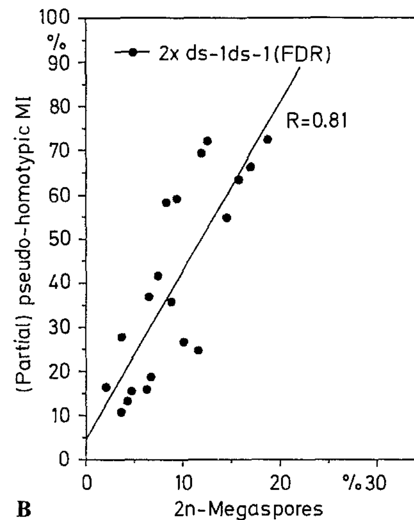
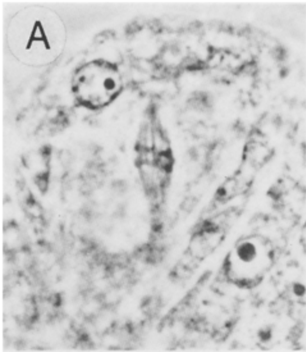


Fig. 3A and B. Relationship between the frequency of 2n megaspores and **A** the number of seeds/fruit following testcrossing in normal synaptic (*Ds-1.*; SDR) and desynaptic (*ds-1ds-1*; FDR) diploid potato clones, **B** the frequency of (partial) pseudohomotypic metaphase I stages in desynaptic (*ds-1ds-1*; FDR) diploid potato clones

and high seed set following 2x × 4x and 2x × 2x(*ds-1*; FDR) testcrosses. Normal synaptic clones and desynaptic ones without any seed set collectively served as experimental controls. To avoid bias generated by asynchronous development of reduced and unreduced megaspores, cytological quantification of 2n megaspore formation was based on the frequencies of dyad megaspores and, whenever present, well-developed uni- to tetranucleate embryo sacs with average nucleolar sizes exceeding 3.5 μm (Stelly and Peloquin 1985; cf. Fig. 2A-D). The frequencies of 2n megaspores so established closely correlated with seed set following 2x × 4x and 2x × 2x(*ds-1*;FDR) testcrosses (Fig. 3A).

The normal course of female meiosis and embryo sac formation in potato has been described extensively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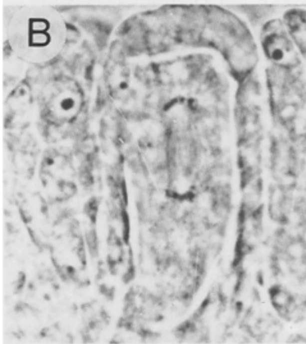
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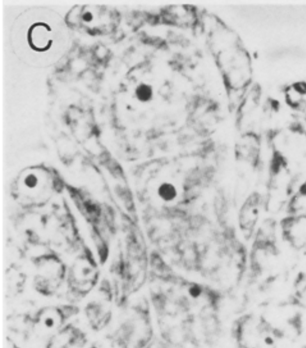
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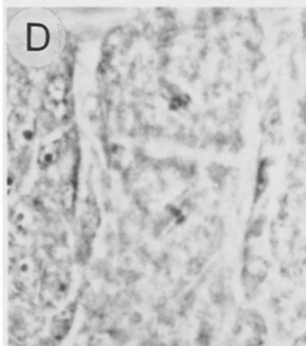
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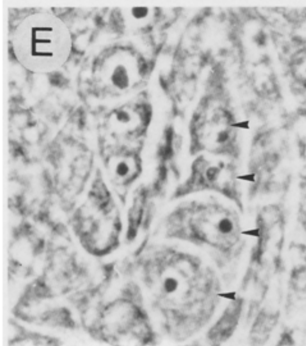
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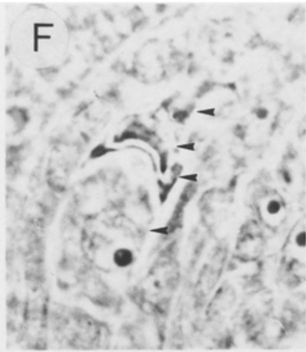
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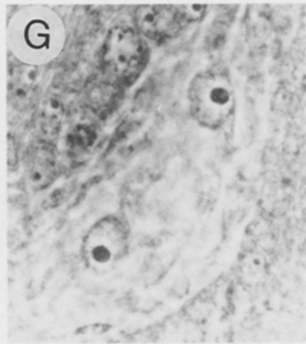
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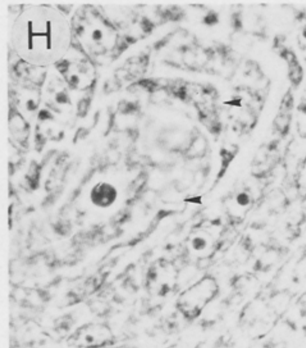
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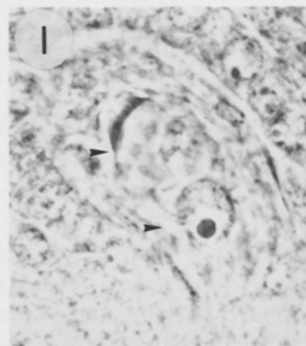
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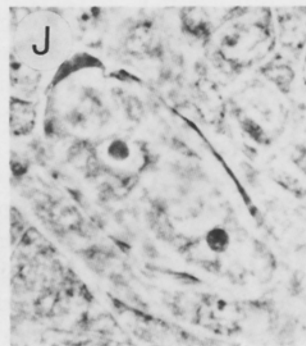
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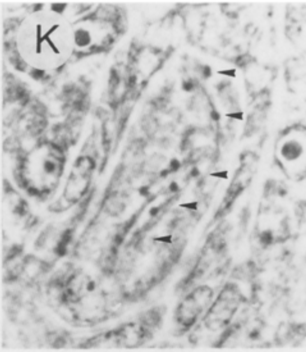
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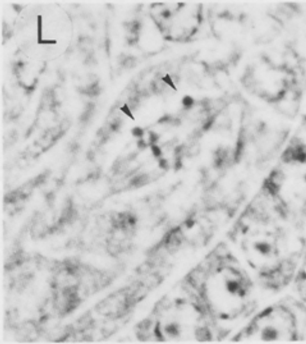
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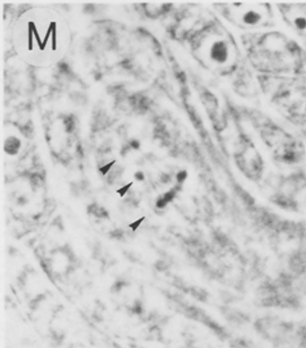
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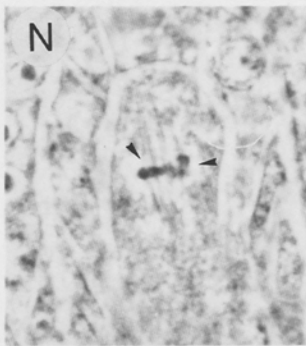
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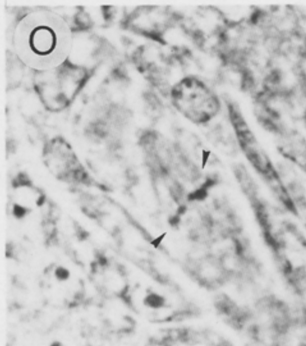
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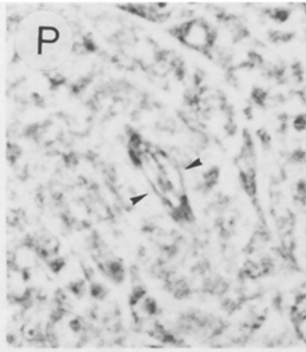
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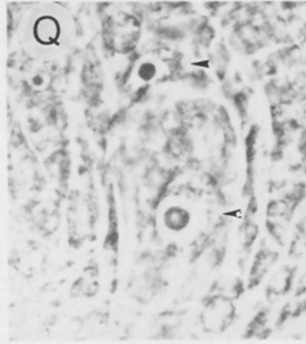
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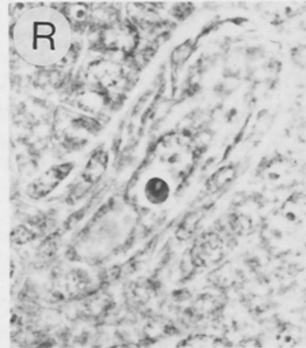
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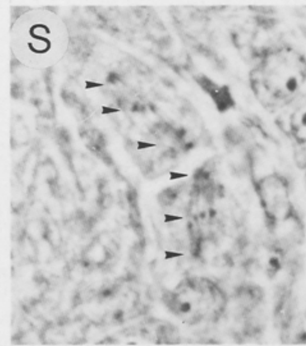
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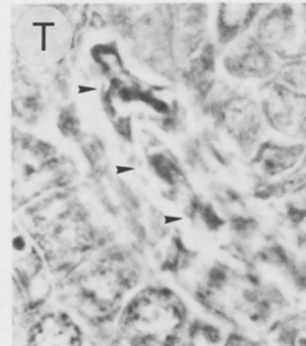
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(Rees-Leonard 1935; Jongedijk 1985). Briefly, in female meiosis cytokinesis is of the successive type. Following disjunctional separation of homologous chromosomes at anaphase I, a cell plate is formed across the persisting phragmoplast. The haploid chromosome complements of the resulting daughter cells subsequently divide equationally, giving rise to a tetrad of reduced megaspores. Megagametogenesis in the functional chalazal megaspore follows the *Polygonum* type of embryo sac development. (Fig. 4A-G).

In normal synaptic clones with consistent 2n megaspore formation the developmental sequence differed from normal in the occasional occurrence of irregular chromosome movement at late anaphase I and the relatively frequent incidence of dyad formation, unreduced embryo sacs (Fig. 4H-J), and irregular metaphase II-anaphase II stages (Fig. 4S). These aberrations were interpreted as resulting from precocious separation of sister chromatids at late anaphase I-prophase II. When all chromosomes are involved, such a precocious chromosome division obviously results in the omission of the second division and thus in the formation of dyads of numerically unreduced megaspores which are genetically equivalent to SDR. If incomplete, however, the daughter cells may enter into metaphase II, resulting in random segregation of univalent sister chromatids and subsequent abortion of predominantly aneuploid megaspores. Where irregular second division stages were virtually absent in normal synaptic clones lacking 2n megaspore formation, their incidence among clones with consistent 2n megaspore formation amouned to 3.7–25.9% of all cases, high levels of 2n megaspore formation invariably being associated with high frequencies of irregular metaphase II stages. The low and insignificant correlation between the frequencies of second division irregularities and the overall levels of female sterility ($r=0.39$) indicated that the latter is largely determined by factors other than the observed second division irregularities. High levels of female sterility (19.4–43.6%) were observed in both normal synaptic plants with and without consistent 2n megaspore formation. Premeiotic and postmeiotic doubling or doubling by failure of cytokinesis in chalazal megaspores, followed by fusion of reduced nuclei prior to megagametophyte development, were not observed.

Desynaptic mutants are characterized by normal chromosome pairing throughout pachytene and a falling apart of bivalents at diakinesis (Ramanna 1983; Jongedijk and Ramanna 1988). In *ds-1* mutants lacking 2n megaspore formation female sterility was nearly complete, and not a single pollen parent was successful in inducing seed set. Meiotic abnormalities further included the occurrence of disfigured spindles (Fig. 4K), random distribution of univalents, occasional univalent division (Fig. 5B), the formation of micronuclei, and abortion of megaspore mother cells cells before the onset or completion of the second meiotic division.

As expected with the *ds-1* gene being an effective sieve against the formation of functional reduced (and SDR) gametes, among *ds-1* mutants with 2n megaspore formation highly significant positive correlations were observed between the level of female fertility (0.9–19.3%), on the one hand, and the frequency of 2n megaspores ($r=0.98$) and seed set following 2x × 4x and 2x × 2x (*ds-1;* FDR) testcrosses ($r=0.94$), on the other. Meiotic studies in these mutants typically revealed substantial frequencies of megaspore mother cells with an exceptionally strong tendency to univalent division at late metaphase I-early anaphase I. Univalent division was often, but not necessarily, preceded by an approximate orientation of most univalents at the equatorial region (Fig. 4L-O), suggesting amphitelic orientation of sister chromatid kinetochores and thus that centromere division takes place at early metaphase I-anaphase I. Amphitelic orientation of sister chromatid kinetochores was confirmed in acetocarmine squashes of enzyme-digested megaspore mother cells (Fig. 5C–G) and proved to be particularly apparent in male meiosis of the *ds-1* mutants under consideration (Fig. 5H). As indicated by the 'bivalent-like' structure of equationally dividing univalents and the frequent occurrence of "chromatin tails" at anaphase I (Fig. 4P), sister chromatid cohesiveness typically persisted beyond metaphase I. Second division stages, if present, were highly irregular (Fig. 4S). Obviously, the equational division of the entire chromosome complement at late metaphase I-early anaphase I (pseudohomotypic division) resulted in the omission of the second division and thus the formation of dyads of numerically unreduced megaspores, which are genetically equivalent to FDR. However, if incomplete (i.e., partial pseudohomotypic division), preponderantly aneuploid daughter cells arise that either abort directly or, again, enter into an abortive second division with randomly segregating, univalent sister chromatids. The percentage of 2n megaspores closely correlated with the frequency of megaspore mother cells with (partial) pseudohomotypic metaphase I stages

Fig. 4A–T. Methyl-salicylate-cleared ovules with typical stages of female meiosis in normal synaptic SDR clones (**A–J**) and desynaptic FDR clones (**K–T**). **A** Metaphase I; bivalents. **B** Anaphase I. **C** Interkinesis. **D** Metaphase II. **E–F** Tetrad of reduced megaspores. **G** Reduced binucleate embryo sac. **H–I** Dyad of SDR 2n megaspores. **J** Unreduced (SDR) binucleate embryo sac. **K** Desynaptic metaphase I; univalents. **L–O** Pseudohomotypic metaphase I; predominant congregation of univalents at equatorial plate. **P** Pseudohomotypic anaphase I; persisting chromatid cohesiveness. **Q** Dyad of FDR 2n megaspores. **R** Unreduced (FDR) uninucleate embryo sac. **S** Typical irregular metaphase II; scattered sister chromatids. **T** Degenerating megaspores ($\pm \times 700$)

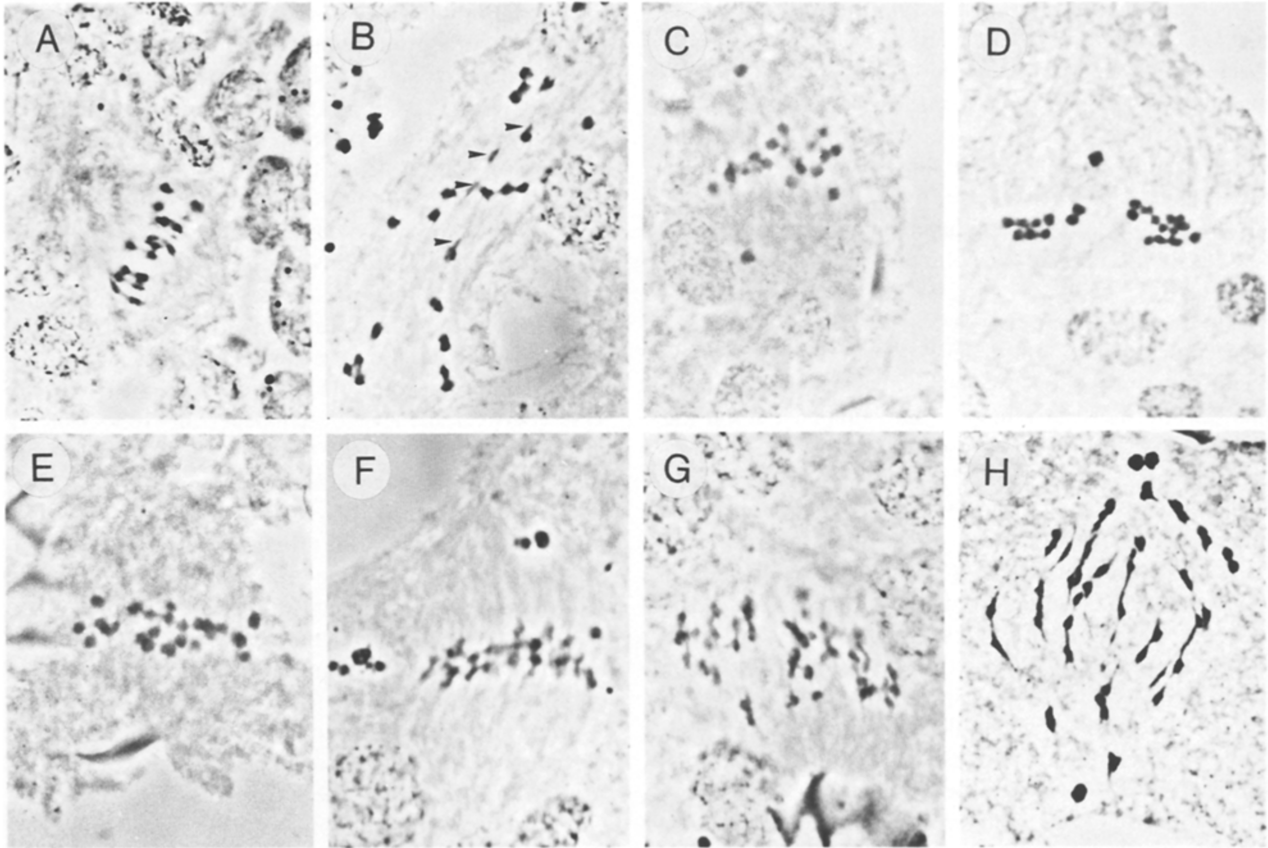

Fig. 5A–H. Acetocarmine squashes of enzyme-digested megaspore mother cells (**A–G**) and pollen mother cells (**H**). **A** Metaphase I; bivalents. **B** Desynaptic metaphase I; univalents scattered, two dividing univalents (*arrows*). **C–E** Pseudohomotypic metaphase I; predominant congregation of univalents at equatorial plate. **F–H** Pseudohomotypic anaphase I; (predominant) univalent division, persisting chromatid cohesiveness (± × 1,000)

(Fig. 3B). Neither megaspore mother cells with largely mitotic divisions (mitotized meiosis) nor restitution nucleus formation following desynaptic or (partial) pseudohomotypic anaphase I and subsequent division of univalent chromosomes in the second division (semiheterotypic division) were observed in any clone.

Discussion

Mechanisms of SDR and FDR 2n megaspore formation

Studying the normal sequence of female meiosis in diploid potato, Jongedijk (1985) inferred that functional 2n megaspores produced under normal synaptic and under mutant synaptic conditions are likely to originate through SDR and FDR, respectively. Cytological and genetical analyses of 2n megaspore formation have since shown that 2n megaspore formation in normal synaptic potato clones is by SDR, and that the prevailing mechanism of SDR 2n megaspore formation consists of omission of the second meiotic division following chromosome doubling after anaphase I (Stelly and Peloquin 1986a, b; Werner and Peloquin 1987, 1991; Douches and Quiros 1988b). The present study confirms that SDR 2n megaspore formation in normal synaptic, diploid potato is caused by the precocious separation of sister chromatids at late anaphase I-prophase II. Precocious chromosome division at late anaphase I-prophase II associated with occasional SDR 2n megaspore formation and high ovule sterility due to second division irregularities has previously been observed in *pc* mutants of tomato (Clayberg 1959). Frequent second division irregularities in normal synaptic, diploid potato clones with substantial SDR 2n megaspore formation through omission of the second division was previously observed by Werner and Peloquin (1991). However, presuming subsequent nuclear restitution in both daughter cells, they considered it an independent abnormality of the second division and thus a distinct mechanism of SDR 2n megaspore formation. Also, their observation that in typically delayed megaspore mother cells some equational chromosome division may occur at anaphase I ('delayed meiotic division'), might be attributed to division precocity.

'Subsexual' processes resulting in FDR 2n megaspores typically occur in diplosporic apomictic plant species (Gustafsson 1946; Rutishauser 1967, Nogler

1984). One essentially consists of omission of female meiosis in archesporial cells, which instead directly develop into mature gametophytes through successive mitotic divisions (mitotized meiosis or mitotic diplospory). The others typically involve a nearly complete lack of chromosome association at metaphase I, and basically consist of (i) equational division of the entire chromosome complement in the second meiotic division following nuclear restitution at anaphase I (semiheterotypic division), or (ii) direct equational division of univalent chromosomes at metaphase I and subsequent omission of the second meiotic division (pseudohomotypic division). It has been claimed that the latter mechanism occurs in *Taraxacum, Erigeron, Archieracium,* and *Chondrilla* (Gustafsson 1935; Bergman 1944) and, as reported here, it was often noted that it was preceded by an approximate orientation of initially scattered univalents at the equatorial plate. The actual existence of the pseudohomotypic division has been disputed, however, and still is considered to be 'very improbable' (Rutishauser 1967; Nogler 1984).

Studying 2n megaspore formation in synaptic mutants of potato, Iwanaga and Peloquin (1979) and Werner and Peloquin (1987, 1991) noted the occasional formation of a restitution nucleus including all chromosomes following anaphase I. However, conclusive evidence for FDR 2n megaspore formation by subsequent equational division of all chromosomes in the second meiotic division was not provided. In the *ds-1* mutants studied here, nuclear restitution following either typical desynaptic or (partial) pseudohomotypic anaphase I stages was consistently absent. The close correlation between the observed percentage of 2n megaspores, on the one hand, and the percentage of (partial) pseudohomotypic metaphases and seed set following 2x × 4x testcrosses, on the other (Fig. 3A, B), thus imply that FDR 2n megaspores in these mutants do originate through pseudohomotypic division. In male meiosis of the same plant material, an exceptionally strong tendency for funtional FDR 2n pollen formation through univalent division at late metaphase I-early anaphase I and consistent absence of nuclear restitution following (partial) pseudohomotypic metaphases has previously been reported by Ramanna (1983). Recently, both 2n pollen and 2n megaspore formation through pseudohomotypic division have also been found in desynaptic tomato lines (M. S. Ramanna, personal communication). As far as the authors are aware, these are the first and only documented cases of functional FDR 2n megaspore formation through pseudohomotypic division in otherwise typically sexual plant species. The exceptionally strong tendency for univalent division, however, is by no means unique. Though generally associated with extreme sterility, it has previously been noted in (a)synaptic mutants of *Brassica campestris* (Stringham 1970), *Zea mays* (Golubovskaya and Mashnenkov 1975), *Crepis capillaris* (Richardson 1935), *Oenothera decipiens* (Catcheside 1939), and *Alopecurus myosuroides* (Johnsson 1944) and in interspecific hybrids of several plant species (Meurman 1928; Lamm 1941; Maan and Sasakuma 1977).

The striking similarities of chromosome behavior in normal synaptic SDR and desynaptic FDR clones suggests that the observed mechanisms of SDR and FDR 2n megaspore formation are closely interrelated. Both basically consist of precocious chromosome division. Depending on whether or not all chromosomes are involved, this precocious chromosome division results either in the omission of the second division and subsequent dyad formation or in the abortion of largely aneuploid megaspores following random segregation of sister chromatids in the second division. In fact, the simultaneous occurrence of normal synaptic and of desynaptic progeny, both with and without consistent 2n megaspore formation through precocious chromosome division in all crosses (Table 3; Fig. 1), indicates that division precocity per se is controlled by a common genetic factor, with the gene or genes involved being independent of *ds-1* and exerting a similar effect on chromosome division in both categories of clones. However, where in normal synaptic plants SDR 2n megaspores consisting of 'sister' chromatids result from postreductional division precocity, division precocity in *ds-1* mutants in basically prereductional and 'non-sister' chromatids are included in FDR megaspores.

This apparent difference in the relative timing of division precocity might simply be attributed to the mutant synaptic condition. Synaptic abnormalities, including those conferred by the *ds-1* gene, have been noted to considerably prolong the duration of metaphase I-anaphase I (Clayberg 1958; Wagenaar 1961 a b; Jongedijk and Ramanna 1989). Such prolongation, when associated with chromosome division largely proceeding along the normal time lines, generally causes a loss of synchrony between cell cycle and chromosome division (Wagenaar 1968; Golubovskaya 1979; Koduru and Rao 1981; Kaul and Murthy 1985). According to Wagenaar (1968) under such conditions, "time is a limiting factor for chromosome division" and "metabolic processes imposing interphase on dividing cells" interrupt and effectively terminate chromosome division by the breakdown of the spindle and the formation of nuclear membranes enclosing chromosomal material wherever located in the cell. Obviously, genes causing precocious chromosome division as early as late anaphase I-prophase II in case of normal synapsis, if similarly expressed in *ds-1* mutants, are likely to take effect in the typically prolonged metaphase I-anaphase I. In *ds-1* mutants, they might thus allow for univalent orientation and for largely regular, but basically prereductional, univalent division (pseudohomotypic division) before the cell is interrupted by "imposition of interphase." In this perspective, the observed

mechanisms of SDR (i.e., 'omission of the second division') and FDR (i.e., pseudohomotypic division) 2n megaspore formation should be considered extremes of a continuum that is being brought about by common genes for division precocity which, depending on the relative timing of cell cycle and chromosome division, may impose postreductional (SDR) or prereductional (FDR) 'restitution' of the sporophytic chromosome number under normal and mutant synaptic conditions, respectively.

Studying chromosome behavior in the semidominant meiotic mutants *ord* and *mei-S332* and in wild-type *Drosophila*, Goldstein (1980) and Lin and Church (1982) concluded that the predominantly postreductional division precocity in the former mutants resulted from reduced chromatid cohesiveness rather than from kinetochore properties. The unmistakably amphitelic orientation of sister chromatid kinetochores, but frequent persistence of some degree of sister chromatid cohesion in (partial) pseudohomotypic metaphases from *ds-1* mutants (Fig. 5C-H), indicates that the same holds true for the precocious chromosome division in normal and desynaptic potato clones reported here. Among plant species, reduced sister chromatid cohesiveness has previously been inferred to cause division precocity in *pc* mutants of tomato (Clayberg 1959). In addition, it might also be involved in SDR 2n megaspore formation in the *dy*, *el*, and *tri* mutants from *Datura*, maize, and barley, respectively (Satina and Blakeslee 1935; Rhoades and Dempsey 1966; Finch and Bennett 1979), and in FDR 2n megaspore formation through pseudohomotypic division observed in some diplosporous apomicts (Gustafsson 1946; Rutishauser 1967).

Genetic basis of 2n megaspore formation

Whereas desynapsis is controlled by a single recessive gene and similarly affects male and female meiosis (Ramanna 1983; Jongedijk and Ramanna 1988, 1989; Jongedijk et al. 1991), the expression and genetic basis of precocious chromosome division and, thus, 2n megaspore formation appear to be more complicated. As indicated by the substantial frequencies of megaspore mother cells with largely normal meiosis in SDR clones and of typical desynaptic meiosis in FDR clones, expression is only partial. In addition, levels of precocious chromosome division in different cells from a single clone and the overall frequency of cells affected in male and in female meiosis may differ considerably (unpublished results). The reason for these sex differences in the expression pattern are as yet obscure. However, sex differences in the overall meiotic sequence [e.g., simultaneous (♂) versus successive (♀) cytokinesis], in the timing of meiosis and meiotic stages (e.g., earlier onset and relatively synchronous course of male meiosis), and/or in the fine tuning of the cycles of cell division and chromosome division might be of crucial importance.

Recently, Werner and Peloquin (1991) claimed that SDR 2n megaspore formation through omission of the second division in normal synaptic potato clones is controlled by a single recessive gene, *os*. Obviously the *os* gene may be presumed to cause postreductional division precocity which, if affecting all chromosomes, results in the formation of SDR 2n megaspores. However, in view of the present results from testcrossing of normal synaptic segregants (Table 3; Fig. 1), monogenic control of SDR 2n megaspore formation is questionable. Firstly, the large variation in seed set within single cross progenies suggests involvement of additional genetic factors that control the frequency and the level of postreductional division precocity and, thus, functional SDR 2n megaspore formation. Secondly, assuming homozygosity (*osos*) of the high seed set parents B and D (12.3 and 25.3 seeds/fruit, respectively), and heterozygosity (*Osos*) of the low seed set parents C and E (1.1 and 1.8 seeds/fruit, respectively), only 50% of the normal synaptic BE/EB and DE/ED progeny and 25% of the normal synaptic CE/EC progeny is expected to form some SDR 2n megaspores. Assuming single gene control, the observed large excess of normal synaptic SDR clones in the latter crosses (Table 3) would indicate semidominant expression of *os* rather than recessiveness. Regardless of the exact genetic basis of 2n megaspore formation, the seed set data demonstrate the possibility to select for increased rates of SDR and consistent FDR-2n megaspore formation in normal synaptic and desynaptic cross progeny, respectively.

FDR 2n megaspore formation in evolution and breeding

The high frequency of 2n megaspore-producing clones among the normal synaptic and the desynaptic progeny supports the hypothesis that sexual polyploidization is the driving force behind the origin and evolution of polyploid *Solanum* species (Den Nijs and Peloquin 1977). The evolutionary significance of SDR 2n megaspores has only recently been recognized (Stelly and Peloquin 1986a, b; Douches and Quiros 1988b). The present results suggest that FDR 2n megaspore formation, though admittedly less frequent, may also be more important than commonly thought.

Whereas diploid potato clones with consistently high levels of SDR 2n megaspore formation have previously been identified among a variety of *Solanum* species (Stelly and Peloquin 1986b; Werner and Peloquin 1987, 1991; Douches and Quiros 1988b), levels of FDR 2n megaspore formation reported so far are quite low (Iwanaga and Peloquin 1979; Werner and Peloquin 1991). Obviously, this may be attributed to the low number of synaptic mutants included in studies on 2n megaspore formation thus far. In the present study, systematic screening of desynaptic mutants revelaed FDR 2n megaspore formation in 45.2% of all *ds-1* mutants.

Although the majority of *ds-1* mutants on the average formed less than five seeds/fruit following testcrossing, 14% produced FDR 2n megaspores in frequencies that resulted in consistent seed set within the 5–25 seeds/fruit range and allowed for routine production of nearly exclusive tetraploid progeny from 2x(*ds-1*; FDR) × 4x, 2x(*ds-1*;FDR) × 2x(*Ds-1*;SDR/FDR), and 2x(*ds-1*;FDR) × 2x(*ds-1*;FDR) crosses. The present availability of diploid potato clones with consistent FDR 2n megaspore formation thus extends the opportunities for direct transfer of enhanced diploid germ plasm to tetraploids. In particular, it facilitates the application of 2x(*ds-1*;FDR) × 2x(*ds-1*;FDR) breeding schemes in cultivar development and in the production of relatively vigorous and uniform true potato seed (TPS) varieties, because FDR 2n gametes from *ds-1* mutants are very efficient in preserving the genetic constitution of the parental clone (Jongedijk et al. 1991).

It should be recognized that mutant synaptic genes also impose certain limitations on their use in breeding. They must be manipulated in a heterozygous condition, because they are generally expressed in both male and female meiosis and thus are either largely sterile or produce only functional FDR 2n gametes resulting in polyploidization upon crossing. As outlined by Hermsen et al. (1985), breeding schemes that consist of (i) introducing mutant synaptic genes and genes for FDR 2n gamete formation in advanced diploids through backcrossing, and (ii) subsequent selection of improved mutant synaptic segregants, with FDR 2n gamete formation following intercrossing of advanced heterozygotes, would be appropriate but laborious. Furthermore it should be realized that mutant synaptic conditions are actually required for FDR 2n megaspore formation. Since heterozygous diploid clones are normal synaptic and thus at best form SDR 2n megaspores, the question remains how to predict whether or not such clones carry genes that will cause substantial FDR 2n megaspore formation in derived synaptic mutants. If indeed SDR and FDR 2n megaspore formation are caused by common genes for division precocity, the occurrence of SDR in normal synaptic heterozygotes, particularly if associated with substantial precocious chromosome division as early as anaphase I, might be a helpful criterion.

As to the proposed breeding for diplosporous apomixis in potato by combining genes for asynapsis/desynapsis, FDR 2n megaspore formation, and pseudogamous seed development (Hermsen 1980; Hermsen et al. 1985; Jongedijk 1985), largely similar limitations are encountered. Nevertheless, some desynaptic clones formed substantial amounts of FDR 2n megagametophytes of diplosporic origin, and pseudogamous seed development from FDR (and SDR) 2n eggs could be induced using marked *S. phureja* pollinators (unpublished results). These observations strongly support the hypothesis that gametophytic apomixis comprises a number of distinct and genetically controlled elements (Petrov 1970; Asker 1980; Hermsen 1980; Matzk 1982). In addition, they demonstrate the feasibility of their combination to attain pseudogamous diplosporic apomixis, allowing approximately identical reproduction in largely sexual plant species. The application of this approach to produce uniform true potato seed varieties obviously requires breeding for increased levels of FDR 2n megaspore formation in synaptic mutants, and either introduction of genes for pseudogamy in these clones or the development of an efficient system for pseudogamous seed production using marked *S. phureja* pollinators.

Acknowledgements. The assistance of P. Wisman, M. Osiecka, M. van de Ven, S. Schuurmans Stekhoven, R. Hutten, C. van der Wolk, W. Beekman, and R. Nijboer in crossing and ploidy determination is highly appreciated. We thank Prof. E. Jacobsen (Department of Plant Breeding, Agricultural University Wageningen) and Dr. J.H. de Jong (Department of Genetics, Agricultural University Wageningen) for their critical comments on the manuscript.

References

Asker S (1980) Gametophytic apomixis: elements and genetic regulation. Hereditas 93:277–293

Bergman B (1944) A contribution to the knowledge of the embryo sac mother cell and its development in two apomicts. Svensk Bot Tidskr 38:249–259

Catcheside DG (1939) An asynaptic *Oenothera*. New Phytol 38:323–334

Clayberg CD (1958) Cytogenetic studies of two meiotic abnormalities in *Lycopersicon esculentum* Mill. PhD thesis, University of California, Berkeley.

Clayberg CD (1959) Cytogenetic studies of precocious meiotic centromere division in *Lycopersicon esculentum* Mill. Genetics 44:1335–1346

Den Nijs TPM, Peloquin SJ (1977) 2n gametes in potato species and their function in sexual polyploidization. Euphytica 26:585–600

Douches DS, Quiros CF (1988a) Genetic recombination in a diploid synaptic mutant and a *Solanum tuberosum* × *S. chacoense* diploid hybrid. Heredity 60:183–191

Douches DS, Quiros CF (1988b) Genetic strategies to determine the mode of 2n egg formation in diploid potatoes. Euphytica 38:247–260

Finch RA, Bennett MD (1979) Action of triploid inducer (*tri*) on meiosis in barley (*Hordeum vulgare* L.). Heredity 43:87–93

Frandsen NO (1968) Die Plastidenzahl als Merkmal bei der Kartoffel. Theor Appl Genet 38:153–167

Goldstein LSB (1980) Mechanisms of chromosome orientation revealed by two meiotic mutants in *Drosophila melanogaster*. Chromosoma 78:79–111

Golubovskaya IN (1979) Genetic control of meiosis. Int Rev Cytol 58:247–290

Golubovskaya IN, Mashnenkov AS (1975) Genetic control of meiosis. 1. A meiotic mutation in maize (*Zea mays* L.) causing the elimination of the first meiotic division. Genetika USSR 7:11–17

Gustafsson A (1935) Studies on the mechanism of parthenogenesis. Hereditas 21:1–111

Gustafsson A (1946) Apomixis in higher plants. I. The mechanisms of apomixis. Lunds Univ Arsskr 42:1–66

Harlan JR, Wet JHJ de (1975) On Ö. Winge and a prayer: The origins of polyploidy. Bot Rev 41:361–390

Hermsen JGTh (1980) Breeding for apomixis in potato: persuing a utopian scheme? Euphytica 29:595–607

Hermsen JGTh (1984a) The potential of meiotic polyploidization in breeding allogamous crops. Iowa State J Res 58:435–448

Hermsen JGTh (1984b) Mechanisms and genetic implications of 2n gamete formation. Iowa State J Res 58:421–434

Hermsen JGTh, Ramanna MS, Jongedijk E (1985) Apomictic approach to introduce uniformity and vigour into progenies from true potato seed (TPS). In: Page OT (ed) Present and future strategies for potato breeding and improvement. Rep 26th Plan Conf CIP, December 12–14, 1983, Lima, Peru, pp 99–114

Iwanaga M, Peloquin SJ (1979) Synaptic mutant affecting only megasporogenesis in potatoes. J Hered 70:385–389

Jacobsen E (1978) Die Chromosomen-Verdopplung in der Züchtung dihaploider Kartoffeln. PhD thesis, Rheinische Friedrich-Wilhelm-Universität, Bonn, 159 pp

Johnsson H (1944) Meiotic aberrations and sterility in *Alopecurus myosuroides* Huds. Hereditas 30:469–566

Jongedijk E (1985) The pattern of megasporogenesis and megagametogenesis in diploid *Solanum* species hybrids; its relevance to the origin of 2n eggs and the induction of apomixis. Euphytica 34:599–611

Jongedijk E (1987a) A rapid methyl salicylate clearing technique for routine phase-contrast observations on female meiosis in *Solanum*. J Microsc 146:157–162

Jongedijk E (1987b) A quick enzyme squash technique for detailed studies on female meiosis in *Solanum*. Stain Technol 62:135–141

Jongedijk E, Ramanna MS (1988) Synaptic mutants in potato, *Solanum tuberosum* L. I. Expression and identity of genes for desynapsis. Genome 30:664–670

Jongedijk E, Ramanna MS (1989) Synaptic mutants in potato, *Solanum tuberosum* L. II. Concurrent reduction of chiasma frequencies in male and female meiosis of *ds-1* (desynapsis) mutants. Genome 32:1054–1062

Jongedijk E, Hutten RCB, Wolk JMASA van der, Schuurmans Stekhoven SIJ (1991) Synaptic mutants in potato, *Solanum tuberosum* L. III. Effect of the *Ds-1/ds-1* locus (desynapsis) on genetic recombination in male and female meiosis. Genome 34:121–130

Kaul MLH, Murthy TGK (1985) Mutant genes affecting higher plant meiosis. Theor Appl Genet 70:449–466

Koduru PRK, Rao MK (1981) Cytogenetics of synaptic mutants in higher plants. Theor Appl Genet 59:197–214

Lamm R (1941) Varying cytological behaviour in reciprocal *Solanum* crosses. Hereditas 27:202–208

Lin HPP, Church K (1982) Meiosis in *Drosophila melanogaster*. III. The effect of orientation disruptor (*ord*) on gonial mitotic and the meiotic divisions in males. Genetics 102:751–770

Maan SS, Sasakuma T (1977) Fertility of amphihaploids in *Triticinae*. J Hered 68:87–94

Marks GE (1966) The enigma of triploid potatoes. Euphytica 15:285–290

Matzk F (1982) Vorstellungen über potentielle Wege zur Apomixis bei Getreide. Arch Zuechtungsforsch 12:183–195

Mendiburu AO, Peloquin SJ, Mok DWS (1974) Potato breeding with haploids and 2n gametes. In: Kasha K (ed) Haploids in higher plants. University of Guelph, Guelph, Ontario, pp 249–258

Meurman O (1928) Cytological studies in the genus *Ribes*. Hereditas 11:289–356

Mok DWS, Peloquin SJ (1975) Three mechanisms of 2n pollen formation in diploid potatoes. Can J Genet Cytol 17:217–225

Nogler GA (1984) Gametophytic apomixis. In: Johri BM (ed) Embryology of a angiosperms. Springer, Berlin Heidelberg, pp 475–518

Peloquin SJ (1982) Meiotic mutants in potato breeding. Stadler Genet Symp 14:1–11

Petrov DF (1970) Genetically regulated apomixis as a method of fixing heterosis and its significance in breeding. In: Khoklov SS (ed) Apomixis and breeding. Amerind, New Delhi New York, pp 18–28

Pfeiffer TW, Bingham ET (1983) Abnormal meiosis in alfalfa, *Medicago sativa*: cytology of 2n egg and 4n pollen formation. Can J Genet Cytol 25:107–112

Ramanna MS (1974) The origin of unreduced microspores due to aberrant cytokinesis in the meiocytes of potato and its genetic significance. Euphytica 23:20–30

Ramanna MS (1979) A re-examination of the mechanisms of 2n gamete formation in potato and its implications for breeding. Euphytica 28:537–561

Ramanna MS (1983) First division restitution gametes through fertile desynaptic mutants of potato. Euphytica 32:337–350

Rees-Leonard OL (1935) Macrosporogenesis and development of the macrogametophyte of *Solanum tuberosum*. Bot Gaz 96:734–750

Roades MM, Dempsey E (1966) Induction of chromosome doubling at meiosis by the elongate gene in maize. Genetics 54:505–522

Richardson MM (1935) Meiosis in *Crepis*. II. Failure of pairing in *Crepis capillaris* L. Wallr. J Genet 31:119–143

Rutishauser A (1967) Fortpflanzungsmodus und Meiose apomiktischer Blütenpflanzen. Protoplasmatologia, Band VI F3. Springer, Vienna New York, 245 pp

Satina S, Blakeslee AF (1935) Cytological effects of a gene in *Datura* which causes dyad formation in sporogenesis. Bot Gaz 96:521–523

Stelly D, Peloquin SJ (1985) Screening for 2n female gametophytes, female fertility, and 2x × 2x crossability in potatoes (*Solanum* spp.). Am Potato J 62:519–529

Stelly D, Peloquin SJ (1986a) Formation of 2n megagametophytes in diploid tuber-bearing *Solanums*. Am J Bot 73:1351–1363

Stelly D, Peloquin SJ (1986b) Diploid female gametophyte formation in 24-chromosome potatoes: genetic evidence for the prevalence of the second meiotic division restitution mode. Can J Genet Cytol 28:101–108

Stringham GR (1970) A cytogenetic analysis of three asynaptic mutants in *Brassica campestris* L. Can J Genet Cytol 12:743–749

Veilleux RE, McHale NA, Lauer FI (1982) 2n gametes in diploid *Solanum:* Frequency and types of spindle abnormalities. Can J Genet Cytol 24:301–314

Wagenaar EB (1961a) Cytological studies of the development of metaphase I in hybrids between *Triticum timopheevi* Zhuk. and *T. durum* Desf. Can J Bot 39:81–108

Wagenaar EB (1961b) Cytological studies of the development of metaphase I in *Triticum* hybrids. II. The behaviour of univalents in meiotic cell division. Can J Genet Cytol 3:204–225

Wagenaar EB (1968) Meiotic restitution and the origin of polyploidy. II. Prolonged duration of metaphase I as causal factor of restitution induction. Can J Genet Cytol 10:844–852

Werner JE, Peloquin SJ (1987) Frequency and mechanisms of 2n egg formation in haploid *tuberosum*-wild species F_1 hybrids. Am Potato J 64:641–654

Werner JE, Peloquin SJ (1991) Occurrence and mechanisms of 2n egg formation in 2x potato. Genome (in press)