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MECHANISMS AND GENETIC IMPLICATIONS OF 2n-GAMETE FORMATION

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ABSTRACT. Gametes with the unreduced number (= 2n) of chromosomes may originate from different abnormal events during meiosis or arise in apomeiotic somatic cells of the ovule (apospory). Meiotic nuclear restitution leading to 2n-gametes may be associated with fused or parallel second metaphase spindles (First Division Restitution, FDR) or with incomplete second meiotic division (Second Division Restitution, SDR). Its association with failing or reduced homologous pairing leads to 2n-gametes which are genetically equivalent to FDR. The basic differences between FDR and SDR are described and explained. Several mechanisms of 2n-gamete formation occurring in association with failing or reduced homologous pairing, as they occur in megaspore mother cells of apomictic species and in spore mother cells of synaptic mutants, interspecific hybrids, amphimonoploids, and anorthoploids, are described and discussed.

Different mechanisms of 2n-gamete production are compared as to their genetic implications. Data about genetic control of processes leading to 2n-gametes are presented along with a discussion on the effects of environmental factors on occurrence and frequency of 2n-gametes.

Index Descriptors: nuclear restitution, 2n-gametes, dyads, apomixis, synaptic mutants, premeiotic doubling, autobivalents, fused spindles, endomitosis, and apospory.

INTRODUCTION

Meiosis is an integrated system consisting of an orderly sequence of events. During the first or heterotypic division, homologous chromosomes pair and exchange genes, then disjoin to form two groups of n chromosomes (= reduction 2n → n). During the second or homotypic division, the chromosomes of each group divide equationally and the sister chromatids move to opposite poles, which results in four groups of n chromosomes. The divisions are completed by cytokinesis, the formation of a reductional cell wall and two equational cell walls. In the pollen mother cell, cytokinesis may be simultaneous or successive and the haploid microspores are arranged in a tetraeder. In the embryosac mother cell, cytokinesis is successive (reduction cell wall first), and the arrangement of the haploid megaspores as a rule is linear. The products of normal meiosis are reduced spores or n-spores, which develop n-gametes.

Gametes with the unreduced number of chromosomes (= 2n) may originate from different abnormal events during meiosis or arise in apomeiotic somatic cells of the ovule (apospory). Also pre- and post-meiotic doubling may give rise to 2n-gametes.

The first example of 2n-gametes was reported by Gates (1909) in an interspecific *Oenothera* hybrid. Rosenberg (1927) was the first to describe

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meiotic nuclear restitution leading to 2n-gametes. Meiotic nuclear restitution is associated either with an incomplete first meiotic division (= First Division Restitution or FDR) or with an incomplete second meiotic division (= Second Division Restitution or SDR). Its association with failing or reduced homologous pairing is genetically equivalent with FDR. Although in all cases dyads are formed consisting of two 2n-spores, FDR and SDR are basically different in cytology and in genetic consequences (Mendiburu and Peloquin, 1979; and Ramanna, 1979). This is obvious, because FDR 2n-gametes comprise the non-sister chromatids of each homologous pair of chromosomes whereas in SDR 2n-gametes the sister chromatids are included.

The following discussion on 2n-gamete formation will focus on abnormal events during meiosis in spore mother cells. As indicated already, the main distinction to be made is between FDR and SDR, where homologous pairing, gene recombination, and reduction during first meiotic division proceed in the normal way. Apart from FDR and SDR, a number of different processes of 2n-gamete formation have been reported (Ramanna, 1979) where homologous pairing is mainly restricted to first prophase stages of meiosis with predominantly univalents at diakinesis and metaphase I, or where homologous pairing is completely absent. These processes and their genetic implications will also be discussed.

FDR AND SDR

Cytogenetic investigations on FDR and SDR have been carried out by many researchers, mainly in potato (*Solanum tuberosum*) (Mendiburu, 1971; Ramanna, 1974, 1979; Mok and Peloquin, 1975a, 1975b; Mendiburu and Peloquin, 1977a, 1977b; Jacobsen, 1976, 1980; Iwanaga, 1980; and Veilleux et al., 1982), but also in alfalfa (*Medicago sativa*) (Vorsa and Bingham, 1979; and McCoy, 1982), in *Datura* (Satina and Blakeslee, 1935), and also in maize (*Zea mays*) (Rhoades and Dempsey, 1966; and Nel, 1975). The mechanisms underlying 2n-megasporogenesis and leading to 2n-eggs are much more difficult to analyze than those underlying 2n-microsporogenesis and leading to 2n-pollen.

Figures 1, 2, and 3 illustrate the processes FDR and SDR and their genetic implications in a diploid. Fig. 1 shows in a highly simplified way normal meiosis, FDR, and SDR with no crossing over. One pair of homologous chromosomes is drawn and given the numbers 1 and 2. The reduction cell wall (R) and the equational cell wall (E) are the horizontal and vertical lines, respectively. It can be seen, that without crossing over, normal meiosis leads to a tetrad with four spores, which are genetically equal two by two; that FDR produces a dyad consisting of two 2n-spores, which are genetically identical to the parent plant; and that SDR gives rise to a dyad with two completely homozygous, genetically different 2n-spores.

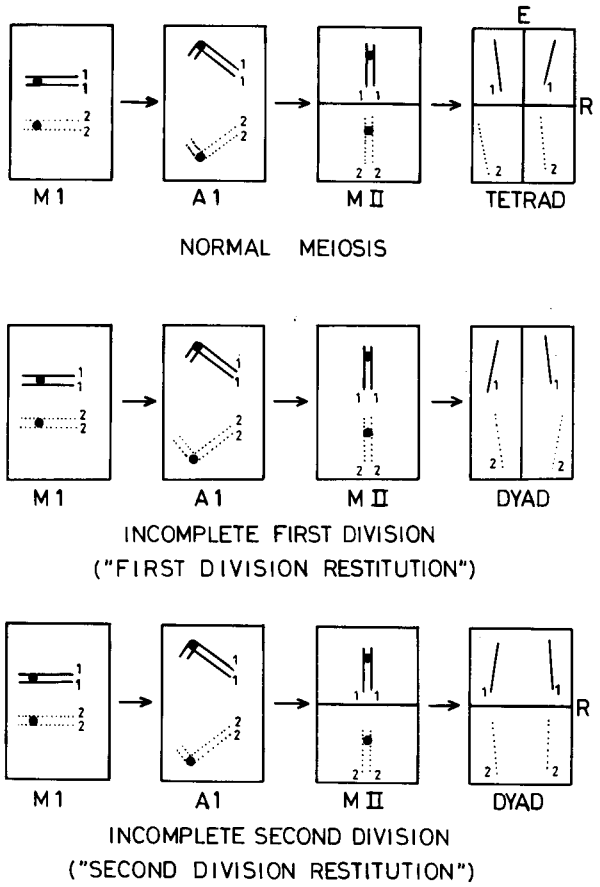


Figure 1. Schematic representation of normal meiosis. First Division Restitution and Second Division Restitution assuming one pair of homologous chromosomes and no crossing over.

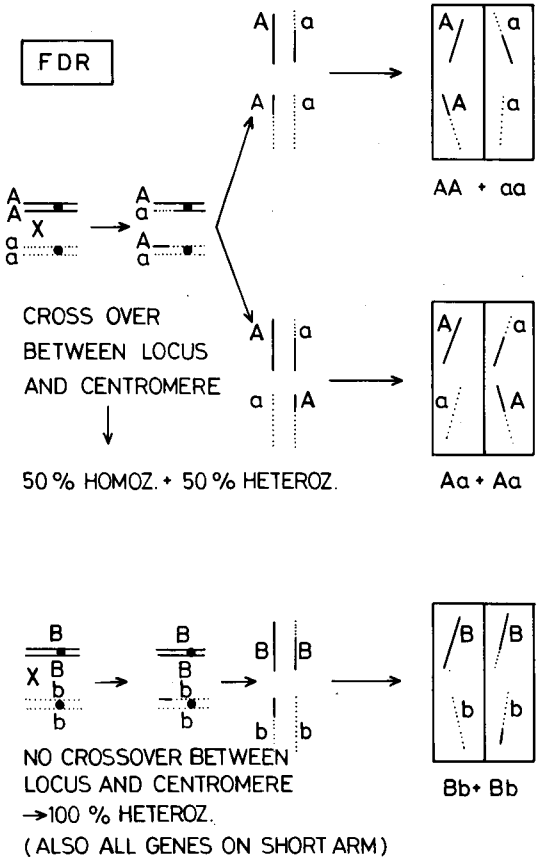


Figure 2. Genetic consequences of FDR assuming one crossover per pair of homologous chromosomes. *Aa* represents all heterozygous loci affected by the crossover, *Bb* those not affected by the crossover.

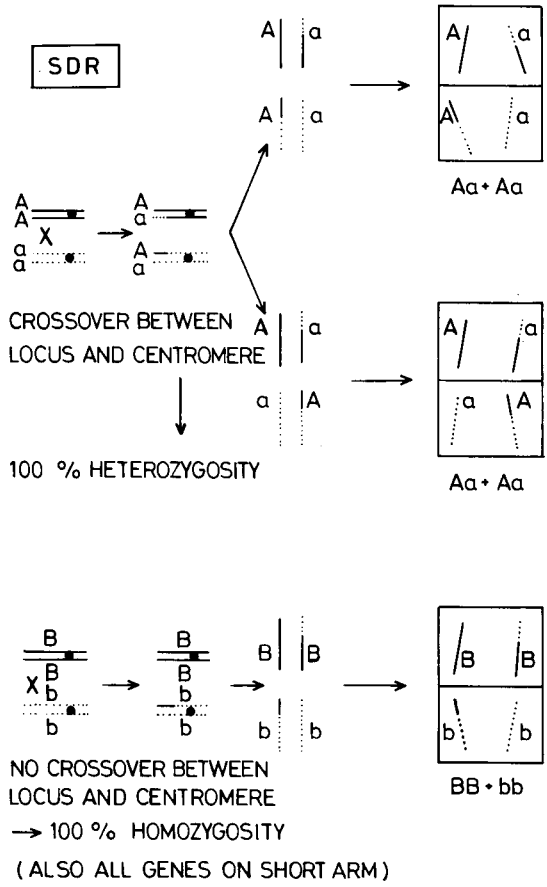


Figure 3. Genetic consequences of SDR assuming one crossover per pair of homologous chromosomes. *Aa* represents all heterozygous loci affected by the crossover, *Bb* those not affected by the crossover.

Obviously, crossing over and gene recombination normally occur during homologous pairing. The genetic implications are shown in Figs. 2 and 3, respectively. One crossover per pair of chromosomes is assumed, which is realistic in plants with small chromosomes pairing as rod bivalents. In both figures, Aa stands for all heterozygous loci distal to the chiasma and thus affected by it, whereas Bb stands for all heterozygous loci not affected by a crossover. The genetic result of FDR (Fig. 2) can be summarized as (i) among the heterozygous loci affected by the crossover (Aa-group), 50% are still heterozygous and 50% have become homozygous and (ii) the heterozygous loci not affected by the crossover (Bb-group) have retained 100% of the parental heterozygosity. The genetic result of SDR (Fig. 3) is (i) the complete Aa-group of heterozygous loci remains heterozygous and (ii) the complete Bb-group of heterozygous loci becomes homozygous.

Assuming a regular distribution of the parental heterozygous loci along the chromosomes, it can be calculated for each separate chromosome with known sites of centromere and crossovers, which percentage of the total heterozygosity is transferred to the progeny via FDR- and SDR-gametes, respectively (Table 1). For chromosomes of potato, the calculated retained heterozygosity on the basis of the above assumptions is about 80% in FDR-gametes and nearly 40% in SDR-gametes. The corresponding percentage in reduced gametes of a potato cultivar is maximally 67%.

FDR-gametes, in addition to all homozygous loci, transfer an average of about 80% of the parental heterozygosity to the progeny. Heterozygous parents produce heterozygous FDR-gametes, and these may confer many multiallelic loci and thus a high vigor on the autotetraploid progeny. FDR-gametes are genetically largely equal to the parent and therefore also among themselves. They thus contribute greatly to the uniformity of the progeny.

SDR-gametes, also those from a highly heterozygous parent, have a relatively high level of homozygosity. On an arbitrarily assumed 1:1 ratio of homo- and heterozygous loci in the parent, the SDR-gametes have 50% + 0.6 x 50% = 80% homozygosity and thus may increase the dosage of many genes. This may be important for improving desirable simply-inherited dominant characters. Furthermore, SDR-gametes from a heterozygous parent are highly heterogeneous and therefore contribute to the heterogeneity and thus to the potential for selection in the progeny.

The cytological processes leading to FDR and SDR were greatly simplified in Figs. 2 and 3. In fact, the origin of FDR is associated with the occurrence of fused (Ramanna 1979; and Veilleux et al., 1982) or parallel (Mok and Peloquin, 1975c; and Vorsa and Bingham, 1979) second metaphase spindles, which with normal meiosis are separated and at an angle of 60°. The origin of SDR is through endomitosis in interphase nuclei between first and second meiotic division and premature formation of the reduction cell wall. In this case, second metaphase, anaphase, and telophase are lacking (Mok and Peloquin, 1975c).

● = centromere

X = crossover

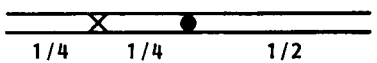
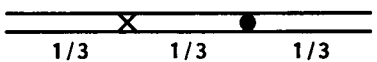
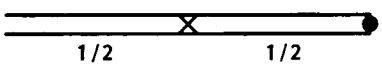
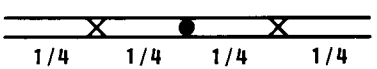
Type of chromosome	Percentage of parental hyterozygosity	
	in FDR-gametes	in SDR-gametes
 1/4 1/4 1/2	3/4 · 1 + 1/4 · 1/2 = 7/8	3/4 · 0 + 1/4 · 1 = 1/4
 1/3 1/3 1/3	2/3 · 1 + 1/3 · 1/2 = 5/6	2/3 · 0 + 1/3 · 1 = 1/3
 1/2 1/2	1/2 · 1 + 1/2 · 1/2 = 3/4	1/2 · 0 + 1/2 · 1 = 1/2
 1/4 1/4 1/4 1/4	2/4 · 1 + 2/4 · 1/2 = 3/4	2/4 · 0 + 2/4 · 1 = 1/2
Average	80.2%	39.6%

Table 1. Percentage of the parental heterozygosity present in FDR- and SDR-gametes for four types of chromosome.

FORMATION OF 2n-GAMETES FOLLOWING RESTRICTED OR LACKING HOMOLOGOUS PAIRING

Restricted or lacking homologous pairing is generally observed in megaspore mother cells of apomictic species but also in spore mother cells of synaptic mutants, anorthoploids (monoploids, triploids, etc.), and in F_1's from wide crosses. The following five mechanisms have been reported:

1. *Premeiotic doubling with autobivalent formation.* Some apomictic species of the genus *Allium* may undergo endomitotic chromosome doubling during early prophase in spore mother cells. Then the sister chromatids of each chromosome stick firmly together as pairs ("autobivalents") forming chiasmata. No pairing of homologous chromosomes occurs and consequently, the resulting 2n-spores are genetically identical to the parent (Hakansson and Levan, 1957; and Gohil and Kaul, 1981). A low frequency of premeiotic doubling among spore mother cells has been reported in some potato species (Lam, 1974; and Iwanaga, 1980), but further details on possible 2n-spores or autobivalents have not been presented by these authors.

2. *Mitotized meiosis.* Mitotized meiosis occurs when the division of the chromosomes in an embryosac mother cell is similar to a mitotic division resulting in a dyad with two 2n-megaspores. This mechanism has been reported in apomictic species of *Poa* (Müntzing, 1940) and *Antennaria* (Stebbins, 1932).

3. *Semi-heterotypic division.* Semi-heterotypic division is involved when homologous chromosomes in an embryosac mother cell hardly pair or do not pair at all. The univalents at metaphase I are distributed irregularly along the spindle figure. A new nuclear wall is formed around the entire spindle figure, resulting in a large restitution nucleus. The chromosomes of this nucleus undergo the second meiotic division, which gives rise to a dyad consisting of two 2n-megaspores. This mechanism was first described by Rosenberg (1927) in *Hieraceum* and later by Okabe (1932) in *Ixeris*. Also, semi-heterotypic division invariably leads to 2n-gametes which to a great extent are genetically identical to the parent.

4. *Pseudohomotypic division.* With pseudohomotypic division, the homologous chromosomes in an embryosac mother cell display hardly any pairing. During metaphase I, univalents predominate and divide equationally. The resulting megaspores thus are 2n-spores genetically equivalent to FDR. This mechanism has been observed in the genera *Taraxacum, Erigeron,* and *Archieraceum* (Gustafson, 1935). Pseudohomotypic division has also been reported in pollen mother cells of some *Solanum* species (Lamm, 1941; and Hermsen and Ramanna, 1981). The 2n-spores, though genetically equivalent to FDR, are not identical with the parental genotype, because homologous pairing is normally found at first prophase stages of meiosis (Ramanna, 1983).

5. *Synaptic mutants, interspecific hybrids, and (amphi)monoploids associated with nuclear restitution.* Homologous pairing may be reduced or

even absent in synaptic mutants (mostly monogenic recessives), in F_1 hybrids between remotely related species, in haploids from allopolyploid species, and in anorthoploids. If, in such plants, lack of homologous pairing is associated with some of the aforementioned restitution mechanisms, the resulting 2n-spores will become functional FDR-gametes. The degree of genetic identity between such gametes and their parent is dependent on the degree of homologous pairing and chiasma formation, if any. On the other hand, if in such plants a restitution mechanism is missing, the reduced gametes will predominantly be aneuploid and sterile.

In asynaptic mutants, homologous pairing and hence gene recombination does not occur at all. In desynaptic mutants, homologous pairing occurs at pachytene, which may result in a limited amount of gene recombination (Sybenga, 1968). Such so-called synaptic mutants are regularly found in many species and may be largely sterile. The terms asynapsis and desynapsis are also used for failure of homologous pairing owing to lack of homology between the chromosomes in intergeneric or interspecific hybrids and in monoploids. Failure of pairing may provide a major stimulus for the formation of restitution nuclei and consequently for the output of well-balanced 2n-gametes. Therefore, it is not surprising that there are numerous reports on nuclear restitution occurring in such plants (Jahr, et al., 1963; Skiebe, 1966; and Harlan and de Wet, 1975). The frequency of 2n- or restitution gametes in such plants is usually low but sufficient to prevent the plants from becoming extinct.

A clear example is the classical hybrid made by Karpechenko (1927) between diploid *Raphanus sativus* (2n = 18) and diploid *Brassica oleracea* (2n = 18). The F_1 produced no seed in the first year. In the second year, 19 out of 90 F_1 plants displayed some fertility. Open pollination of these F_1 plants yielded 821 seeds. Chromosomes were counted in 301 progeny plants, of which 218 were tetraploid and 19 higher than tetraploid. This shows that predominantly restitution male and female gametes were functional.

Wagenaar (1968), Maan et al. (1980), and Islam and Shepherd (1980), from their experimental results with intergeneric hybrids within the *Triticinae*, concluded that restitution gametes almost exclusively arise from spore mother cells with the highest degree of desynapsis. Wagenaar (1961) found that the duration of metaphase I is positively correlated with the degree of desynapsis. So in 1968, he suggested that in cells with the most prolonged metaphase I, the first-division activities are interrupted and effectively terminated such that reduction cannot occur anymore, which then leads to FDR-restitution gametes including all 2n chromosomes.

DIFFERENT MECHANISMS OF 2n-GAMETE FORMATION COMPARED

The merits of FDR and SDR have been discussed at length. All other processes explained in the previous section are genetically equivalent to FDR.

They may differ from FDR in the percentage of parental heterozygosity that is transferred to the progeny. In potato, FDR-gametes transfer about 80% to the progeny together with a reasonably high uniformity, whereas the situation may differ when other mechanisms are involved.

Premeiotic doubling + autobivalent formation and those cases where there is no gene recombination at all (non-reduction, complete asynapsis) lead to 2n-gametes, of which the genotype is completely identical to that of the parent. Therefore, such gametes transfer 100% of the parental genotype intact to the progeny. Because they all have one and the same genotype, they confer even more uniformity on the progeny as FDR-gametes do.

All other mechanisms, including pseudohomotypic division, semi-heterotypic division, desynapsis associated with fused spindles, or other kinds of restitution, take an intermediate position. It should be pointed out that in desynaptic mutants, homologous pairing was observed at pachytene (Ramanna, 1983). Chiasmata need not necessarily be formed, but they may be. So gene recombination is not precluded. Therefore, the assumption by Okwuagwu and Peloquin (1981) and Peloquin (1982) of a 100% intact transfer of the parental genotype to the progeny is controversial, although it is hard to establish as to what degree the assumption of these authors is inaccurate.

The mechanisms described are also different in respect to the frequency of 2n-gametes among the total of functional gametes produced by a plant. In all those plants where homologous pairing is hampered or prevented due to synaptic genes, lack of homology, unbalanced ploidy, or some other cause, 2n-gametes as a rule are the only functional gametes. The n-gametes, if formed, are predominantly sterile. However, with FDR and SDR, both functional 2n-gametes as well as functional n-gametes may be produced. In these cases, the proportion of 2n-gametes, which is important for breeding, is greatly affected by genetic and environment factors.

GENETIC AND ENVIRONMENTAL CONTROL OF 2n-GAMETE FORMATION

All meiotic processes are regulated by genes but are also sensitive to environmental factors. The origin of 2n-gametes is due to minor or major deviations of normal meiosis. These may be caused by different mutants affecting specific stages of the meiotic system. Several recessive mutant genes are known. In *Datura*, a recessive gene *dy* affects both micro- and megasporogenesis in a way that nearly all spores formed are 2n-SDR-spores (Satina and Blakeslee, 1935). The elongate gene in maize apart from despiralization of the chromosomes at both meitoic anaphases also causes the production of 2n-SDR-eggs in varying proportions among the functional eggs. Microsporogenesis is not affected (Rhoades and Dempsey, 1966). Nel (1975) found genetic evidence for both SDR- and FDR-eggs due to the elongate gene. Golubovskaya and Mashnenkov (1975), also for maize (*Zea mays*), described a recessive mutant