

## Cytomixis in *Lolium perenne*

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*Abstract.* Cytomixis is a spontaneous process occurring through the formation of cytoplasmic bridges between adjacent pollen mother cells. This phenomenon was observed in the pollen mother cells of 3 genotypes of *Lolium perenne* which had been subjected to directional selection for productivity of green material. — The process has led to the formation of up to 34.8% of PMCs with chromosome numbers deviating from the normal diploid number  $2n = 14$ . The abnormal PMCs contained chromosome numbers ranging from 2–56 as observed at first metaphase. — Abnormal PMCs were also observed with approximately the same frequency in Meiosis II. This phenomenon is under genetic control. The evolutionary significance of cytomixis and the cytological consequences are discussed.

### Introduction

Since Gates (1911) introduced the term “cytomixis” to describe the mechanism of chromatin passage from one pollen mother cell (PMC) to an adjacent PMC, reports have been published of this phenomenon in many plant species (see Kamra, 1960). Cytomixis was recorded in grasses by Church (1929), who stated that “the occurrence of this abnormality in connection with hybrids is very striking. In contrast, the examination of considerable material in the case of *Phalaris arundinacea*, a normal diploid, has revealed no cytomixis”.

Kamra (1960) in reviewing the occurrence of cytomixis reported that it could be found in normal species, hybrids and apomicts. More recently, however, Salesses (1970) established its occurrence in both natural and artificial triploid hybrids of *Prunus spinosa* ( $2x = 32$ )  $\times$  *P. cerasifera* ( $2n = 16$ ), but not in diploid, tetraploid or hexaploid species of *Prunus*. Similarly, Semyarkhina and Kuptsou (1974) found that cytomixis occurred more frequently in the anthers of triploid and autotetraploid than in diploid sugar beet. It can be clearly seen that cytomixis occurs in a whole range of plants although it is difficult to establish precise conditions under which the phenomenon occurs. The evolutionary significance of the process is similarly open to question (see Brown and Bertke, 1969). The present report records the occurrence of cytomixis in the diploid species *Lolium perenne*.

### Materials and Methods

Out of 403 plants examined from within a series of lines of *Lolium perenne*, which had been subjected to directional selection for productivity of green material (Vivero, 1975), three were found which showed cytomixis. Genotype number B424-6 derived from an unselected control population which had been maintained by the random mating of forty genotypes collected in the wild and was the same as population 6 used by Hayward and Breese (1966). Genotypes B512-67/1 and B512-761/3 came from the first and third selection cycles, respectively, of two lines, the former (67/1) being a line from population 7 and the latter (761/3) originated from an interpopulation hybrid (pop. 1  $\times$  pop. 7) of Hayward and Breese (1966).

Panicles were fixed in Carnoy's solution (6:3:1), and the anthers stained in alcoholic hydrochloric acid carmine (Snow, 1963) followed by aceto-orcein. Chromosome pairing and chiasma frequency were recorded in 90 to 135 PMCs.

### Results

The spontaneous migration of chromatin from one pollen mother cell (PMC) to another was observed in each of the three genotypes. Bridges joining two adjacent cells, sometimes three, were clearly visible, with bivalents moving from one cell into another (Figs. 1 and 2). As a result of this passage of bivalents PMCs occurred within each plant with chromosome numbers deviating from the basic diploid complement. The frequency distribution of the PMCs with different chromosome numbers in the three plants is presented in Table 1.

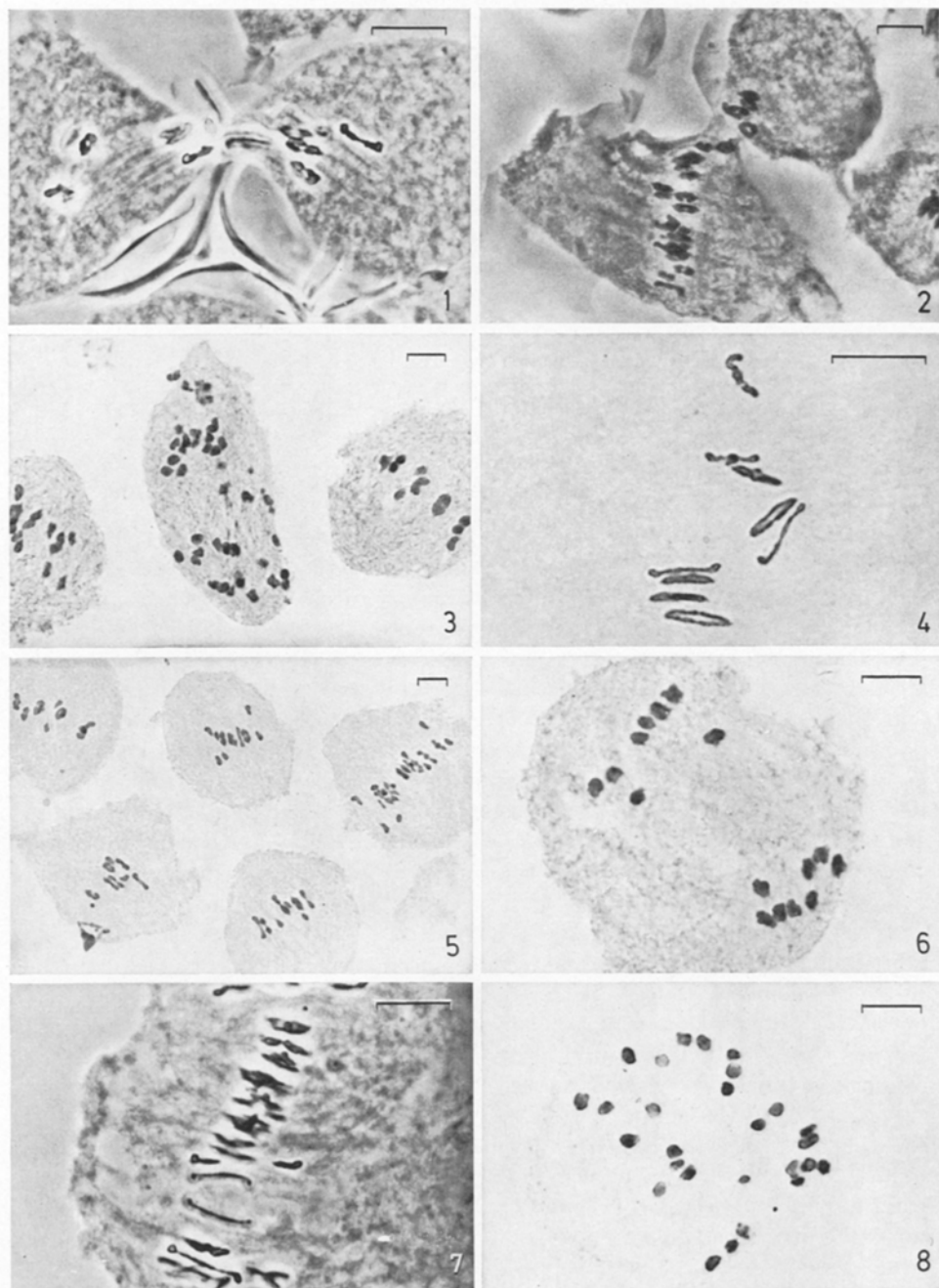
In genotype B424-6, a plant derived directly from a wild population, 24.4% of the PMCs were abnormal with chromosome numbers ranging from 2 to 26 per cell (Fig. 4). The remaining two plants, B512-67/1 and B512-761/3 showed a higher frequency of aberrant cells, — 33.3% and 34.8% respectively. In both of these plants the majority of the deviant cells were tetraploid with 28 chromosomes, together with a few hexaploid cells (Figs. 5 and 7) but in B512-761/3 cells with up to 58 chromosomes were observed (see Table 1).

In all aneuploid cells, the chromosomes paired as bivalents and orientation on the metaphase plate was regular. Anaphase 1 analysis revealed that some cells contained variable separations such as 13:13, 11:15 and 8:8 (see Figs. 3, 6 and 8) in addition to 7:7, 14:14 and 21:21 separations. At the second meiotic division, cells with abnormal chromosome numbers were observed with approximately the same frequency as found in meiosis I. The occurrence of cells with more than 28 chromosomes would indicate that in some instances more than two cells had been involved in the migration of chromatin. Associated with the differing ploidy levels were marked differences in size of PMCs suggesting that the process of transfer involved cytoplasmic as well as chromatin material.

### Discussion

Several explanations for the process of cytomixis have been put forward. Maréchal (1963) proposed that nuclear fragments and entire nuclei pass from one cell to another via plasmodesmata, whilst Heslop-Harrison (1964) suggested the existence of "communicating channels". With regard to the former explanation it is difficult to envisage how nuclear material could pass through such fine pores and, furthermore, implies the random nature of the phenomenon. The present observations indicate that "communicating channels" (Figs. 1 and 2) are formed which allow the complete or partial migration of cell contents from one or more PMCs to another. That this phenomenon was found in only three plants, which

Figs. 1—8. The bars represent 10  $\mu$ m. Fig. 1. Cytomixis in Metaphase I — Note the cytoplasmic bridge between two PMCs and the chromatin migration from one PMC to the other. Fig. 2. Cytoplasmic bridge between two PMCs. Note the high number of bivalents in one cell which indicate that more than two cells were involved in the process. Fig. 3. Two normal cells with 7 bivalents at Metaphase I and one cell at Anaphase I with 21:21 separation. Note the



size difference between the two cell types. Fig. 4. Cell with 9 bivalents as a result of incomplete transfer of cell contents. Fig. 5. Normal cells with 7 bivalents, one cell with 14 bivalents and a cell with chromatin passing into it. Fig. 6. Abnormal Anaphase I with 8:8 separation. Fig. 7. Abnormal cell with 21 bivalents. Fig. 8. Abnormal Anaphase I with 26 chromosomes 13:13 separation

Table 1. Diploid PMCs with abnormal numbers of chromosomes

Number of chromosomes	No. of PMCs of each genotype		
	B <sub>424-6</sub>	B <sub>512-67/1</sub>	B <sub>512-761/3</sub>
2-12	6	0	0
14 normal	68	82	88
16-26	16	8	6
28	0	29	24
30-40	0	0	3
42	0	4	8
44-54	0	0	3
56	0	0	2
58 and over	0	0	1
Total	90	123	135
Percentage of abnormal cells	24.4%	33.3%	34.8%

formed part of an extensive cytological study, together with the fact that two of the genotypes (67/1 and 761/3) had a common parent, would suggest that this mechanism is subject to genetic control as proposed by Brown and Bertke (1969). The absence of any multivalent configurations in the hyperploidy cells suggests that cytomixis in this instance occurs after the initiation of chromosome pairing since it would be reasonable to expect multivalents to be formed if the aberrant chromosome numbers had arisen before the beginning of synapsis. This is also supported by the fact that there was an even number of chromosomes in the deviant cells indicating that chromosomes had migrated in pairs. This would also indicate that these communicating channels probably occur after the early phases of meiosis and could be associated with possible changes in the structure of the cell wall at this time.

The significance of cytomixis as a force in evolution is not clear. Sarvella (1958) suggested cytomixis as a possible mechanism which could explain the origin of aneuploid plants. Maréchal (1963) claimed that although the result of cytomixis is the production of a certain amount of pollen with a chromosome number less than the haploid number the viability and competitive ability of such aneuploid gametes would probably be reduced and therefore unlikely to be of any significance. On the contrary, Salesses (1970) mentioned some possible genetic consequences of cytomixis. He speculated that the production of aneuploid PMCs and unreduced gametes could be an important factor leading to spontaneous variation in *Prunus*. For example the coincidence of unreduced gametes and total cytomixis could give rise to hexaploid plums by direct hybridization between triploid hybrids.

In the present case it is arguable whether unreduced and aneuploid gametes would be able to compete effectively with euploid pollen and participate freely in fertilization. However, if the same phenomenon occurs in the female side as suggested by Sarvella (1958) the absence of competition would mean that these aneuploid gametes would function and produce aneuploid genotypes. The signifi-

cance of such genotypes would be less in a crop which reproduces by sexual reproduction than in species which are naturally maintained by vegetative propagation or apomixis. Therefore the evolutionary significance of this phenomenon would probably be more meaningful in genera in which a polyploid series exists as being one of the methods for producing unreduced gametes and polyploid forms in nature. However, considering the total absence of polyploid species in *Lolium* such a role has been of little consequence in speciation in this genus.

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