

Cytomixis—a unique phenomenon in animal and plant

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Abstract Cytomixis is reported to be a uniform phenomenon in the context of fertilization during spermatogenesis of animals and in some lower groups of plants where oogamous reproduction prevails. However, the phenomenon is versatile in flowering taxa as it lacks uniformity in occurrences, causes, formation of intercellular bridges, involvement of number of cells in a cluster, evolutionary significance among others. A review on cytomixis is conducted with an objective that it may offer a scope to unravel some of the ambiguities associated with it and provide further information on cell, reproductive, structural and evolutionary biology.

Keywords Spermatogenesis · Flowering plants · Chromatin migration · Intercellular bridges · Syncytium · Ambiguity

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Abbreviations

AI	Anaphase I
CCs	Cytoplasmic channels
DMYPT	<i>Drosophila melanogaster</i> myosin binding subunit of myosin phosphatase
EM	Electron microscope
IBs	Intercellular bridges
MI	Metaphase I
PMCs	Pollen mother cells
RBM44	RNA binding motif protein 44
TEX14	Testis-expressed gene 14

Introduction

Körnicker (1901) first studied cytomixis in microsporogenesis of *Crocus sativus* and Gates (1911) defined the phenomenon in *Oenothera gigas* as the process of extrusion of chromatin from the nucleus of one PMC into the cytoplasm of the adjacent mother cell. Omara (1976) considered cytomixis to be a spontaneous process occurring through the formation of cytoplasmic bridges between adjacent PMCs. Cell-to-cell contact in cytomixis achieved through cellular bridging provided direct routes for transfer of signals and components, thereby allowing evolution of complex forms in multicellular organisms (Zani and Edelman 2010; Haglund et al. 2011).

Haglund et al. (2011) suggested that the role of incomplete cytokinesis, in certain tissues and developmental stages, give rise to cells interconnected in syncytia by stable intercellular bridges and it occurs in both female and male germ lines in species ranging from insects to humans as well as in some somatic tissues in invertebrates. Plasmodesmata in plants and the septal pore in fungi also represent intercellular bridges formed by incomplete cytokinesis (Robinson and Cooley 1996; Lucas and Wolf 1993).

Considering different views in plant and animal systems, the term cytomixis may be defined as mixing of cytoplasm in somatic and reproductive cells through cellular bridging, thereby forming a syncytia involving two to many cells. Sharing of cytoplasmic constituents during cytomixis seems to be predominant in male reproductive cell lines. Intercellular bridge formation is a key event during the process, irrespective of the cause of its occurrence. Intercellular bridges (IBs; Dym and Fawcett 1971; Hime et al. 1996; Haglund et al. 2011), cytoplasmic channels (CCs; Whelan 1974) and wide open plasmodesmal canal (Paolillo and Cukierski 1976) are terminologies referred for interconnection between cells in animals, higher plants and in lower groups of plants, respectively. In *Drosophila melanogaster*, IBs are reported to be as ring canals (Haglund et al. 2011). The present review is aimed at discussing the potential significance of cytomixis in animals, lower groups of plants and more specifically in male germ cells in angiosperms for its effective exploration.

Animals

Cytomixis involving intercellular transfer of the organelles and other cytoplasmic constituents seems to be a general phenomenon during spermatogenesis of animals (Roosen-Runge 1977; Carlson and Handel 1988; Ventela et al. 2003; Guo and Zheng 2004). In oogamous reproduction of multicellular organisms, the male germ cells were reported to be interconnected within a common syncytium by wide IBs (0.1–2.0 μm) to achieve best gamete quality and synchrony in the process for the exchange of a few molecular signals as well as mixing of protoplasm between connected cells of similar levels/states of mRNA, proteins and organelles (Guo and Zheng 2004). The formation of large and/or rapidly developing syncytia with more connected cells and the IB sizes were suggested to possess intensified cytotoxic activity during the process of spermatogenesis of animals (Weber and Russell 1987). In animals, IBs ('ring canals') appear in the first mitotic division of spermatogonia and persist through several rounds of premeiotic mitosis and the two subsequent meiotic divisions until the later stages of spermiogenesis (Roosen-Runge 1977).

Giorgi (1978) detected IBs in ovarian follicle cells of *Drosophila*, which played a key role in synchronous differentiation of the linked follicle relating to the behavior of these cells in the secretion of the egg covering precursors. Robinson (1996) observed assembly of IBs called ring canals in the *Drosophila* germline and IBs were reported to play role in intercellular transport of cytoplasm. Weber and Russell (1987) suggested that IBs are not static structures but show size variations and considerable structure related diversity during spermatogenesis. Gondos (1973) reported IBs between developing germ cells in human fetal ovaries at 10 to 20 weeks' gestation. Bridges were frequently found in early stages of degeneration but less

frequent and distorted as well as disrupted during advanced stages of cellular degeneration. Chang et al. (2010) were of opinion that during mouse testis maturation centrosomal protein 55 (Cep 55) together with pericentrin (centrosomal protein) were localized to the IBs interconnecting spermatogenic cells in a syncytium. The centrosomal proteins are therefore significant for the stable bridge between germ cells during spermatogenesis and spermiogenesis. Greenbaum et al. (2011) reported that IBs in mammalian germ cells are composed of general cytokinesis components with additional germ cell-specific factors TEX14. TEX14 was reported to be an inactive kinase essential for the maintenance of stable IBs in gametes of both sexes but whose loss specifically impairs male meiosis. TEX14 associated with protein RBM44 was suggested to be associated with RNA transport.

Ong et al. (2010) suggested that physical resistance and inhibition of myosin II activity via DMYP (Drosophila melanogaster myosin binding subunit of myosin phosphatase) might be responsible in *Drosophila* to prevent the contractile rings (formed due to incomplete cytokinesis during oogenesis) from being completely closed. Therefore, the process of cytokinesis is rather significant for fertilization events. Haglund et al. (2011) also emphasized the role of incomplete cytokinesis during development for the formation of stable IBs, which give rise to cells interconnected in syncytia. Incomplete cytokinesis and stable IBs were reported to be the key events in male germ lines for fertility in insects and mammals (Robinson and Cooley 1997; Greenbaum et al. 2006) and also played significant role in synchronizing mitotic cell divisions and entry into meiosis (Fawcett et al. 1959; Guo and Zheng 2004; Caldwell and Handel 1991; Braun et al. 1989). Female germ line IBs were reported to be required for fertility in *Drosophila* but not in mice (Robinson and Cooley 1996; Greenbaum et al. 2009).

Lower plant groups

The haploid male gametes in lower groups of plants showing IBs were found to survive the whole process of spermatogenesis from the early mitosis to the very late stage of sperm differentiation. Cytomixis is reported in algae (Deason et al. 1969; Kwiatkowska and Maszewski 1976; Kwiatkowska et al. 2003), fungi (Dong and Junying 1988), bryophytes (Paolillo and Cukierski 1976; Renzaglia and Garbary 2001), and fern species (Carr 1976; Renzaglia and Garbary 2001; Dong et al. 2004; Heng-Chang et al. 2007) among others. Hoops et al. (2000) reported cytoplasmic bridges in *Volvox* spp. from embryonic cells, which played a central role in inversion by providing the physical framework against which the cells exerted force to reverse the curvature of the cellular sheet. The cytoplasmic bridges disappeared soon after inversion in smaller volvocaceans but in other lineages of *Volvox*

the bridges were retained throughout the life of the adult providing source of nutrient transfer. In *Funaria hygrometrica* synchronously dividing spermatogenous cells were found to be connected with each other by numerous cytoplasmic bridges and at the late stage of the antheridial development when cells began to degrade all the spermatids within an antheridium seemed to be connected with each other by the expanded cytoplasmic bridges (Dong et al. 2004). The IBs formed between microspore mother cells of *Isoetes sinensis* were narrow, stretched, and extending from prophase I to telophase II, and it has also been reported that cytomixis and irregular chromosomal phenomenon might not be a natural process (Heng-Chang et al. 2007). Paolillo and Cukierski (1976) correlated protoplasmic changes with the changing nature of the walls and associated spaces around each spermatogenous cell during spermatogenesis in the moss *Polytrichum*, thereby reflecting a significance for dispersal of the sperms at maturity. Cytomixis noted in shoot apex of *Picea abies* was due to variations in the osmotic pressure in cell wall or more probably due to disturbances in mitotic division (Guzicka and Wozny 2005).

Angiosperms

In flowering plants, electronic microscopic observation revealed that large anther species like lily (*Lilium* spp.) developed wide (0.5–2.0 μm) IBs with strong cytotoxic activity compared to species like *Arabidopsis* possessing small anthers and narrow (0.1–0.15 μm) IBs (Owen and Makaroff 1995). This observation led to the contention that all flowering plants during microsporogenesis might form IBs and possess cytotoxic activity to an extent depending on the size of the anthers. Though the phenomenon is reported predominantly during microsporogenesis in countless of flowering plants it is also studied in root meristems (Sarvella 1958; Tarkowska 1960; Bobak and Herich 1978), leaves and epidermal scales (Tarkowska 1960), tapetal cells (Cooper 1952; Sapre and Deshpande 1987) and ovary cells (Koul 1990), but from available reports it seems that it is not a universal phenomenon among the flowering taxa. Therefore, the assumption of anther size playing significant role in cytomixis seems to be rather ambiguous. Considering the versatility of the phenomenon, cytomixis is described in flowering taxa emphasizing some of the key attributes like occurrences, possible causes, formation of cytotoxic channels, number of cells involved in a cluster, environmental influence and evolutionary significance with an objective to highlight some of the variability associated to it for its further exploration.

Occurrences

Cytomixis is encountered in natural population of flowering plants (Kravets 2012) as well as induced by mutagen treatments

(Datta and Biswas 1984; Sharma and Kumar 2004; Rai et al. 2010; Srivastava and Kumar 2011). More often the phenomenon is observed in plant species possessing genetically disturbed background like haploids, triploids, polyploids, nullisomics and apomictics (Kihara and Lilienfield 1934; de Nettancourt and Grant 1964; Gottschalk 1970; Salesses 1970; De and Sharma 1983; Gayen and Sarkar 1996; Peng et al. 2003), hybrids (Morikawa and Leggett 1996; Maity and Datta 2008; Li et al. 2009; Souza et al. 2010) and in few secondary polyploids (Datta et al. 2005; Iqbal and Datta 2007; Mandal and Datta 2011, 2012). Cytomixis is reported to be more common in diploid (Pagliarini and Pereira 1992; Malallah and Attia 2003; Kumar and Singhal 2008) than polyploid (Basavaiah and Murthy 1987; Sheidai and Attia 2005) species.

Possible causes

The possible causes for cytomixis are reported to be variable. Faulty fixation (Sinoto 1922; Woodworth 1931; Gelin 1934; Jacob 1941; Maheshwari 1950; Takats 1959), chemical and herbicide effects (Haroun 1995; Ajay and Sarbhoy 1987), effect of chemical mutagens (Kumar and Sharma 2002; Bhat et al. 2006), changes in biochemical processes (Koul 1990), consequence of physiological control (Bell 1964; Bhal and Tyagi 1988) mechanical injury (Takats 1959; Tarkowska 1965, 1966; Morisset 1978), pathological consequences (Weiling 1965; Bobak and Herich 1978; Morisset 1978), nutritional deficiency (Miljajev 1967; Bobak and Herich 1978), environmental stresses and pollution (Malallah and Attia 2003; Haroun et al. 2004; Kumar and Tripathi 2008) and temperature effects (Basavaiah and Murthy 1987) were suggested to be the possible causes for cytomixis. Morikawa and Leggett (1996) performed cytological and morphological studies of wild population of *Avena canariensis* and were of opinion that cytomixis is an isolating mechanism.

Cytomixis as natural phenomenon

Apart from the above-mentioned causes of cytomixis, recent evidences suggest that it is a natural phenomenon under genetic control influenced by physiological and environmental factor(s) (Soodan and Wafai 1987; Zheng et al. 1987; Bedi 1990; Bellucci et al. 2003; Boldrini et al. 2006; Ghaffari 2006; Lattoo et al. 2006; Himshikha et al. 2010; Mandal and Datta 2011; Kumar et al. 2012; Kaur and Singhal 2012). Weiling (1965) in tomato reported that cytomixis under certain conditions was a normal cellular process but on the intensity of effective stimuli it might often be a pathological process, however the boundary between differentiations was not clear. Soodan and Wafai (1987) presumed the involvement of specific genes for cytomixis, which expressed only under particular environmental conditions. Song and Li (2009) reported cytomixis in *Salvia miltiorrhiza* where freshly collected anthers

were squashed and stained to avoid any fixation effects and the occurrence of the phenomenon was considered to be a natural cytological event under direct genetic control and physiological factors. Datta et al. (2005) reported that cytomixis was persistently present in *Ocimum basilicum* and *Withania somnifera*, and the phenomenon was unaltered under mutational background, thereby indicating that cytomixis is a natural process in the studied species. Yun-sheng and Yong-ping (2006) studied cytomixis on PMCs of “Arbo” wheat nullisomic lines and thought that the phenomenon to be controlled by poly-gene or key-gene which exists in all of the allopolyploid wheat’s three genomes. Iqbal and Datta (2007) reported the preponderance of cytomixis in wild germplasm of *W. somnifera*, but Das et al. (2009) found incipient cytomictic behavior of chromosomes in two recommended varieties (Poshita and Jawahar 22) of the species. This observation indicated that under selection, possibly cytomictic potentiality decreased. A similar result was also studied in *Corchorus* spp. when assessed over the generations (Mandal and Datta 2011). Mandal and Datta (2012) suggested that cytomixis noted in *C. fascicularis* is possibly genetically controlled and to an extent influenced by environmental factor(s) and the genes involved in the process may be differentially expressed or repressed under given set of condition(s).

Formation of intercellular bridges

Wang et al. (2002) from electron microscopic observations revealed that IBs possibly initiated at leptotene by local removal of the primary wall and the middle lamella keeping plasmalemma intact, thereby yielding a cluster of unopened gaps/channels (0.11 to 1.1 μm wide) and subsequently at zygotene the plasmalemma of two adjacent meiocytes at these sites ruptured, curved towards and fused with each other leading to the formation of IBs. As the IBs grew in their length at late zygotene, cytomixis was more active and after pachytene IBs gradually occluded by callose deposition. On the contrary, Heslop-Harrison (1966) argued against the initiation of callose wall formation during early prophase occluding the usual pathway of movements. Wang et al. (2002) reported that microfilament/microtubule system also played pivotal role in the nuclear and cytoplasmic intercellular movement through IBs. However, Sidorchuk et al. (2007) in tobacco reported that microtubular cytoskeleton played no obvious role in the process of cytomixis, rather suggested that the elevated level of cytomixis was due to disturbances of synthesis of callose wall. Xiu-Wan et al. (1991) were of opinion that ATP-ase possibly provided the force in chromatin migration between PMCs. Wang et al. (2004) reported the existence of IBs in epidermis and vascular parenchyma of wheat, tobacco, and onion anthers, but the channels appeared singly rather than in groups. Whelan (1974) observed cytomixis in 13 out of 30 taxa of monocotyledons and dicotyledons and with the exception of *Helianthus* spp.

variable-sized holes were found to be localized in two to four areas of the callose wall. Irregular callose wall deposition (Sidorchuk et al. 2007) and existence of actin in intercellular connections between meiocytes (Dong et al. 2005) were also reported to play significant role in cytomixis. The existence of IBs between adjoining PMCs permitting the transfer of chromatin materials were confirmed from different reports (Zheng et al. 1987; Yen et al. 1993; Falistocco et al. 1995; Mclean et al. 1997; Boldrini et al. 2003; Tyagi 2003). de Nattancourt and Grant (1964) reported that IBs were formed before the onset of meiosis by non-synchronous premeiotic mitotic division where cytokinesis and karyokinesis were out of phase resulting in incomplete cell wall formation. Baquar and Husain (1969) in *Armebia hispidissima*, Risueño et al. (1969) in *Scilla non-stricta* and *Allium cepa*, Gupta and Gupta (1973) in F_1 hybrid of *Nicotiana suaveolens* and *N. glutinosa* among others suggested that IBs arisen de novo and chromatin migration occurred as a natural phenomenon. IBs formed between tobacco PMCs could be (1) on the basis of plasmodesmata, (2) de novo, neither interfering with each other (Mursalimov et al. 2010). However, Li et al. (2003) doubted whether IBs alone could sufficiently render the occurrence of cytomixis rather some meiotic genes must be involved to control the process. Withers and Cocking (1972) mentioned that enzymatic degradation of the walls between adjacent cells that were addressed to each other removed constrictions on the plasmodesmata, thereby permitted their expansion, and result in mixing of the cytoplasm. However, Liu et al. (2007) were of opinion that enzymatic digestion repressed rather than stimulate cytomictic activity thereby indicating that the phenomenon is a normal cellular process.

Cells in a cluster

During the early stage of anther development, PMCs occur in a compact group within a single microsporangium, the individual meiocytes being closely addressed and may facilitate chromatin migration through IBs formed between PMCs. If so, then only cells in two to four groups may be formed but a preponderance of two to eight cells or rarely 16 cells in a cluster to form coenocyte were mostly reported (Datta et al. 2005; Himshikha et al. 2010; Mandal and Datta 2011; Massoud et al. 2011; Saggoo et al. 2011). Levan (1941) reported 2 to ± 30 PMCs in a cluster that give rise to large plasmodia in haploid *Phleum pratense* ($2n=3x=21$). Gayen and Sarkar (1996) reported a maximum of 48 PMCs in a cluster with interconnections in maize haploids. Boldrini et al. (2006) suggested that in polyploidy accessions of *Brachiaria humidicola* (Poaceae) cell cluster of two to ten cells were involved during the process of cytomixis extending from prophase I to telophase II. Cell cluster forming syncyte is reported to be a monogenic character (Beadle 1932; Smith 1942; Pantulu and Manga 1971; Rao and

Koduru 1978), thereby indicating involvement of genetic factor(s) in the process.

The occurrence of more than four cells in a cluster suggested that meiocytes of different microsporangia must be involved in the process. It is interesting to note that in a single microsporophyll squash preparation cytomixis was exhibited only by few groups of cells, thereby suggesting that most of the meiocytes present in the microsporangium either vary in their cytotoxic potential or fail to get exposed to the specific condition(s) required to trigger the process. Cell clusters which were formed during early microsporogenesis and persisted possibly signal the phenomenon. Few to several cell clusters were formed in a single microsporophyll squash preparation but all PMCs in an antheridium do not form a single cluster and therefore lack meiotic synchrony that prevails during the process of spermatogenesis in animals and in some lower groups of plants.

Cells in cluster were anucleated or with deficient or extra chromosomes than normal thereby demonstrating the degree and direction of chromosome transfer. The intensity of cytotoxic behavior of chromosomes was assessed in *Corchorus* spp. from the frequency of hypo- and hyperploidy cells formed and it was found to be significantly higher in metaphase I (MI) than anaphase I (AI) cells (Mandal and Datta 2011; 2012). Baquar and Husain (1969) reported that the tendency of chromatin to migrate seemed to be higher in the early prophase stages and reduced as the meiosis advanced. The chromosomes were sticky, agglutinated (Datta et al. 2005; Kumar and Singhal 2008) and underwent structural alteration prior to the phenomenon (Boldrini et al. 2003; Utsunomiya et al. 2004). Jeelani et al. (2011) suggested that cytomixis induced the disturbance of spindle formation resulting into irregular distribution of chromatin within the PMCs as well as between the sharing PMCs. In this context, it would be logical to mention that aneuploid variation in chromosome number may also occur due to mutations in the spindle apparatus causing the overexpression of SKT15 protein in human (PB6211) cancerous cell line (Duesberg 1999).

Migration of nucleoli

Transmigration of nucleoli during cytomixis was noted (Li et al. 2009; Himshikha et al. 2010; Mursalimov and Deineko 2011). On the contrary, nucleolus disintegrates into many parts whilst entering in a neighboring cell through IBs in *Cucurbita* and *Lycopersicon esculentum* (Weiling 1965). This observation is rather variable to the concept of organelle transfer during the process of cytomixis of animals. It may be relevant to mention that lack of IBs in the walls between tapetum (tissue possessing a nutritional role) did not affect nutritional transport in angiosperms (Bhojwani and Bhatnagar 1974; Johri 1984). Moreover, the absence of IBs between spore mother cells of bryophytes (Brown

and Lemmon 1980) and PMCs of some gymnosperms (Vasil and Aldrich 1970) unaffected nutrient transfer where callose wall is also initiated during the early prophase.

Environmental influence

de Souza and Pagliarini (1997) reported that high temperature and lack of water during summer season might possibly account for cytomixis in *Brassica napus* and *B. campestris*, normally grown in winter. The high temperature was also considered to be the cause of the high incidence of cytomixis in *Hemerocallis* (Narain 1979), *Ervatamia divaricata* (De and Sharma 1983), *Helicanthes elastic* (Soman and Bhavanandan 1993), Rose (Pécricx et al. 2011), among others. Malallah and Attia (2003) suggested that cytomixis observed in *Diplotaxis harra* might be associated with temperature and drought stress phases prevailing during the growth season in Kuwait. Kaur et al. (2010) observed cytomixis in *Poa annua* growing wild in higher altitudes but the species depicted normal meiosis in plains. Mandal and Datta (data unpublished) studied distinctive influence of high temperature (>39 °C) on the intensity of cytomixis in *Corchorus fascicularis*. Cytomixis was restricted up to first meiotic division (Fig. 1). Apart from normal ($2n=14$; Fig. 1dII, eI, hII) chromosome number cytomixis included hypo- and hyperploidy cells (Fig. 1dI, eII, f–g, hI, i, k), differential condensation of chromosomes, formation of minute fragments (Fig. 1c, f–g, i, k–l) of variable number (1 to 5) and sizes (0.04–0.09 μm) with possible constrictions, enhanced univalent frequency (14I formation, complete desynapsis; Fig. 1j), PMCs with two nucleoli (Fig. 1d) and irregular AI separation (Fig. 1l), without affecting pollen fertility. Meiotic abnormalities namely, chromatin stickiness, pycnotic chromatin, interbivalent connections, laggards, bridges, micronuclei, among others have been associated to cytomixis in different plant species (Baptista-Giacomelli et al. 2000; Singhal and Kaur 2011).

Possibility of the origin of B-chromosome

Cytomixis resulting in fragmentation of A-chromosomes in *Lilium* was reported to be the mechanism for the origin of B-chromosomes (Cheng et al. 1975). Yanyou and Jiemei (1996) put forward the supposition of cytomixis for the origin of B-chromosomes in plants. B-chromosomes reported in cytotoxic population of *Papaver somniferum* were of sub-metacentric and telocentric types (Patra et al. 1988). Sheidai (2007) also suggested that B-chromosomes pioneered cytomixis possibly played significant role in the evolution of plant species.

Evolutionary significance

Cytomixis resulting in the formation of hypo- and hyperploidy cells along with other meiotic abnormalities possibly resulted in

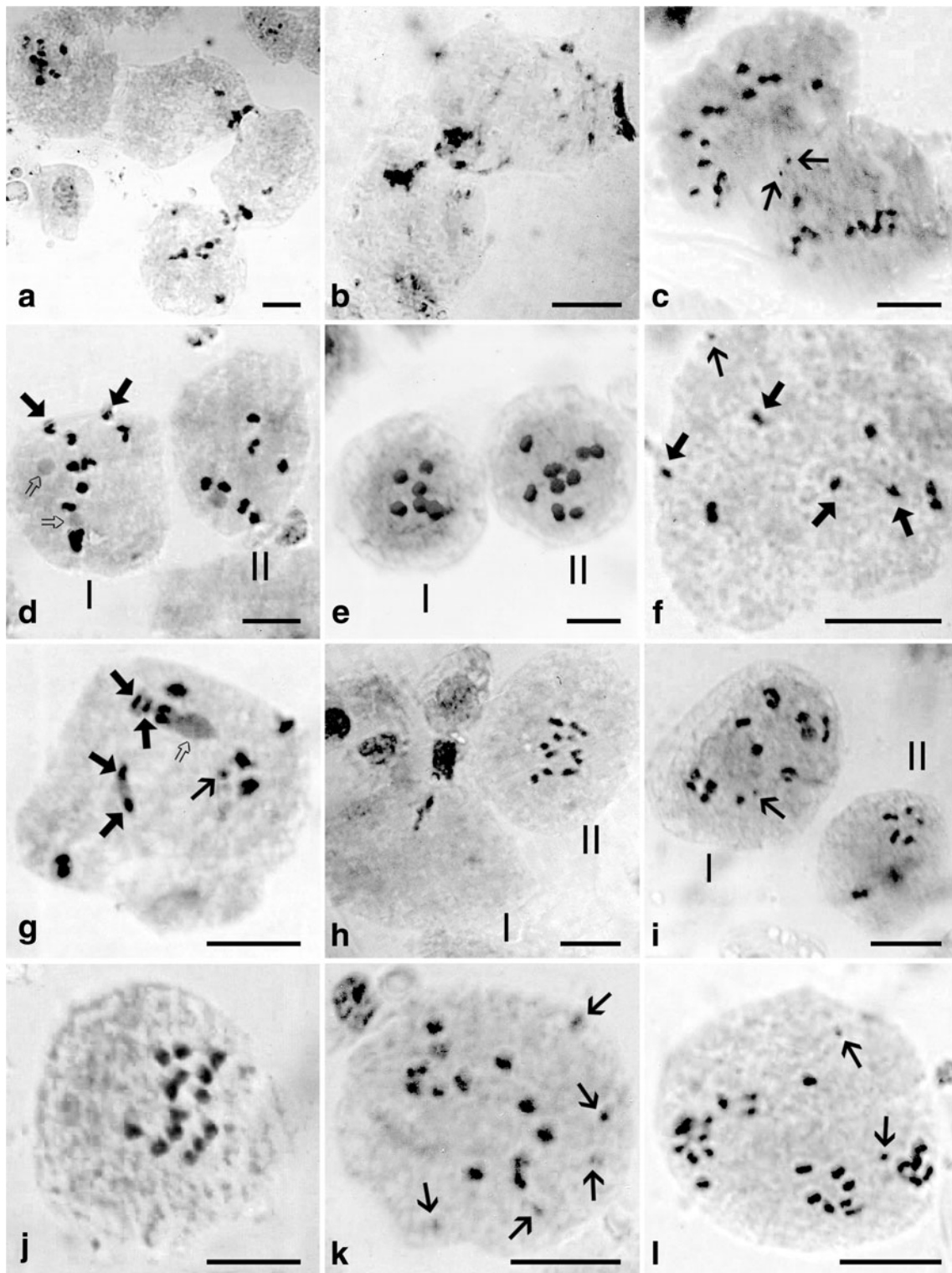


Fig. 1 Meiotic configurations in *Corchorus fascicularis* showing cytomixis (a–c) and aneuploid PMCs at diplotene-diakinesis (d, g), metaphase I (e–f, h–k) and anaphase I (l) (a) cytomixis involving four meiocytes with cytoplasmic connections (b) differential condensation, agglutination and fragmentation of chromatins during transfer between cells (c) cell fusion between two PMCs with both showing $2n=14$ chromosomes (d) I: hyperloid PMC with two nucleoli ($8\text{II}+2\text{I}$, $2n=18$); II: 7II ($2n=14$); (e) I: 7II ; II: hyperloid PMC

(10II , $2n=20$); (f) hypoploid cell ($3\text{II}+4\text{I}$, $2n=10$); (g) hyperloid PMC ($9\text{II}+4\text{I}$, $2n=22$) with a fragment; (h) I: hypoploid cell (2II , $2n=4$), II: $4\text{II}+6\text{I}$ ($2n=14$); (i) I: tetraploid meiocyte (14II , $2n=28$) with a fragment, II: hypoploid meiocyte ($4\text{II}+2\text{I}$, $2n=10$); (j) 14I ($2n=14$); (k) $4\text{II}+6\text{I}$ ($2n=14$) + 5 fragments (l) irregular groupings in a hyperloid cell. Scale bar = $10\ \mu\text{m}$; nucleolus (open arrow), univalents (thick right arrow), and fragments (right arrow) are marked

the formation of heterogenous pollen grains, thereby reducing pollen fertility and viability in different plant species (Baptista-Giacomelli et al. 2000; Bellucci et al. 2003; Haroun et al. 2004; Ghaffari 2006; Kumar et al. 2008; Singhal et al. 2008; Gupta et al. 2009; Rani et al. 2010; Saggoo et al. 2011; Srivastava and Kumar 2011). The origin of aneuploid and polyploid gametes in cytomixis was emphasized as of evolutionary significance (Sarvella 1958; Shkutina and Kozlovskaya 1974; Omara 1976; Datta and Biswas 1984; Bhal and Tyagi 1988; Falistocco et al. 1995; Sheidai et al. 2003; Sheidai and Fadaei 2005; Sheidai and Nouroozi 2005; Ghaffari 2006; Sheidai and Bagheri-Shabastari 2007; Fadaei et al. 2010; Rai et al. 2010) in that it can lead to the production of plants with higher ploidy through polyploidization (Villeux 1985). Datta and Biswas (1984) detected a trisomic ($2n=13$) in the M_3 mutant generation (lax branching) of *Nigella sativa* (black cumin). The mutant showed cytotoxic behavior of chromosomes forming polyploid and aneuploid PMCs at M_2 . Singhal et al. (1990) performed meiotic counts in different woody species of the family Rosaceae and reported variation in chromosome number in PMCs of *Cotoneaster acuminata* ($n=17$), *Prunus domestica* spp. *insitita* ($n=8$) and *Rosa leschenaultiana* ($n=7$), and that such variation was attributed to chromatin transfer during cytomixis. Singhal et al. (2008) suggested the formation of heterogenous sized fertile pollen grains in *Hippophae rhamnoides* (Elaeagnaceae) as the consequence of cytomixis and predicted in the origins of plants with aneuploid and polyploid chromosome number. Srivastava and Kumar (2011) reported that there exist no correlation between cytomixis and

decrease in pollen fertility percentage. Kumar et al. (2012) found cytomixis in the PMCs of *Papaver dubium*, *Mecanopsis latifolia*, *M. Mexicana* and *M. aculeata*, resulting in the formation of unreduced gametes and presumed that it may lead to the production of aneuploid plants.

The IBs persisting up to telophase II (Boldrini et al. 2006; Li et al. 2009; Song and Li 2009) may account for heterogenous pollen grains formation. Most reports indicated that the phenomenon is more prevalent at meiosis I (Datta et al. 2005; Mandal and Datta 2011, 2012) rather than throughout meiosis II (Omara 1976; He et al. 2004; Boldrini et al. 2006; Kumar and Tripathi 2008; Himshikha et al. 2010; Kumar et al. 2010; Singhal and Kaur 2011). Mandal and Datta (2011, 2012) reported that the aberrant meiocytes formed due to cytomixis failed to survive beyond meiosis I (specifically MI) and did not affect pollen fertility and viability, microgametogenesis and seed set per capsule in *Corchorus* spp. thereby, corroborating the reports of Koul (1990) in *Alopecurus arundinaceus*, Morikawa and Leggett (1996) in *Avena canariensis*, Datta et al. (2005) in *Ocimum basilicum* and *W. somnifera*, Das et al. (2009) in *W. somnifera* varieties among others. Interesting to note that pollen fertility enhanced by 15- to 20-folds in maize haploids with extensive cytomixis (Gayen and Sarkar 1996). Negrón-Ortiz (2007) suggested that cytomixis provides a mechanism whereby gametes with variable chromosome number influence reproduction and promote speciation. Improvement in the sense of diversity and yield potential was also predicted in inbred lines of *Zea mays* undergoing cytomixis (Rai et al. 2010).

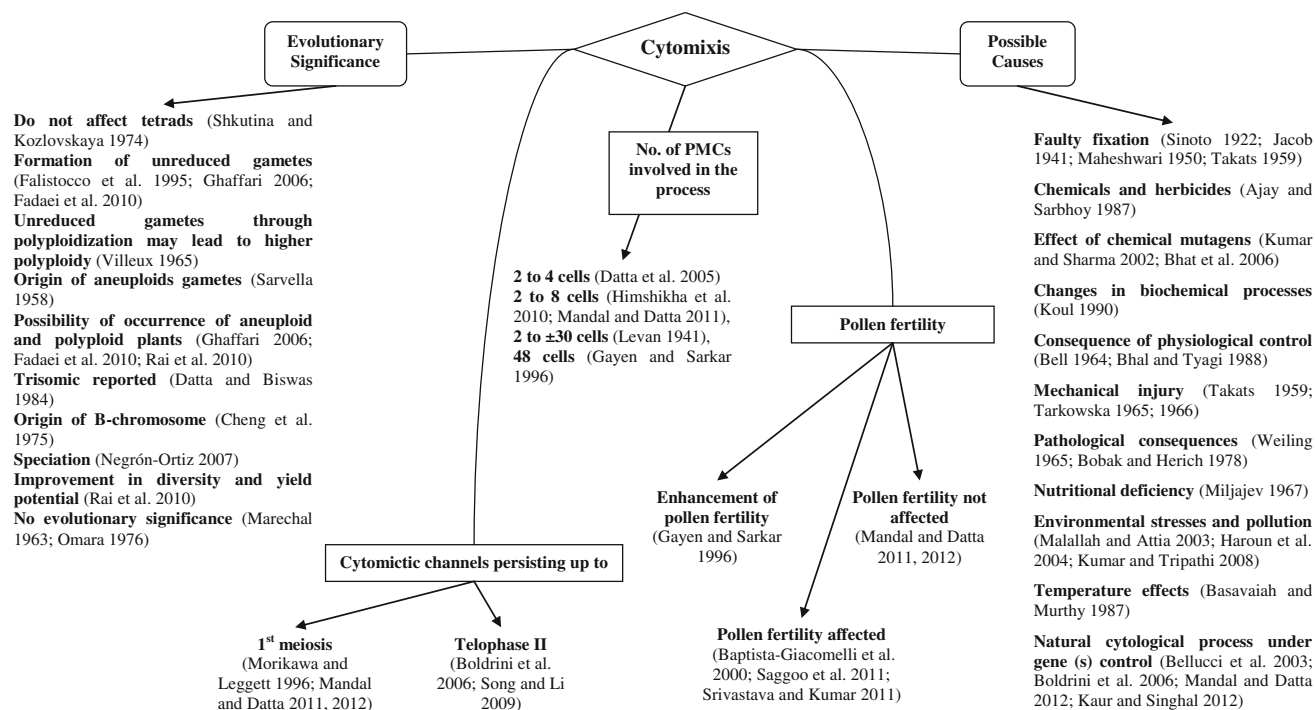


Fig. 2 Diagrammatic representation of cytomixis showing its variation in flowering taxa

Summary and prospect

In oogamous reproduction, the role of IBs for the elimination of heterogeneity during male gamete development, formation of syncytium, meiotic synchrony for simultaneous maturation and production of high-quality male gametes and sharing of protoplasm were essential and key events for successful fertilization in animals and in some lower group of plants (Larson and Barrett 2000; Magnard et al. 2001). However, meiotic synchrony relating to fertilization events is rather not uniform in flowering plants. A diagrammatic representation (Fig. 2) is presented to depict similarities and differences in the process of cytomixis in flowering taxa. From the perusal of the literature, it seems that the phenomenon of cytomixis in male reproductive cells is quite variable in angiosperms in relation to the following aspects: (1) lack of uniformity in occurrence, (2) possible cause(s), (3) persistence of IBs in meiotic stages, (4) variation in number of cells in a cluster, (5) reduction/enhancement of pollen fertility and (6) possible significance. In this context, several key questions may be pointed out. (1) If the phenomenon is a normal cellular event under gene(s) control, then why it is not uniformly present in all flowering plants? (2) Why the intensity of cytomixis is found to decrease under cultivation/acclimatization? (3) If only a few PMCs in an antheridium are undergoing cytotoxic behavior — is there any signal transduction pathway controlling the phenomenon? (4) Why all PMCs of a sporangium are not interconnected to form a common cluster and provide meiotic synchrony? (5) Is meiotic asynchrony affects fertilization? (6) Is movement of cell organelles through the channels during the process, regulated? (7) Is cytomixis in flowering plants representing evolutionary advancement by not allowing meiotic synchrony and persisting heterogeneity to prevail?

Conclusion

Although the phenomenon of cytomixis is rather uniform in the context of fertilization event in animals and in some lower groups of plants where oogamous reproduction prevails, in flowering taxa it is unique because of its versatility.

In angiosperms, formation of polyploid/aneuploid PMCs due to cytomixis has been emphasized as of evolutionary significance. However, to justify it the following sequential events need to occur: (1) gametes with hypo- and hyperploid chromosome numbers should be tolerant to undergo fertilization process, (2) viability of the formed zygote is essential for next generation plants and (3) raised seedling should be adaptive under appropriate ecological niche. However, synchrony of all the above-mentioned events is rather difficult to ascertain. On the contrary, as many plant species (30% to 80%) are reported to be polyploid in origin (Leitch and Bennett 1997;

Ramsey and Schemske 1998; Soltis and Soltis 1999; Parisod et al. 2010), the role of cytomixis (if any) in chromosome duplication and polyploidization needs to be more specifically evaluated. In the context it would be relevant to suggest that the phenomenon is more predominant in polyploid species than diploids. The female germ lines of angiosperm species should also be explored to assess whether cytomixis prevails there or not, which may be significant in the proper understanding of the process not only in flowering taxa but as a whole. With this in view, the phenomenon of cytomixis has been discussed in animals, lower groups of plants and specifically in angiosperms with an objective that it may provide scope of further exploration to unravel some of the ambiguity associated to it. There seems to be a general ignorance about cytomixis. Proper knowledge of the phenomenon may be effective in understanding cell, reproductive, structural and evolutionary biology.

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References

- Ajay KJ, Sarbhoy RK (1987) Cytogenetical studies on the effect of some chlorinated pesticides: II. Effect on meiotic chromosomes of *Lens* and *Pisum*. *Cytologia* 52:55–61
- Baptista-Giacomelli FR, Pagliarini MS, de Almeida JL (2000) Meiotic behavior in several Brazilian oat cultivars (*Avena sativa* L.). *Cytologia* 65:371–378. doi:10.1508/cytologia.65.371
- Basavaiah D, Murthy TCS (1987) Cytomixis in pollen mother cells of *Urochloa panicoides* P. Beauv. (Poaceae). *Cytologia* 52:69–74
- Baquar SR, Husain SA (1969) Cytoplasmic channels and chromatin migration in the meiocytes of *Arnebia hispidissima* (Sieb.) DC. *Ann Bot* 33:821–831
- Beadle GW (1932) A gene in *Zea mays* for failure of cytokinesis during meiosis. *Cytologia* 3:142–155
- Bedi YS (1990) Cytomixis in woody species. *Proc Indian Acad Sci (Plant Sci)* 100:233–238. doi:10.1007/BF03053475
- Bell CR (1964) Cytomixis in *Tauschia nudicaulis* Schlecht (Apiaceae). *Cytologia* 29:369–398
- Bellucci M, Roscini C, Mariani A (2003) Cytomixis in pollen mother cells of *Medicago sativa* L. *J Hered* 94:512–516. doi:10.1093/jhered/esg096
- Bhal JR, Tyagi BR (1988) Cytomixis in pollen mother cells of *Papaver dubium* L. *Cytologia* 53:771–775
- Bhat TA, Parveen S, Khan AH (2006) MMS-induced cytomixis in pollen mother cells of Broad Bean (*Vicia faba* L.). *Turk J Bot* 30:273–279
- Bhojwani SS, Bhatnagar SP (1974) The embryology of angiosperms. Vikas Publishing House, Delhi
- Bobak M, Herich R (1978) Cytomixis as a manifestation of pathological changes after the application of trifluralin. *Nucleus* 21:22–26
- Boldrini KR, Bione NCP, Pagliarini MS (2003) Chromosome transfer among pollen mother cells of garden crotons (*Codiaeum variegatum* Blume). *Cytologia* 68:341–344

- Boldrini KR, Pagliarini MS, do Valle CB (2006) Cell fusion and cytomixis during microsporogenesis in *Brachiaria humidicola* (Poaceae). *South Afr J Bot* 72:478–481
- Braun RE, Behringer RR, Peschon JJ, Brinster RL, Palmiter RD (1989) Genetically haploid spermatids are phenotypically diploid. *Nature* 337:373–376
- Brown RC, Lemmon BE (1980) Ultrastructure of sporogenesis in a moss. *Ditrichum pallidum*: I. meiotic prophase. *Bryologist* 83:137–152
- Caldwell KA, Handel MA (1991) Protamine transcript sharing among postmeiotic permatids. *Proc Natl Acad Sci* 88:2407–2411
- Carlson JG, Handel MA (1988) Intercellular bridges and factors determining their patterns in the grasshopper testis. *J Morphol* 196:173–185
- Carr DJ (1976) Plasmodesmata in growth and development. In: Gunning BES, Robards AW (eds) *Intercellular communication in plants: studies on plasmodesmata*. Springer, Berlin, pp 243–288
- Chang YC, Chen YJ, Wu CH, Wu YC, Yen TC, Ouyang P (2010) Characterization of centrosomal proteins Cep55 and pericentrin in intercellular bridges of mouse testes. *J Cell Biochem* 109:1274–1285
- Cheng KC, Zheng GC, Nie XW, Yang QL, Wang YX, Zhou YS, Chen JS (1975) Light and electron microscopical observation on cytomixis and the study of its relation to variation and evolution. *Acta Botanica Sinica* 17:60–69
- Cooper DD (1952) The transfer of deoxyribose nucleic acid from the tapetum to the sporocytes at the onset of meiosis. *Am Nat* 86:271–274
- Das A, Datta AK, Ghose S (2009) Cytogenetical studies on two varieties of *Withania somnifera*. *J Trop Med Plants* 10:249–256
- Datta AK, Biswas AK (1984) Cytomixis and a trisomic in *Nigella sativa* L. *Cytologia* 49:437–445
- Datta AK, Mukherjee M, Iqbal M (2005) Persistent cytomixis in *Ocimum basilicum* L. (Lamiaceae) and *Withania somnifera* (L.) Dun (Solanaceae). *Cytologia* 70:309–313
- De M, Sharma AK (1983) Cytomixis in pollen mother cells of an apomictic ornamental *Ervatamia divaricata* (Linn.) Alston. *Cytologia* 48:201–207
- de Nettancourt D, Grant WF (1964) La cytogenetics de Lotus (Leguminosae) III. Un cas de cytomixie dans un hybride interspecific. *Cytologia* 29:191–195
- de Souza AM, Pagliarini MS (1997) Cytomixis in *Brassica napus* var. Oleifera (Brassicaceae). *Cytologia* 62:25–29
- Deason TR, Darden WH Jr, Ely S (1969) The development of sperm packets of the M5 strain of *Volvox aureus*. *J Ultrastruct Res* 26:85–94
- Dong LZ, Junying YX (1988) By isolating single spore for determine genotype of *Pleurotus sapidus* and *Lentinus edodes*. *J Agric Univ Hebei*. doi:cnki:ISSN:1000-1573.0.1988-03-014
- Dong W, Li W, Guo GQ, Zheng GC (2004) Ultrastructural aspects of plasmodesmata and cytoplasmic bridges during spermatogenesis in *Funaria hygrometrica*. *Acta Botanica Sinica* 46:988–996
- Dong W, Li W, Zhang CG, Guo GQ, Zheng GC (2005) Three dimensional immunolocalization of actin in pollen mother cells of David lily. *Act Bot Bor-Occ Sinica* 25:8–13
- Duesberg P (1999) Are centrosomes or aneuploidy key to cancer? *Science* 284:770–771. doi:10.1126/science.284.5423.2089f
- Dym M, Fawcett DW (1971) Further observations on the numbers of spermatogonia, spermatocytes, and spermatids connected by intercellular bridges in the mammalian testis. *Biol Reprod* 4:195–215
- Fadaei F, Sheidai M, Asadi M (2010) Cytological study the genus *Arenaria* L. (Caryophyllaceae). *Caryologia* 63:149–156
- Falisto E, Tosti N, Falcinelli M (1995) Cytomixis in pollen mother cells of diploid *Dactylis* one of the origins of 2n gametes. *J Hered* 86:448–453
- Fawcett DW, Ito S, Slautterback D (1959) The occurrence of intercellular bridges in groups of cells exhibiting synchronous differentiation. *J Biophys Biochem Cytol* 5:453–460
- Gates RR (1911) Pollen formation in *Oenothera gigas*. *Ann Bot* 25:909–940
- Gayen P, Sarkar KP (1996) Cytomixis in maize haploids. *Indian J Genet* 56:79–85
- Gelin OE (1934) Embryologische und cytologische studiem in Heliantheae—Coreopsidineae. *Acta Horti Bergiani* 11:99–128
- Ghaffari SM (2006) Occurrence of diploid and polyploidy microspores in *Sorghum bicolor* (Poaceae) is the result of cytomixis. *Afr J Biotech* 5:1450–1453
- Giorgi F (1978) Intercellular bridges in ovarian follicle cells of *Drosophila melanogaster*. *Cell Tissue Res* 186:413–422
- Gondos B (1973) Germ cell degeneration and intercellular bridges in the human fetal ovary. *Z Zellforsch Mikrosk Anat* 138:23–30
- Gottschalk W (1970) Chromosome and nucleus migration during microsporogenesis of *Pisum sativum*. *Nucleus* 13:1–9
- Greenbaum MP, Yan W, Wu MH, Lin YN, Agno JE, Sharma M, Braun RE, Rajkovic A, Matzuk MM (2006) TEX14 is essential for intercellular bridges and fertility in male mice. *Proc Natl Acad Sci* 103:4982–4987
- Greenbaum MP, Iwamori N, Agno JE, Matzuk MM (2009) Mouse TEX14 is required for embryonic germ cell intercellular bridges but not female fertility. *Biol Reprod* 80:449–457
- Greenbaum MP, Iwamori T, Buchold GM, Matzuk MM (2011) Germ cell intercellular bridges. *Cold Spring Harb Perspect Biol* 3:a005850. doi:10.1101/cshperspect.a005850
- Guo GQ, Zheng GC (2004) Hypotheses for the functions of intercellular bridges in male germ cell development and its cellular mechanisms. *J Theo Biol* 229:139–146
- Gupta RC, Himshikha P, Kumar P, Dhaliwal RS (2009) Cytological studies in some plants from cold deserts of India, Lahaul and Spiti (Himachal Pradesh). *Chromosome Bot* 4:5–11
- Gupta SB, Gupta P (1973) Selective elimination of *Nicotiana glutinosa* chromosomes in the F₁ hybrids of *N. suaveolens* and *N. glutinosa*. *Genetics* 73:605–612
- Guzicka M, Wozny A (2005) Cytomixis in shoot apex of Norway spruce [*Picea abies* (L.) Karst.]. *Trees* 18:722–724
- Haglund K, Nezis IP, Stenmark H (2011) Structure and functions of stable intercellular bridges formed by incomplete cytokinesis during development. *Commun Integr Biol* 4:1–9
- Haroun SA (1995) Cytomixis in pollen mother cells of *Polygonum tomentosum* Schrank. *Cytologia* 60:257–260
- Haroun SA, Al Shehri AM, Al Wadie HM (2004) Cytomixis in the microsporogenesis of *Vicia faba* L. (Fabaceae). *Cytologia* 69:7–11
- He Z, Wang H, Li J, Ye Q, Taylor CW (2004) Chromosome behavior during meiosis and development of spore mother cells in the Chinese quillwort *Isoetes sinensis* T. C. Palmer (Isoetaceae). *Am Fern J* 94:183–195
- Heng-Chang W, Li JQ, He ZC (2007) Irregular meiotic behavior in *Isoetes sinensis* (Isoetaceae), a rare and endangered fern in China. *Caryologia* 60:358–363
- Heslop-Harrison J (1966) Cytoplasmic continuity during spore formation in flowering plants. *Endeavour* 25:65–72
- Hime GR, Brill JA, Margaret TF (1996) Assembly of ring canals in the male germ line from structural components of the contractile ring. *J Cell Sci* 109:2779–2788
- Himshikha P, Kumar R, Gupta C, Kumari S, Singhal VK (2010) Impact of chromatin transfer and spindle abnormalities on pollen fertility and pollen size in *Plantago lanceolata* L. *Cytologia* 75:421–426
- Hoops HJ, Nishii I, Kirk DL (2000) Cytoplasmic bridges in *Volvox* and its relatives. *Madame Curie Bioscience Database* [Internet]. Austin (TX): Landes Bioscience

- Iqbal M, Datta AK (2007) Cytogenetic studies in *Withania somnifera* (L.) Dun. (Solanaceae). *Cytologia* 72:43–47
- Jacob KT (1941) Certain abnormalities in the root tips of cotton. *Curr Sci* 10:174–175
- Jeelani SM, Rani S, Kumar S, Kumari S, Gupta RC (2011) Evaluation of cytomorphological diversity in *Filipendula vestita* (Wall. ex G. Don) Maxim., (Rosaceae) from Western Himalayas. *Cytologia* 76:403–410
- Johri BM (1984) Embryology of angiosperms. Springer, Berlin
- Kaur H, Gupta A, Kumari S, Gupta RC (2010) Meiotic studies in *Poa annua* L. from different altitudinal ranges of North India. *Cytologia* 75:313–318
- Kaur D, Singhal VK (2012) Phenomenon of cytotoxicity and intraspecific polyploidy (2x, 4x) in *Spergularia diandra* (Guss.) Heldr. & Sart. in the cold desert regions of Kinnaur district (Himachal Pradesh). *Cytologia* 77:163–171
- Kihara H, Lilienfeld F (1934) Kerneinwanderung und Bildung syndiploider pollennutterzellen bei dem F₁-Bastard *Triticum aegilopoides* × *Aegilops squarrosa*. *Jap J Genet* 10:1–28
- Körnicker M (1901) Über ortsveränderung von Zellkernern S B Niederhein Ges Natur-U Heilkunde Bonn A, pp 14–25
- Koul KK (1990) Cytotoxicity in pollen mother cells of *Alopecurus arundinaceus* Poir. *Cytologia* 55:169–173
- Kravets EA (2012) Nature, significance, and cytological consequences of cytotoxicity. *Cytol Genet* 46:188–195
- Kumar G, Sharma V (2002) Induced cytotoxicity in chickpea (*Cicer arietinum* L.). *Nucleus* 45:24–26
- Kumar G, Tripathi R (2008) Induced cytotoxic variations through abiotic stresses in grasspea (*Lathyrus sativus* L.). *Indian J Genet* 68:58–64
- Kumar P, Singhal VK (2008) Cytology of *Caltha palustris* L. (Ranunculaceae) from cold regions of western Himalayas. *Cytologia* 73:137–143
- Kumar P, Singhal VK, Kaur J (2008) Cytotoxicity induced meiotic abnormalities in pollen mother cells of *Clematis flammula* L. (Ranunculaceae). *Cytologia* 73:381–385
- Kumar P, Singhal VK, Kaur D, Kaur S (2010) Cytotoxicity and associated meiotic abnormalities affecting pollen fertility in *Clematis orientalis*. *Biol Plantarum* 54:181–184
- Kumar S, Jeelani SM, Rani S, Gupta RC, Kumari S (2012) Cytology of five species of subfamily Papaveroideae from the Western Himalayas. *Protoplasma*. doi:10.1007/s00709-012-0413-7
- Kwiatkowska M, Maszewski J (1976) Plasmodesmata between synchronously and asynchronously developing cells of the antheridial filaments of *Chara vulgaris* L. *Protoplasma* 87:317–327
- Kwiatkowska M, Popłońska K, Wojtczak A (2003) *Chara tomentosa* antheridial plasmodesmata at various stages of spermatogenesis. *Biol Plantarum* 46:233–238
- Larson BMH, Barrett SCH (2000) A comparative analysis of pollen limitation in flowering plants. *Biol J Linn Soc* 69:503–520
- Lattoo SK, Khan S, Bamotra S, Dhar AK (2006) Cytotoxicity impairs meiosis and influences reproductive success in *Chlorophytum comosum* (Thunb.) Jacq.—an additional strategy and possible implications. *J Biosci* 31:629–637
- Leitch IJ, Bennett MD (1997) Polyploidy in angiosperms. *Trends Plants Sci* 2:470–476
- Levan A (1941) Syncyte formation in the pollen mother cells of haploid *Phleum pratense*. *Hereditas* 27:243–252
- Li W, Yang J, Pan YF, Guo GQ, Zheng GC (2003) Chromosome localization of genes that control synchronous development of pollen mother cells in wheat. *Caryologia* 56:275–279
- Li XF, Song ZQ, Feng DS, Wang HG (2009) Cytotoxicity in *Thinopyrum intermedium*, *Thinopyrum ponticum* and its hybrids with wheat. *Cereal Res Commun* 37:353–361
- Liu H, Guo GQ, He YK, Lu YP, Zheng GC (2007) Visualization on intercellular movement of chromatin in intact living anthers of transgenic tobacco expressing histone 2B-CFP fusion protein. *Caryologia* 60:1–20
- Lúcas WJ, Wolf S (1993) Plasmodesmata: the intercellular organelles of green plants. *Trends Cell Biol* 3:308–315
- Magnard JL, Yang M, Chen YCS, Leary M, McCormick S (2001) The Arabidopsis gene *Tardy asynchronous meiosis* is required for the normal pace and synchrony of cell division during male meiosis. *Plant Physiol* 127:1157–1165
- Maheshwari P (1950) An introduction to the embryology of angiosperms. McGraw-Hill, New York
- Maity S, Datta AK (2008) Cytomorphological studies in F₁ hybrids (*Corchorus capsularis* L. × *Corchorus oltorius* L.) of jute (Tiliaceae). *Comp Cytogenet* 2:143–149
- Malallah GA, Attia TA (2003) Cytotoxicity and its possible evolutionary role in a Kuwaiti population of *Diplotaxis harra* (Brassicaceae). *Bot J Linn Soc* 143:169–175
- Mandal A, Datta AK (2011) Secondary chromosome associations and cytotoxicity in *Corchorus* spp. *Cytologia* 76:337–343
- Mandal A, Datta AK (2012) Inter- and intra-plant variations in cytotoxic behavior of chromosomes in *Corchorus fascicularis* Lamk. (Tiliaceae). *Cytologia* 77:269–277
- Massoud R, Karamian R, Nouri S (2011) Impact of cytotoxicity on meiosis in *Astragalus cyclophyllus* Beck (Fabaceae) from Iran. *Caryologia* 64:256–264
- Mclean BG, Hempel FD, Zambryski PC (1997) Plant intercellular communication via plasmodesmata. *Plant Cell* 9:1043–1054
- Miljajev EL (1967) Cytochimiceskoje I electron-mikroskopi ceskoje izucenje mikosporogeneza *Citrus sinensis*. Autoreferat Kandidaatskej dizertacije
- Morikawa T, Leggett M (1996) Cytological and morphological variations in wild populations of *Avena canariensis* from the Canary Islands. *Genes Genet Syst* 71:15–21
- Morisset P (1978) Cytotoxicity in the pollen mother cells of *Ononis* (Leguminosae). *Can J Genet Cytol* 20:383–388
- Mursalimov SR, Baiborodin SI, Sidorchuk IV, Shumnyi VK, Deineko EV (2010) Characteristics of the cytotoxicity channel formation in *Nicotiana tabacum* L. pollen mother cells. *Tsitol Genet* 44:19–24
- Mursalimov SR, Deineko EV (2011) An ultrastructural study of cytotoxicity in tobacco pollen mother cells. *Protoplasma* 248:717–724
- Narain P (1979) Cytotoxicity in the pollen mother cells of *Hemerocallis* Linn. *Curr Sci* 48:996–998
- Negrón-Ortiz V (2007) Chromosome numbers, nuclear DNA content, and polyploidy in *Consolea* (Cactaceae), an endemic cactus of the Caribbean Islands. *Am J Bot* 94:1360–1370
- Omara MK (1976) Cytotoxicity in *Lolium perenne*. *Chromosoma* 55:267–271
- Ong S, Foote C, Tan C (2010) Mutations of DMYP1 cause over constriction of contractile rings and ring canals during *Drosophila* germline cyst formation. *Dev Biol* 346:161–169
- Owen HA, Makaroff CA (1995) Ultrastructure of microsporogenesis and microgametogenesis in *Arabidopsis thaliana* (L.) Heynh. Ecotype Wassilewskija (Brassicaceae). *Protoplasma* 185:7–21
- Pagliarini MS, Pereira MAS (1992) Meiotic studies in *Pilocarpus pennatifolius* Lem. (Rutaceae). *Cytologia* 57:231–235
- Pantulu JV, Manga V (1971) Monofactorial ‘multiploid sporocytes’ condition induced by EMS in pearl millet. *Genetica* 42:214–218
- Paolillo DJ Jr, Cukierski M (1976) Wall developments and coordinated cytoplasmic changes in spermatogenous cells of *Polytrichum* (Musci). *Bryologist* 79:466–479
- Parisod C, Holderegger R, Brochmann C (2010) Evolutionary consequences of autopolyploidy. *New Phytol* 186:5–17
- Patra NK, Srivastava HK, Chauhan SP (1988) B chromosome in spontaneous and induced intercellular chromosome migration of *Papaver somniferum* L. *Indian J Genet* 48:31–42
- Peng ZS, Yang J, Zheng GC (2003) Cytotoxicity in pollen mother cells of new synthetic hexaploid amphidiploid (*Aegilops tauschii* × *Triticum turgidum*). *Cytologia* 68:335–340

- Pécricx Y, Rallo G, Folzer H, Cigna M, Gudin S, Le Bris M (2011) Polyploidization mechanisms: temperature environment can induce diploid gamete formation in *Rosa* sp. *J Exp Bot* 62:3587–3597
- Rai PK, Kumar G, Tripathi A (2010) Induced cytotoxic diversity in maize (*Zea mays* L.). *Tsitol Genet* 44:9–14
- Ramsey J, Schemske DW (1998) Pathways, mechanisms, and rates of polyploid formation in flowering plants. *Tree* 14:348–352
- Rani S, Kumar S, Jeelani SM, Gupta RC, Kumari S (2010) Effect of cytotoxicity on male meiosis in populations of *Clematis grata* Wall. from Western Himalayas. *Chromosome Bot* 5:61–64
- Rao MK, Koduru PRK (1978) Cytogenetics as a factor for syncyte formation and male sterility in *Pennisetum americanum*. *Theor Appl Genet* 53:1–7
- Renzaglia KS, Garbary DJ (2001) Motile male gametes of land plants: diversity, development and evolution. *Crit Rev Plant Sci* 20:107–213
- Risueño MC, Giménez-Martín G, López-Sáez JF, R-García MI (1969) Connexions between meiocytes in plants. *Cytologia* 34:262–272
- Robinson DN (1996) Stable intercellular bridges in development: The cytoskeleton lining the tunnel (Short Survey). *Trends Cell Biol* 6:474–479
- Robinson DN, Cooley L (1996) Stable intercellular bridges in development: the cytoskeleton lining the tunnel. *Trends Cell Biol* 6:474–479
- Robinson DN, Cooley L (1997) Genetic analysis of the actin cytoskeleton in the *Drosophila* ovary. *Annu Rev Cell Dev Biol* 13:147–170
- Roosen-Runge EC (1977) The process of spermatogenesis in animals. Cambridge University Press, London
- Saggoo MIS, Gill A, Walia S (2011) Cytotoxicity during microsporogenesis in some populations of *Croton bonplandianum* of north India. *Cytologia* 76:67–72
- Salesses G (1970) Sur le phénomène de cytotoxicité chez des hybrides triploïdes de prunier. Conséquences génétiques possibles. *Ann Amélior Plant* 20:383–388
- Sapre AB, Deshpande DS (1987) A change in chromosome number due to cytotoxicity in an interspecific hybrid of *Coix* L. *Cytologia* 52:167–174
- Sarvella P (1958) Cytotoxicity and loss of chromosomes in meiotic and somatic cells of *Gossypium*. *Cytologia* 23:14–24
- Sharma V, Kumar G (2004) Meiotic studies in two cultivars of *Cicer arietinum* L. after EMS treatment. *Cytologia* 69:243–248
- Sheidai M (2007) B-chromosome variability in pomegranate (*Punica granatum* L.) cultivars. *Caryologia* 60:251–256
- Sheidai M, Bagheri-Shabestari ES (2007) Cytotoxicity and unreduced pollen formation in some *Festuca* L. species of Iran. *Caryologia* 60:364–371
- Sheidai M, Fadaei F (2005) Cytogenetic studies in some species of *Bromus* L. section *Genea* Dum. *J Genet* 84:189–194
- Sheidai M, Attia S (2005) Meiotic studies of some *Stipa* (Poaceae) species and population in Iran. *Cytologia* 70:23–31
- Sheidai M, Nouroozi M (2005) Cytological studies on some species of *Bromus* sect. *Bromus*. *Bot Lithua* 11:141–150
- Sheidai M, Koobaz P, Zehzad B (2003) Meiotic studies of some *Avena* species and populations in Iran. *J Sci IR Iran* 14:121–131
- Shkutina FM, Kozlovskaya VF (1974) Cytotoxicity in meiosis in some grass forms in the subtribe Triticinae. *Genetica* 10:5–12
- Sidorchuk IV, Deineko EV, Shumny VK (2007) The role of microtubular cytoskeleton and callose walls in the cytotoxicity process in tobacco (*Nicotiana tabacum* L.) pollen mother cells. *Tsitologiya* 49:876–880
- Singhal VK, Gill BS, Sidhu MS (1990) Cytology of woody members of Rosaceae. *Proc Plant Sci* 100:17–21
- Singhal VK, Kaur D, Kumar P (2008) Effect of cytotoxicity on the pollen size in ‘seabuckthorn’ (*Hippophae rhamnoides* L., Elaeagnaceae). *Cytologia* 73:167–172
- Singhal VK, Kaur D (2011) Cytotoxicity induced meiotic irregularities and pollen malformation in *Clematis graveolens* Lindley from the cold desert of Kashmir district of Himachal Pradesh (India). *Cytologia* 76:319–327
- Soltis DE, Soltis PS (1999) Polyploidy: recurrent formation and genome evolution. *Trends Ecol Evol* 14:348–352
- Sinoto Y (1922) On the extrusion of the nuclear substance in *Iris japonica* Thumb. *Tokyo Bot Mag* 36:99–110
- Smith L (1942) Cytogenetics of a factor for multiploid sporocytes in barley. *Am J Bot* 29:451–456
- Soman TA, Bhavanandan KV (1993) Temperature sensitive cytotoxicity in *Helicanthes elastica* (Desr) Dans (Loranthaceae). *Cytologia* 58:21–26
- Song ZQ, Li XF (2009) Cytotoxicity in pollen mother cells of *Salvia miltiorrhiza*. *Caryologia* 62:213–219
- Soodan AS, Wafai BA (1987) Spontaneous occurrence of cytotoxicity during microsporogenesis in almond (*Prunus amygdalus* Batsch) and peach (*P. persica* Batsch). *Cytologia* 52:361–364
- Souza VF, Pagliarini MS, Rodvalho M, Faria MV (2010) Meiotic behavior as a selection tool in silage corn breeding. *Genet Mol Res* 9:2096–2103
- Srivastava P, Kumar G (2011) EMS-induced cytotoxicity variability in safflower (*Carthamus tinctorius* L.). *Cytol Genet* 45:240–244
- Takats ST (1959) Chromatin extrusion and DNA transfer during microsporogenesis. *Chromosoma* 10:430–453
- Tarkowska J (1960) Cytotoxicity in the epidermis of scales and leaves and in meristem of root apex of *Allium cepa* L. *Acta Soc Bot Pol* 29:149–168
- Tarkowska J (1965) Experimental analysis of the mechanism of cytotoxicity: I. Cytotoxicity in vegetative tissues. *Acta Soc Bot Pol* 34:27–44
- Tarkowska J (1966) Experimental analysis of the mechanism of cytotoxicity: II. Cytotoxicity in the pollen mother cells of the lily — *Lilium candidum* L. *Acta Soc Bot Pol* 35:25–40
- Tyagi BR (2003) Cytotoxicity in pollen mother cells of spearmint (*Mentha spicata* L.). *Cytologia* 68:67–73
- Utsunomiya KS, Pagliarini MS, do Valle CB (2004) Chromosome transfer among meiocytes in *Brachiaria nigropedata* (Ficalho & Hiern) Stapf. (Gramineae). *Cytologia* 69:395–398
- Vasil IK, Aldrich HC (1970) Histochemical and ultrastructural study of the ontogeny and differentiation of pollen in *Podocarpus macrophyllus* D. Don *Protoplasma* 71:1–37
- Ventela S, Toppari J, Parvinen M (2003) Intercellular organelle traffic through cytoplasmic bridges in early spermatids of the rat: mechanisms of haploid gene product sharing. *Mol Biol Cell* 14:2768–2780
- Villeux R (1985) Diploid and polyploid gametes in crop plants: mechanisms of formation and utilization in plant breeding. In: Janick J (ed) *Plant breeding revision 3*. AVI Publishing Co, Westport, CT, p 442
- Wang XY, Nie XW, Guo GQ, Pan YF, Zheng GC, Cheng KC (2002) Ultrastructural characterization of the cytoplasmic channel formation between pollen mother cells of David lily. *Caryologia* 55:161–169
- Wang SY, Yu CH, Li S, Wang CY, Zheng GC (2004) Ultrastructural aspects and possible origin of cytoplasmic channels providing intercellular connection in vegetative tissues of anthers. *Russ J Plant Physiol* 51:97–106
- Weber J, Russell L (1987) A study of intercellular bridges during spermatogenesis in the rat. *Am J Anat* 180:1–24
- Weiling F (1965) Light and electron microscopical observation on cytotoxicity and its possible relation to cytotocytosis. *Planta* 67:182–212
- Whelan EDP (1974) Discontinuities in the callose wall, intermeiocyte connections and cytotoxicity in angiosperm meiocytes. *Can J Bot* 52:1219–1224

- Withers LA, Cocking EC (1972) Fine-structural studies on spontaneous and induced fusion of higher plant protoplast. *J Cell Sci* 11:59–75
- Woodworth RH (1931) Cytomixis. *J Arnold Arbor Harvard Univ* 12:23–25
- Xiu-Wan N, Xin-Yu W, Shang-wen C, Guo-chang Z (1991) X-ray microanalysis of ATP-ase reaction products in pollen mother cells of lily. *Acta Biol Exp Sinica*. doi: CNKI:SUN:SWSB.0.1991-01-002
- Yanyou W, Jiemei LPX (1996) Investigation to origin way of B chromosomes in plants. *Explor Nature*. doi: cnki:ISSN:10004041.0.1996-04-016
- Yen C, Yang JL, Sun GL (1993) Intermeiocyte connections and cytomixis in intergeneric hybrids of *Roegneria ciliaris* Trin. Nevski with *Psathyrostachys huashanica* Keng. *Cytologia* 58:187–193
- Yun-sheng W, Yong-ping C (2006) Study on cytomixis on pollen-mother-cell (PMC) of "Arbo" wheat nullisomic lines. *J Anhui Agric Sci* 34:25–26, doi: cnki:ISSN:0517–6611.0.2006-01-013
- Zani BG, Edelman ER (2010) Cellular bridges: routes for intercellular communication and cell migration (Short Survey). *Commun Integr Biol* 3:215–220
- Zheng GC, Yang QL, Zheng YR (1987) The relationship between cytomixis and chromosome mutation and karyotype evolution in lily. *Caryologia* 40:243–259