Plant Cell Rep (2017) 36:1641–1653
DOI 10.1007/s00299-017-2180-6

ORIGINAL ARTICLE

Meiotic chromosome behavior of the male-fertile allotriploid lily cultivar 'Cocossa'

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Received: 1 March 2017 / Accepted: 7 July 2017 / Published online: 24 July 2017

Abstract

Key message **Cytological observations of microsporogenesis in the allotriploid lily cultivar 'Cocossa' showed that viable pollen production could be attributed mainly to disoriented spindles, abnormal cytokinesis, and cytomixis during male meiosis.**

Abstract To identify the reasons why the allotriploid lily cultivar 'Cocossa' can produce aneuploid and euploid functional male gametes and can be used as the paternal parent in lily introgression breeding, we performed a detailed investigation of microsporogenesis using the conventional cytological methods. The allotriploid not only produced single pollen grains with variable sizes but also produced adherent pollen grains. Pollen viability was estimated at 50.1% based on staining and 30.8% based on germination. Based on the chromosomal analysis of BC_2 plants derived from Oriental cultivars (♀) crossed with the OOT cultivar 'Cocossa' (♂), it was concluded that the objective allotriploid contributed haploid (x), diploid ($2x$), and aneuploid chromosome complements. Common meiotic abnormalities were observed, indicating the high genetic imbalance of this allotriploid. In addition to normally oriented metaphase II spindles (linear and perpendicular), abnormal spindles, such as parallel, tripolar, fused, and multiple spindles, accounted for 6.21, 6.41, 14.27, and 1.17%, respectively. Tripolar and fused spindles resulted in the production of triads and dyads, which contributed to unreduced pollen production. Some microsporocytes exhibited complete or partial absence of cytokinesis, which led to relatively high frequencies of monads, dyads, and triads. Furthermore, the phenomenon of cytomixis during microsporogenesis occurred mainly in the first meiotic prophase and early development of pollen grains, which we assume is a possible cause of unreduced gamete generation. Our study offers a new resource for lily introgression breeding.

Keywords Lily · Allotriploid · Male gamete fertility · Disoriented spindle · Aberrant cytokinesis · Cytomixis

Introduction

Lilies, which belong to the genus *Lilium* (Liliaceae), are important bulbous flowers that are primarily used as cut flowers and potted plants worldwide. Most lily cultivars originating from intra-sectional hybridizations are classified into four groups: Asiatic (A), Longiflorum (L), Oriental (O), and Trumpet (T) (Van Tuyl et al. 2000). Because each group has its own unique set of horticultural traits, it is desirable to combine valuable traits from different groups into one new cultivar through crossbreeding (Luo et al. 2012). Crossbreeding between Oriental and Trumpet (OT) cultivars revolutionized flower colors in *Lilium* and its flower trade worldwide (Younis et al. 2014). The previous studies reported that most OT lily cultivars have

Communicated by Xian Sheng Zhang.

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diploid, triploid, and aneuploid forms, viz., diploids selected from F_1 hybrids or BC_1 hybrids from n gametes of F_1 and triploids mainly from $2n$ gametes and possibly, though less likely, from diploid × amphidiploid crosses. In contrast, aneuploid progenies resulted from viable aneuploid gametes (Luo et al. 2012, 2013; Zhou et al. 2014).

Since triploid plants have many desirable characteristics, they have attracted increasing attention. At present, many Polygonum-type autotriploid and allotriploid plants have been successfully used for the crossbreeding of many plant species (Ramsey and Schemske 2002; Ramanna and Jacobsen 2003). In contrast, the Fritillaria-type triploid lilies, both autotriploid (AAA) and allotriploid (AOA, LAA, LLO, OOT, MAD), can act as the maternal parent to hybridize with an appropriate diploid or tetraploid paternal parent, giving rise to mass euploid and aneuploid progenies (Lim et al. 2003; Barba-Gonzalez et al. 2006; Zhou et al. 2014; Xi et al. 2015). However, to our knowledge, a few studies on the successful production of BC_2 plants derived from crossing with viable allotriploid pollens have hitherto been reported for LAA-hybrids (Khan et al. 2009; Fang et al. 2014). No cases of allotriploid OOT lilies as the paternal parent have been reported. Thus, we sought to investigate the cytological basis for pollen fertility in the objective allotriploid OOT lily.

Variations in pollen size and fertility are usually attributed to irregular chromosome pairing and abnormal meiotic division, as has been reported in triploid *Populus* (Wang et al. 2010). Aberrations during chromosome pairing, segregation, spindle formation, or cytokinesis that might result in the formation of triads, dyads or monads, which are responsible for unreduced gamete production, have been reported in polyploid plants of *Citrus* (Bosco et al. 1999) and *Brachiaria ruziziensis* (Risso-Pascotto et al. 2005). Cytomixis has also been proposed as a possible mechanism for unreduced gamete production (Dewitte et al. 2012).

The objective of the present study is to investigate the detailed meiotic chromosome behaviors during microsporogenesis. We further discuss the impact of aberrant meiosis on variably sized pollen grains and its relationship with pollen viability in objective allotriploid lily.

Materials and methods

Plant material

The allotriploid OOT lily cultivar 'Cocossa' ($2n = 3x = 36$; Fig. 4a) was imported from The Netherlands and purchased from Hongyue Company, Zhejiang Province, China. The origin of the allotriploid OOT hybrid 'Cocossa' has been reported previously (Zhang et al. 2012). Flower buds used for cytological analysis were sampled from plants grown in a greenhouse at Beijing Forestry University under natural light with temperatures between 16 and 35 °C.

Pollen viability and size observation

For pollen viability staining, a mixed sample of pollen from two to three anthers of each analyzed plant was stained with 2% aceto-carmine and viewed under the Leica DM500 microscope (Heerbrugg, Switzerland). Swollen pollen grains that strongly stained red were assumed to be viable. The diameters of 1000 stained single pollen grains were measured using the attached calibrated micrometer of Leica DM500. Graphs were generated in Excel 2007 (Microsoft Corporation). Male gametes from allotriploid OOT hybrids are fertile based on pollen germination tests of more than 30 flowers per anthesis season (from June to July) for 3 years. Fresh pollen was collected from fully open flowers on the first day of anthesis, and samples were examined by culturing pollen on artificial liquid medium at pH 5.8 as proposed by Chung et al. (2013) in an incubator at 25 °C and 70% humidity with a 24-h light/dark cycle. A pollen grain was considered to have germinated if the pollen tube length was equal to or greater than the pollen diameter. Three microscopic field areas were randomly selected, and 30 plant samples were observed from each area.

Pollen morphological observation

Pollen morphology was studied using scanning electron microscopy (Hitachi S-3400, Tokyo, Japan). Dry pollen grains were directly attached to double-sided adhesive tape and coated with gold. Microscopic observation and image acquisition were conducted following the method described by Du et al. (2014).

Backcrosses and chromosomal counts

Pollen from 'Cocossa' was used to pollinate the diploid Oriental hybrid cultivars 'Chealse' and 'Royal Vanzanten.' Backcrossed seedlings were germinated on seed germination medium. The number of mitotic chromosome in root tip cells of paternal parent and BC_2 progenies was determined according to the technique by Barba-Gonzalez et al. (2006).

Cytological observations of microsporogenesis

Anthers derived from young flower buds at different developmental stages were fixed with freshly prepared

Carnoy's solution (3:1 ethanol-acetic acid) for 24 h at room temperature and then stored in 70% ethanol at 4 °C. Microsporocytes were squeezed out of the anthers into a drop of 1% carbol-fuchsin and squashed. All phases of meiosis were examined using light microscopy. The univalents were dispersed in the cytoplasm and, together with lagging chromosomes, chromosome bridges, micronuclei, and different types of spindle configurations in the meiotic process, were analyzed with variations in the meiotic products.

Results

Pollen viability and size variation

In the allotriploid analyzed here, the frequency of strongly stained red pollen grains was 50.1%, whereas shrunken and unstained pollen grains were observed 49.9% of the time (Table 1). In addition to single pollen grains, including regular smaller ellipsoidal and larger spherical pollen grains (Fig. 3a–h), a few (12.5%) adherent pollen grains were viable (Table 1; Figs. 1a, 3i–l). The diameters of stainable single pollen grains ranged from 72.24 to 180.53 μm, with an average of 126.60 μm (Fig. 2). The sizes of viable pollen grains varied considerably, indicating a wide range of ploidies. Furthermore, some giant pollen grains with diameters of more than 173.76 μm were found, which might indicate a very high ploidy level. Germination of pollen on artificial liquid medium revealed that 21.7% of single pollen grains and 9.1% of adherent pollen grains were viable (Table 1). For most of the adherent pollen grains that germinated, both single pollen grains of an adherent pollen grain were able to form pollen tubes (Fig. 1b).

Production of BC_2 progenies and chromosomal analysis

The results of backcrosses between Oriental hybrids (♀) with the allotriploid hybrid 'Cocossa'(♂) are shown in Table 2. A cytological analysis of 24 BC_2 progeny plants revealed that 7 of the progeny were euploids and 17 were aneuploids (Table 2). The chromosome numbers of the BC_2 progeny ranged from 24 to 36. Eight representative ploidy levels were described. The numbers of chromosomes were 24, 26, 28, 29, 32, 34, 35, and 36 (Fig. 4b–i). These findings demonstrated that the allotriploid hybrid 'Cocossa' produces viable haploid (x), diploid ($2x$) and aneuploid gametes at variable frequencies.

Abnormal meiotic chromosome behaviors

In this lily allotriploid, microsporocytes undergo two successive divisions of the nucleus and cytokinesis, finally forming a tetrad. Meiosis initiates when flower buds are approximately 25 mm in length. Buds ranging from 26 to 33 mm exhibited predominantly prophase I, whereas buds ranging from 34 to 36 mm exhibited metaphase I–telophase II. Most microsporocytes completed meiosis and became microspores when the bud length was >37 mm.

Chromosome behavior across developmental stages is described below. In most cases (60%), microsporocytes from all anthers in the same flower bud were at the same meiotic stage. In others (40%), mixed stages of meiosis were observed in the same flower bud and even in the same anther.

In each stage of meiosis, several abnormal chromosomal behaviors were recorded (Table 3), indicating complex chromosome pairing and unbalanced chromosome segregation. During the first meiotic division, the chromosome configuration at diakinesis was irregular, and univalents, bivalents, trivalents, and multivalents could be observed (Fig. 5a). At metaphase I, chromosomes that did not align on the equatorial plate should be univalents (Fig. 5b). At anaphase I, lagging chromosomes resulted from univalents, and chromosome bridges and lagging chromosomes were found (Fig. 5c, d). In this phase, chromosomal fusions and stickiness were also found (Fig. 5e, f). Chromosome bridges were observed in telophase I (Fig. 5g); chromosome stickiness resulted in thick chromosomal bridges restricting chromosome separation during telophase I (Fig. 5h). Micronuclei derived from lagging chromosomes and/or acentric fragments were found (Fig. 5i). Although some cells completed the first cytokinesis, the chromosome bridge did not break (Fig. 5j–l). Micronuclei were positioned in different regions in the dyad stage (Fig. 5m, n). Unequal chromosome segregation resulting in a dyad with one haploid nuclei and an empty microspore was observed (Fig. 5o), and restitution nuclei (Fig. 5p) were seen in the dyad phase.

During the second meiotic division, chromosome separation was asynchronous in pre-prophase and anaphase II (Fig. 6a, b), and chromosome bridges (Fig. 6c) were still observed. Chromosome disequilibrium segregation

Table 1 Pollen viability of the allotriploid OOT lily cultivar 'Cocossa'

Pollen type	Stained (%)	Unstained (%)	Germination (%)	Non-germination (%)
Single pollen grains	386 (37.6)	459 (44.7)	224 (21.7)	568 (55.1)
Adherent pollen grains	128 (12.5)	53 (5.2)	94 (9.1)	145 (14.1)

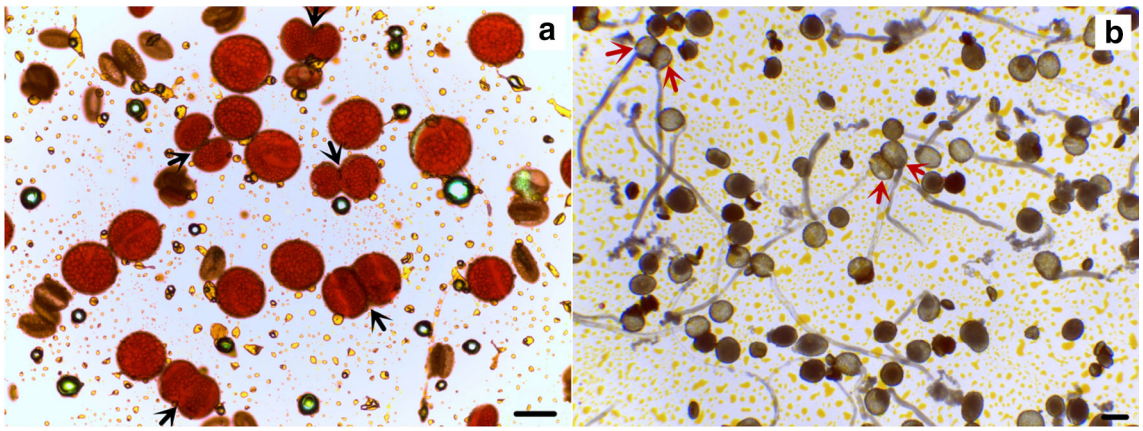

Fig. 1 Pollen stainability and germination test. **a** Pollen grains of different sizes and shapes dyed with aceto-carmine; stained adherent pollens (*black arrows*). **b** Viable pollen grains germinated on an artificial liquid medium; adherent pollen germination (*red arrows*). *Bars* 100 μm (color figure online)

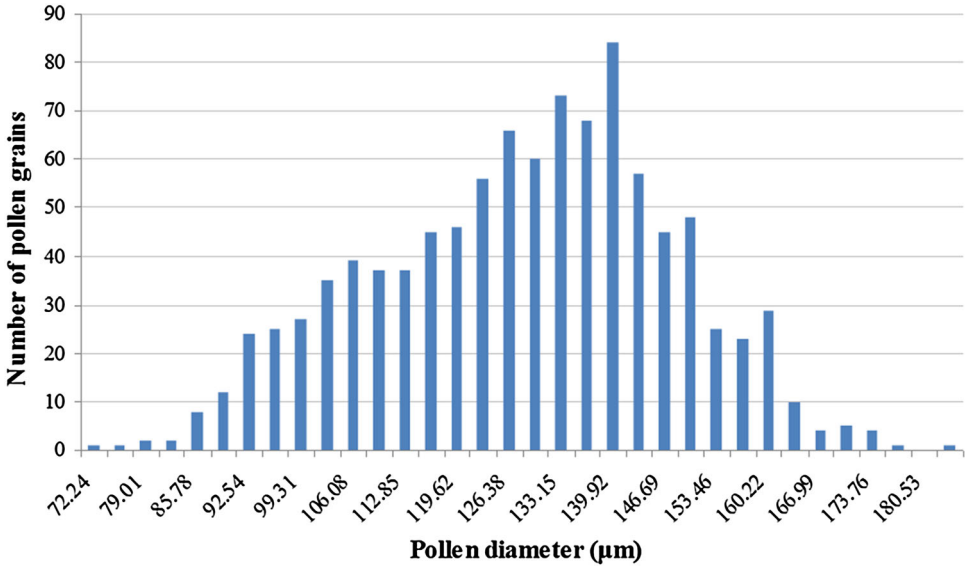

Fig. 2 Frequency distribution of the diameters of stainable single pollen grains

(Fig. 6d, i) and nuclei closely related to each other (Fig. 6e, j) were also found in anaphase II and telophase II. One or two chromosome bridges were observed in telophase II (Fig. 6f, g). Micronuclei were still observed in telophase II (Fig. 6h) and the tetrad stage (Fig. 6l). Unequal chromosome segregation resulting in an empty microspore was found in telophase II (Fig. 6k), as was unequal chromosome distribution in tetrads (Fig. 6m–p).

The abnormal orientation of spindles (Fig. 7) and cytokinesis (Fig. 8) in the second meiotic division varied in this allotriploid lily. In metaphase II, apart from normal spindles that had linear alignment (Fig. 7a) and were perpendicular (Fig. 7b), parallel (Fig. 7c), tripolar (Fig. 7d), fused (Fig. 7e), and multiple spindles (Fig. 7f) were also observed, with frequencies of 6.21, 6.41, 14.27, and 1.17%, respectively (Table 3). Consequently, tetrads (Fig. 7o), triads (Fig. 7p), dyads (Fig. 7q), and polyads (Fig. 7r) were produced. Three types of tetrads, tetragonal (Fig. 7m), tetrahedral (Fig. 7n), and linear arrangements (Fig. 7o), were observed. Furthermore, different types of meiotic products, i.e., polyads with six (Fig. 8a) or five (Fig. 8b) equal microspores and tetrads with one microcyte (Fig. 8c), were observed. Different configurations of triads (Fig. 8d, e), dyads (Fig. 8f–l), and monad products (Fig. 8m–p) were also observed. During microspore formation, microspores including some number of nuclei were found (Fig. 8q–t).

The extent of chromatin migration between cells in cytomixis may vary. A direct fusion of two adjacent cells was observed at prophase I (Fig. 9a, b). Most of the nucleus leave the cell central zone and pass to the adjacent cell through the cytomictic channel (Fig. 9c–e). Some hypoploid (Fig. 9f) and enucleated (Fig. 9g) cells were also observed due to partial and complete chromatin transfer at the dyad stage. In addition, large amounts of different fusion (Fig. 9h–l) and direct fusion between two proximate

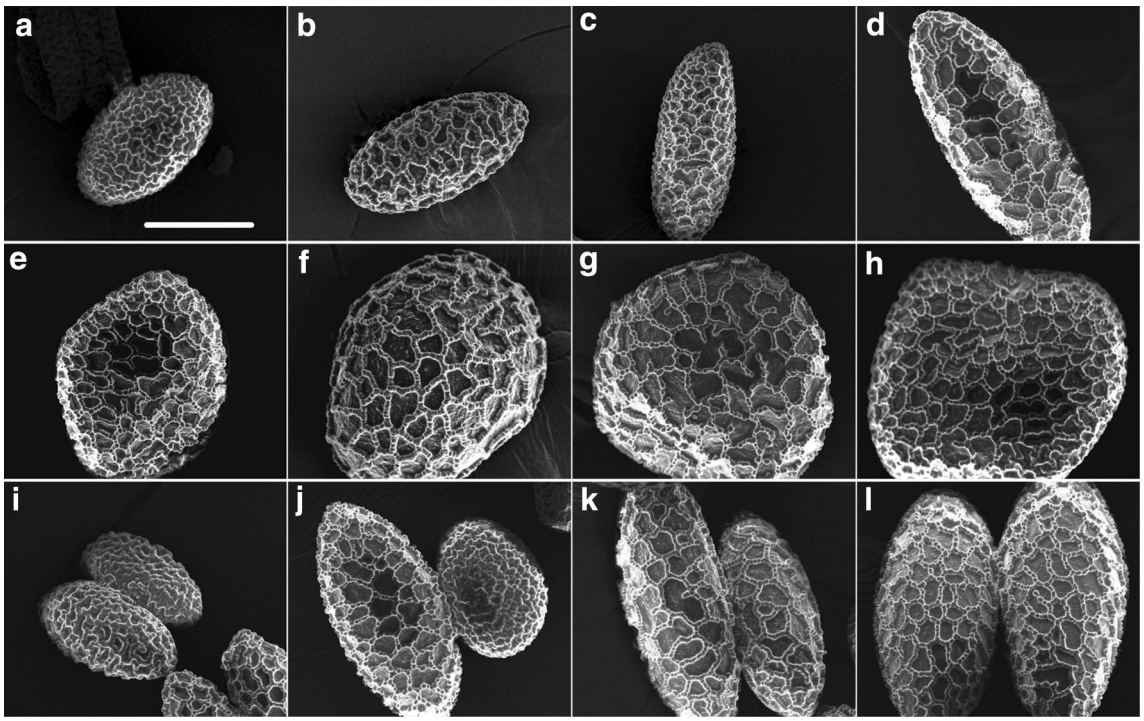

Fig. 3 Scanning electron micrographs of different pollen grains. **a–h** External morphology of single pollen grains. **i–l** External morphology of adherent pollen grains. *Bars* 50 μm

Table 2 Ploidy levels of BC_2 progenies resulting from crossing Oriental parents with allotriploid OOT lily cultivar 'Cocossa'

Parents		No. of plantlets analyzed	Ploidy level		
Female	Male		2*x*	3*x*	Others
Chealse	Cocossa	13	3		2 ($2x + 2$)
					3 ($2x + 4$)
					1 ($2x + 5$)
					3 ($3x - 2$)
					1 ($3x - 1$)
Royal Vanzanten	Cocossa	11	2	2	4 ($2x + 4$)
					2 ($2x + 5$)
					1 ($3x - 1$)

immature pollen grains (Fig. 9m, n) were observed at the early pollen grains development phases, which may result in jumbo-sized pollen grains. The most likely consequence of these events is the formation of unreduced pollen.

Discussion

In general, triploid BC_1 progenies derived via *Lilium* intersectional hybrids are likely highly sterile due to their complex genome constitution. However, the allotriploid OOT cultivar 'Cocossa' ($2n = 3x = 36$) used in this study was partially male fertile.

Pollen size measurement is an easy and commonly used method to screen for $2n$ pollen within a population (Dewitte et al. 2012). The association is caused by the positive correlation between DNA content and cell volume, which in turn influences pollen diameter. In general, the presence of unreduced pollen producers results in a bimodal pollen grain size distribution instead of a normal unimodal distribution (Tondini et al. 1993). In this allotriploid lily, the frequency distribution of the diameters of stainable single pollen grains followed a Gaussian distribution, which is different from the allotriploid white polar, which has a bimodal distribution (Wang et al. 2010). However, according to the triads, dyads and monads were formed in the meiosis products, which indicated the objective allotriploid production of unreduced pollen grains. The wide range of pollen diameters may represent the various chromosome numbers of pollen grains due to

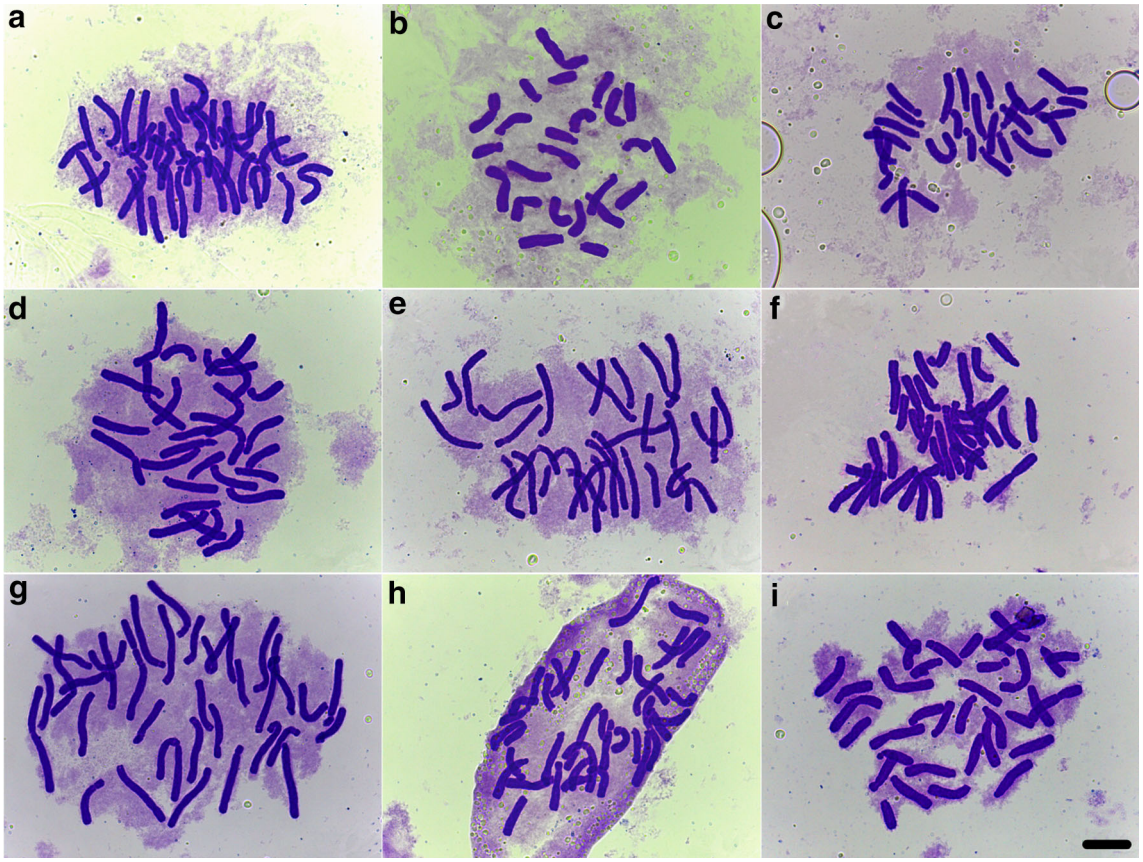

Fig. 4 Mitotic chromosome numbers in root tip cells from paternal parent and BC_2 progenies. **a** Paternal chromosome numbers of allotriploid hybrid 'Cocossa' $2n = 3x = 36$. **b–i** Chromosome numbers of BC_2 progenies. **b** $2n = 2x = 24$. **c** $2n = 2x + 2$=26. **d** $2n = 2x + 4$=28. **e** $2n = 2x + 5$=29. **f** $2n = 3x$-4 = 32. **g** $2n = 3x$-2 = 34. **h** $2n = 3x$-1 = 35. **i** $2n = 3x = 36$. *Bars* 20 μm

Table 3 Meiotic abnormalities recorded in the allotriploid OOT lily cultivar 'Cocossa'

Phases	No. of analyzed cells	No. of abnormal cells (%)	Abnormalities	No. of cells
Metaphase I	890	35.96	Univalents dispersed in cytoplasm	320
Anaphase I	502	60.96	Lagging chromosomes	263
			Bridge	43
Telophase I	742	74.53	Micronuclei	187
			Bridge	132
			Chromosome stickiness	234
Metaphase II	1030	28.06	Parallel spindles	64
			Tripolar spindles	66
			Fused spindles	147
			Multiple spindles	12
Anaphase II	480	9.17	Bridge	44
Telophase II	460	56.52	Micronuclei	192
			Bridge	68
Meiotic product	1056	40.15	Monad	29
			Dyad	261
			Triad	125
			Polyad	9

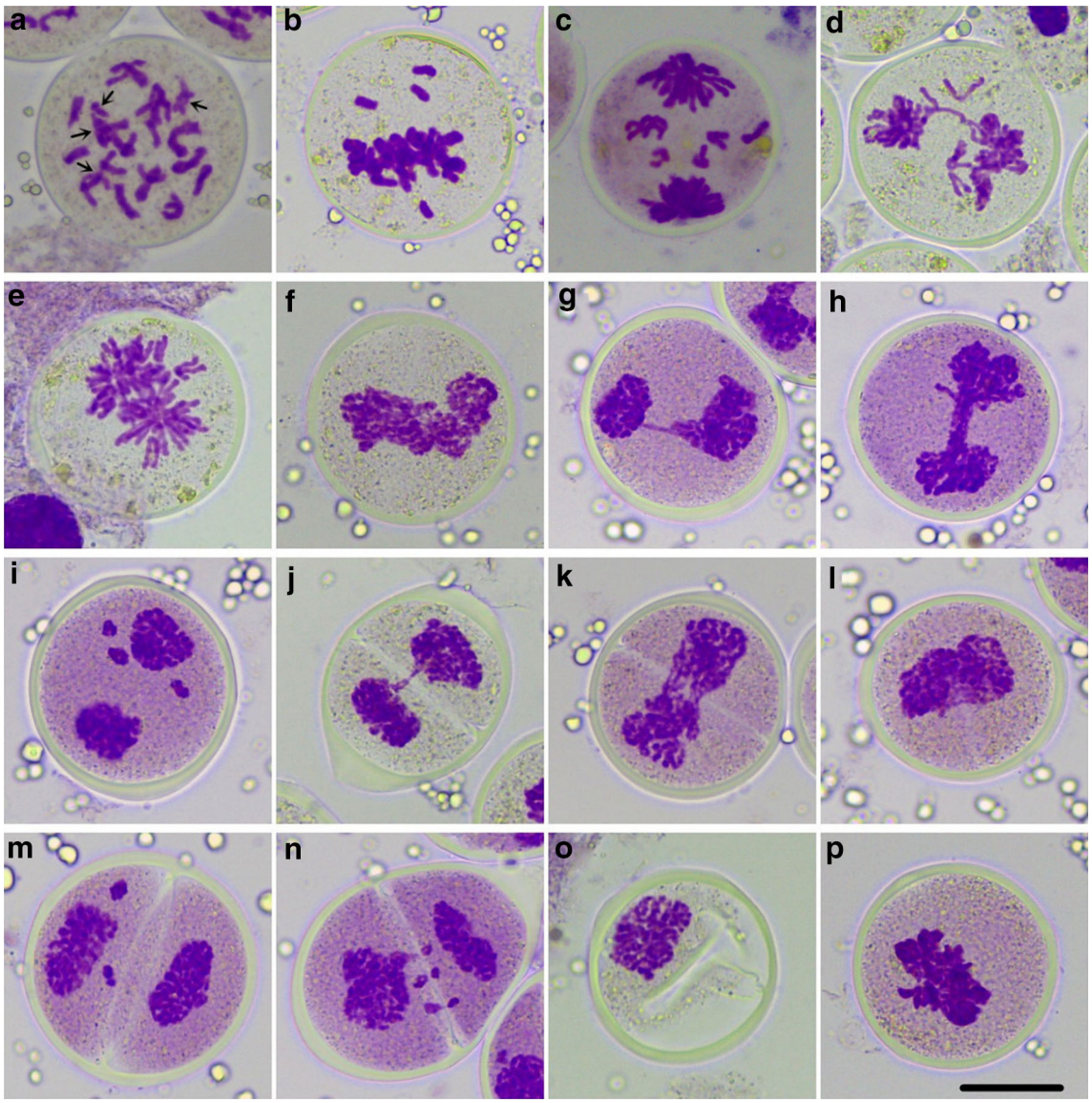

Fig. 5 Meiotic abnormalities in the first meiotic division. **a** Microsporocytes in diakinesis, with univalents, bivalents, trivalents, and multivalents (*arrows*). **b** Univalents did not align on the equatorial plate in metaphase I. **c** Anaphase I with lagging chromosomes at the center of the cell. **d** Anaphase I with lagging chromosomes and a chromosome bridge in the same cell. **e** Chromosome fusion in anaphase I. **f,** Chromosome stickiness in anaphase I. **g** Chromosome bridge in telophase I. **h** Thick chromosome bridge in telophase I. **i** Three micronuclei with different chromosomal contents at telophase I. **j**, **k** Chromosome bridges still remaining at the dyad stage. **l** Incomplete nuclear division. **m** Micronuclei were allocated to a single microspore. **n** Micronuclei were positioned the in equatorial region in telophase I. **o** Unequal chromosome segregation. **p** Restitution nucleus at the dyad stage. *Bars* 20 μm

irregular chromosome pairing and unbalanced chromosome segregation. Therefore, triploid meiosis is hardly expected to give rise to aneuploid gametes with a half triploid chromosome number ($n = 3x/2$), whereas some unreduced pollen grains may not have exactly $3x$ the DNA content. This conclusion is supported by the chromosome numbers of the BC_2 progenies, where chromosome numbers of 30 and 48 were not seen. The chromosome number of BC_2 progenies varied between 24 and 36, which is consistent with the ploidy level of progenies from allotriploid LAA lily cultivar 'Ceb Dazzle' as male (Fang et al. 2014).

Adherent pollen grains were observed in this allotriploid. Several possible causes could explain the release of pollen in cohesion. Adherent pollen may be associated with incomplete degeneration of the primary microspore cell wall beyond the tetrad stage (Volkova et al. 2016). Another reason proposed for the permanent binding of pollen in cohesion could be a lack of callose deposition between the microspores during microspore separation in the tetrad period (Preuss et al. 1994; Rhee et al. 2003) or its early disappearance after the end of the tetrad period (Lora et al. 2009). Pollen tubes emerging from different pollen

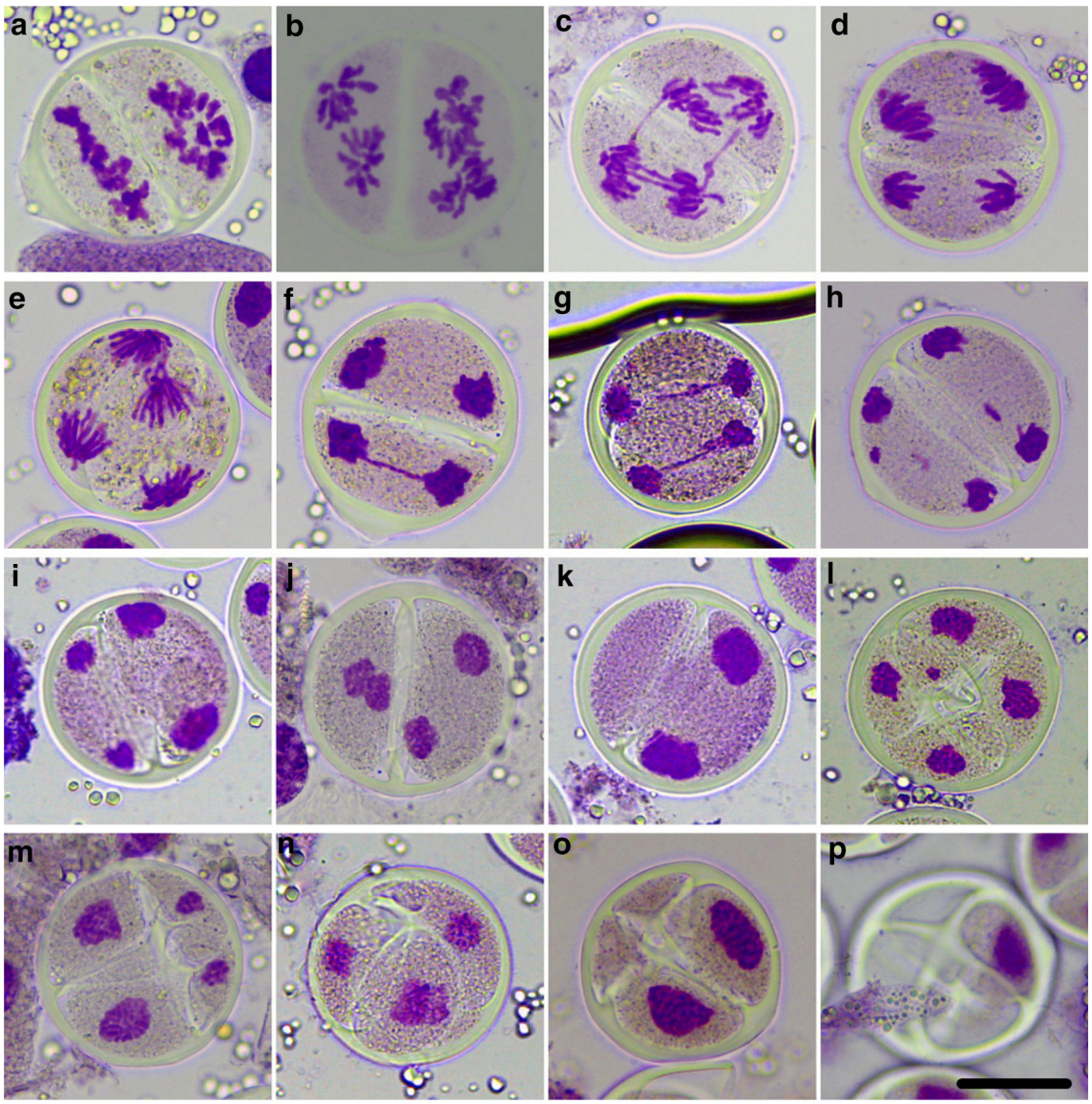

Fig. 6 Meiotic abnormalities in the second meiotic division. **a** Chromosome separation showing asynchronicity in pre-prophase. **b** Chromosome separation showing asynchronicity in anaphase II. **c** Chromosome bridges in anaphase II. **d** Chromosome disequilibrium segregation in anaphase II. **e** Chromosome fusion in anaphase II. **f**, **g** One or two chromosome bridges in telophase II. **h** Micronuclei in telophase II. **i** Chromosome disequilibrium segregation in telophase II. **j** Two nuclei fusion in telophase II. **k** Chromosome asymmetric division in telophase II, leading to an empty spore. **l** Tetrad with one micronucleus. **m** Unbalanced chromosome distribution in tetrad. **n**–**p** Tetrads caused by unequal cytokinesis, leading to one or more empty microspores. *Bars* 20 μm

grains of an adherent pollen pair were observed in this study (Fig. 1b); this phenomenon was also found in *Scheuchzeria palustris* pollen germination, which recognized the primary mechanisms of microspore cohesion, namely, that pollen grains are held together in the dyads due to a simple fusion of tectal layers. This fusion occurs in the late tetrad period (Volkova et al. 2016). Thus, additional studies are required to confirm the primary reason for pollen cohesion and sticking in a broad sample of our objective allotriploid.

In our investigation of microsporogenesis in an objective allotriploid lily, although common abnormalities resulting in pollen sterility were noted, other meiotic abnormalities such as disoriented spindle, aberrant cytokinesis, and cytomixis were also observed, which may be responsible for the pollen fertility.

Accurate spindle positioning is a critical aspect of cell division, as it ensures that each daughter cell contains a single nucleus (Brownfield et al. 2015). Abnormal spindle orientation may lead to meiotic nuclear restitution, resulting in unreduced gamete production (Bretagnolle and Thompson 1995). Tripolar and fused spindles have been identified as reasons for triad formation and dyad formation, respectively, and are currently the most accepted

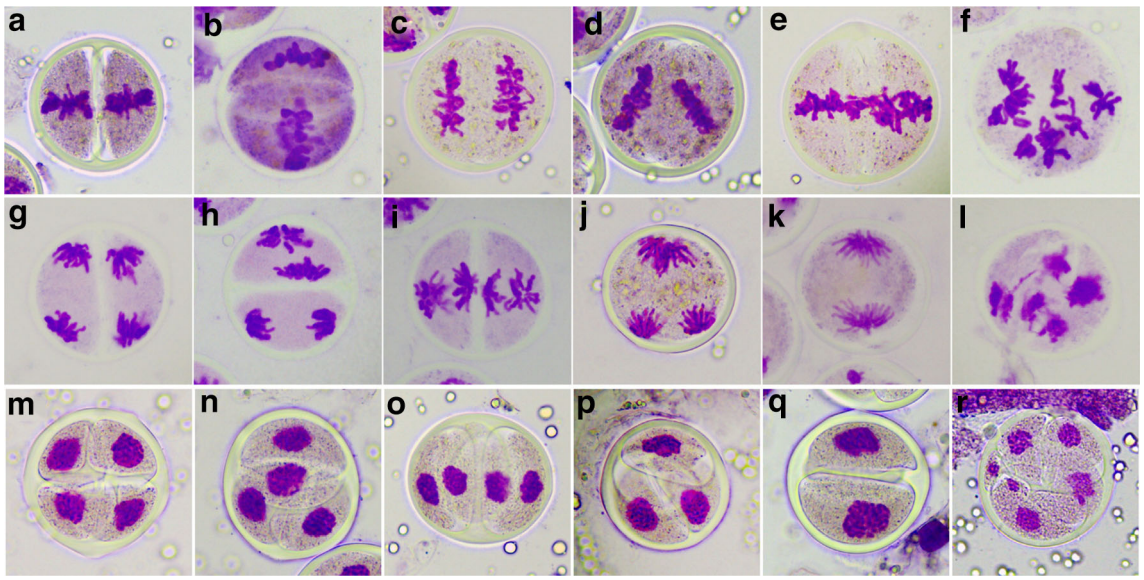

Fig. 7 Aspects of meiotic spindles at metaphase II in microsporocytes. **a** Linear spindles. **b** Perpendicular spindles. **c** Parallel spindles. **d** Tripolar spindles. **e** Fused spindles. **f** Multiple spindles. **g**–**l** Spindles at anaphase II, which formed from **a**–**f**. Meiotic products of telophase II (**m**–**r**). **m** Tetragonal tetrad. **n** Tetrahedral tetrad. **o** Linear tetrad. **p** Triad. **q** Dyad. **r** Polyad with six unequal microspores. *Bars* 20 μm

causes (Zhang and Kang 2010). The orientation of metaphase II spindles determines the tetrad configuration: linear and perpendicular orientation spindles might result in the production of tetragonal and tetrahedral tetrads observed in *Lilium*. In this study, tetragonal, tetrahedral, and linear tetrads were observed in meiotic products, which indicated that linear, perpendicular, and parallel spindles might not contribute to dyad formation. Multiple spindles have been reported in several plant species (Risso-Pascotto et al. 2005; Wang et al. 2010; Luo et al. 2013) and are strongly correlated with abnormal cytokinesis, which leads to polyad formation (Tilquin et al. 1984). Tel-Zur et al. (2005) assumed that most of the products of polyad divisions contain incomplete chromosome complements and are, therefore, unlikely to mature into functional pollen grains. Multiple spindles were observed in the second meiotic division of this allotriploid, and polyads with six or five equal microspores and six unequal microspores occurred with low frequency (0.85%). In contrast, greater frequencies of multipolar spindles by spontaneous occurrence were reported among interspecific and intergeneric hybrids (Risso-Pascotto et al. 2005). In addition, a few polyads, including main microspores with one or more microcytes, were also observed. Wang et al. (2015) analyzed the indirect immunofluorescence of microtubular cytoskeletons and showed that some micronuclei surround by microtubules formed microcytes at the tetrad stage. Microcyte formation likely resulted in the loss of chromosomes from microspores, which may increase the variation in pollen size.

Aberrant cytokinesis can also result in unreduced gamete formation (Dewitte et al. 2012). In meiosis of higher plants, radial microtubule systems (RMS) are thought to play primary roles in the organization and apportionment of the cytoplasm, the location of division planes, and the definition of nuclear cytoplasmic domains (Brown and Lemmon 1991). In the allotriploid lily studied here, the occurrence of different types of monads, dyads, and triads that may be derived from the RMSs were completely or partially lacking in some microsporocytes, as was found in an allotriploid white poplar (Wang et al. 2010).

Several cytokinetic mutants, which result in a single microspore with four nuclei and giant pollen development, have been identified in *Arabidopsis* (Hulskamp et al. 1997; Spielman et al. 1997). In this study, both normal and defective meiotic cytokineses were observed. Defective cytokinesis resulted in multinucleate microspore production, similar to allotriploid white poplar (Wang et al. 2010). This outcome indicated that the meiotic cytokinesis of this triploid is likely to be involved in a similar mechanism at the molecular level.

Cytomixis is defined as the intercellular migration of nuclei through cytoplasmic connection channels (Mursalimov and Deineko 2015). This phenomenon has been more commonly observed in male meiosis of numerous plant species (Singhal and Kumar 2008; Mursalimov et al. 2013; Rani et al. 2016). Cytomixis was assumed as one of the origins of 2*n* gametes, and it has been reported in genetically unbalanced plants (hybrids, aneuploids and

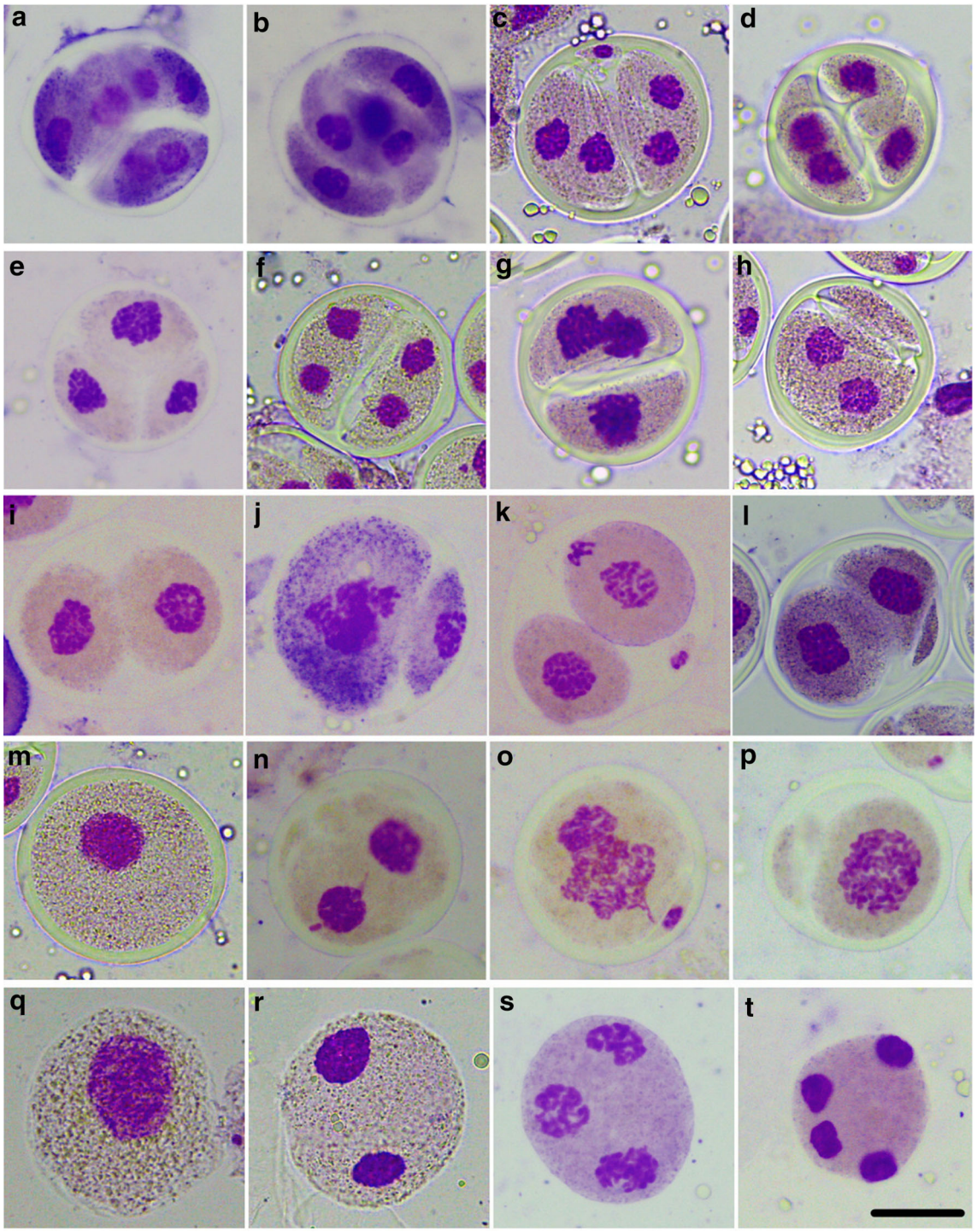

Fig. 8 Aberrant cytokinesis in the tetrad phase. **a** Polyad with six equal microspores. **b** Polyad with five equal microspores. **c** Tetrad with one microcyte. **d** Triad derived from one normally dividing nucleus and two fusion nuclei. **e** Asymmetry triad. **f** Dyad with two fusion nuclei**. g** Dyad with one fusion nucleus. **h** Dyad with one fusion nucleus and one empty microspore. **i** Symmetric dyad. **j** Unbalanced chromosome distribution in dyad. **k** Dyad with one microcyte. **l** Dyad with one empty-nucleus cell. **m** Monad with one nucleus. **n** Monad with two fusion nuclei. **o** Monad with one microcyte. **p** Monad with one empty-nucleus cell. **q** Giant microspore with one primary nucleus. **r** Microspore with two nuclei. **s** Microspore with three nuclei. **t** Microspore with four nuclei. *Bars* 20 µm

polyploids), such as *Dactylis* (Falistocco et al. 1995), *Sorghum bicolor* (Ghaffari 2006), and *Himalayan poppy* (Singhal and Kumar 2008). The previous analyses revealed that cytomixis is most frequently observable in the first meiotic prophase, such as in tobacco (Mursalimov and Deineko 2015), cereals (Sidorchuk et al. 2016), and lily

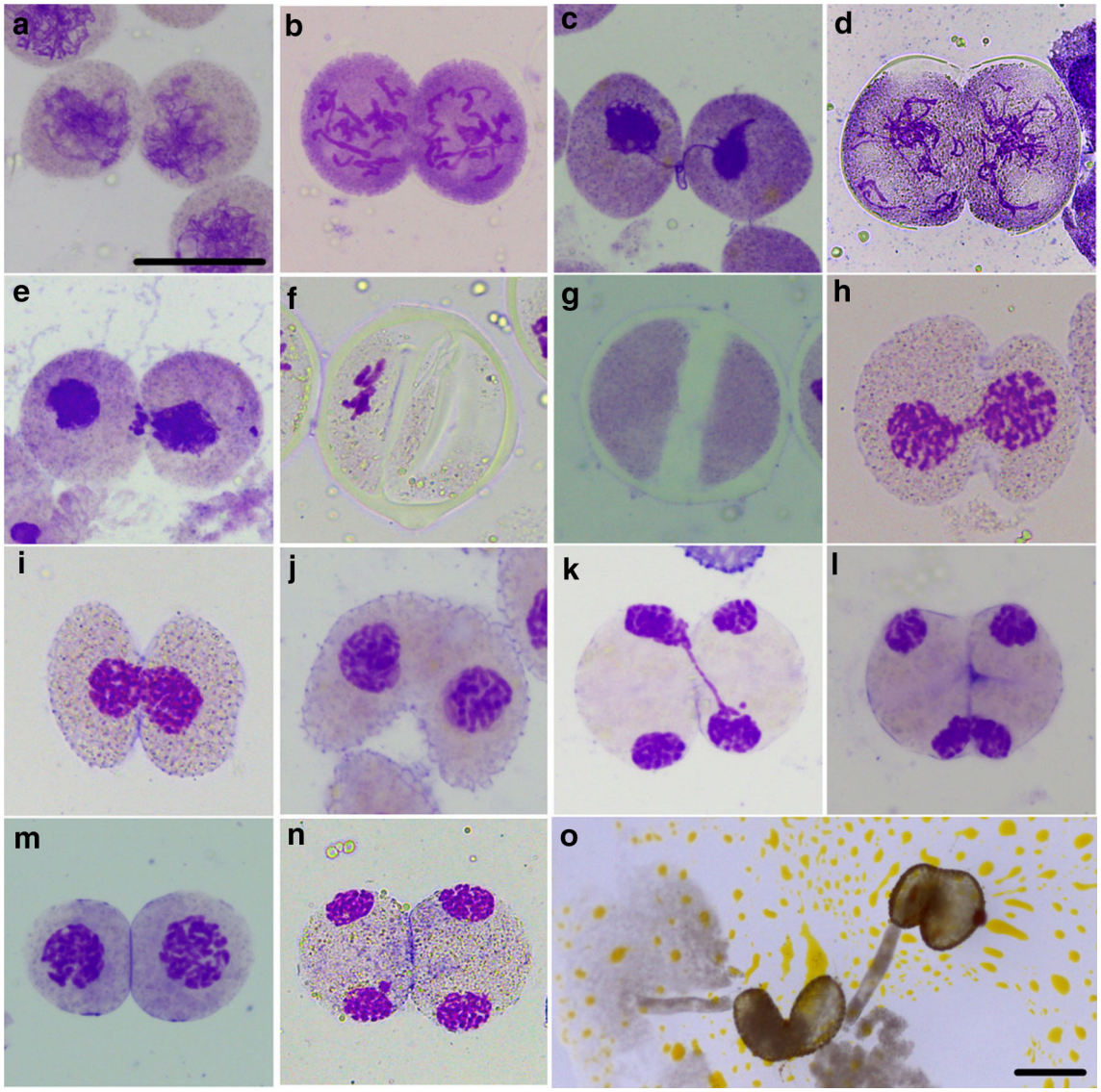

Fig. 9 Cytomixis. **a**, **b** Direct fusion of two microsporocytes at the prophase I stage. **c** Chromatin transfer through narrow cytoplasmic strands. **d** Chromatin transfer through broad cytoplasmic channels. **e** Chromatin transfer through multiple chromatin strands. **f** Hypoploid cell. **g** Enucleated cell. **h**–**l** Chromatin transfer between two immature pollen grains. **m**, **n** Direct fusion of two immature pollen grains. **o** Fused pollen germination. *Bars* 100 μm

(Zheng et al. 1987). In our study, a similar cytomixis phenomenon during microsporogenesis occurring mainly in the first meiotic prophase was observed, and we showed that cytomixis derived from early pollen grain development could produce viable pollen (Fig. 9o).

Conclusion

Based on pollen stainability and germination tests, following the backcross trial confirmation, an objective allotriploid lily was shown to be partially male fertile. The number of chromosomes of the BC_2 progenies ranged from 24 to 36. These findings suggested that the objective allotriploid lily contributed haploid (x), diploid (2*x*), and aneuploid chromosome complements. The male gamete fertility could be attributed mainly to disoriented spindles, abnormal cytokinesis, and cytomixis during microsporogenesis. These naturally occurring functional male gametes in allotriploid hybrid genotypes are highly valuable for introgression breeding of lilies.

Author contribution statement GXJ and XQZ conceived and designed the experiments; XQZ and QZC performed the experiments; XQZ analyzed the data and wrote the manuscript; QZC processed pictures; and PZ conducted chromosomal counts. All authors read and approved the manuscript.

Acknowledgements This work was supported by Grants from the National Forestry Public Welfare Industry Research Project (201204609) and the National Natural Science Foundation of China (31470106).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

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