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# Meiotically asynapsis-induced aneuploidy in autopolyploid *Arabidopsis thaliana*

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**Abstract** The patterns of homologue segregation are the basis for euploidy or aneuploidy formation in diploids and allo-/auto-polyploids. Homologue segregation in diploids resembles that in allopolyploids during meiosis; however, meiotic chromosome behavior in autopolyploids is complicated by multiplication of homologous chromosome components. Obviously, loss of single chromosomes (or segmented chromosomes) frequently leads to abortion of reproductive gametes in diploids and allopolyploids. In contrast, the consequence of chromosome loss in autopolyploids is effortlessly compensated for by the presence of multiplied chromosome complements. Here, we use the meiotically asynaptic gene *asy1*, in combination with polyploidization, to elucidate aneuploidy formation in autotetraploid Arabidopsis. The results indicate that, due to homologous asynapsis in meiotic prophase I, retarded chromosome losses could induce aneuploidy during gametogenesis in autotetraploid *asy1*. The severe loss of individual chromosomes probably reaches the haploid genome among selfed or backcrossed progeny, leading to stochastic chromosome loss in Arabidopsis. Reciprocal crosses of autotetraploid *asy1* with wild-type prove a pathway of duoparental transmission of aneuploidy (hypoploidy and hyperploidy). Viable hypoploids over-transmit via male gametes; conversely, viable hyperploids transmit mainly in female gametogenesis. This result suggests a more stringent maternal restriction of ploidy transmission in autopolyploid Arabidopsis.

**Keywords** Asynapsis · Aneuploidy · Autopolyploid · Chromosome · Arabidopsis

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## Introduction

Change in ploidy is a prominent process in plant speciation and genome diversity. Approximately 70% of flowering plants have undergone polyploidization during evolution (Ramsey and Schemske 1998; Soltis et al. 2008). In plant sexual cycles, the chromosome set is reduced by half meiotically due to the fact that there are two rounds of nuclear division to every one round of DNA replication producing haploid gametes, unequivocally demonstrating that ploidy alternation of chromosome sets ($2n \rightarrow n$) dominates plant reproductive life cycles.

Based on nuclear chromosome sets, the ploidy of flowering plants can be categorized into three distinct groups: euploid, hypoploid and hyperploid; hypoploidy and hyperploidy together are termed aneuploidy. Cytogenetically, euploid sets of chromosomes arise from equal segregation of homologous chromosomes under the strict surveillance of meiotic genes. The malfunction of this machinery can lead to fatal improper segregation of homologous chromosomes and produce aneuploid progeny via microspore cells or embryo-sac cells.

The drastic effects of imbalanced gene-dosage on phenotype (e.g., aborted pollen with reduced fertility) in aneuploid plants are apparently more severe than those in polyploid plants (Birchler et al. 2007; Huettel et al. 2008). Phenotypic effects could be interpreted by the plausible hypothesis that the dosage imbalance of genes encoding

regulatory molecules and/or transcription factors disturbs the stoichiometry of multi-component regulatory complexes and eventually disrupts normal cellular processes (Birchler et al. 2007). Typically, the dosage imbalance involves the products of house-keeping genes and metabolic processes. In contrast, polyploid plants, with their increased dosage of overall genome, exhibit some specific changes in phenotype but seldom exhibit the extreme effects observed in aneuploid plants. Contrasting with the effects caused by polyploidization, aneuploidization engenders more negative than positive effects (Guo and Birchler 1994). For instance, in comparison with diploid plants such as Arabidopsis, tetraploids have reduced fertility and vigorous development, while aneuploids usually exhibit severe infertility and some developmental defects. The aneuploidy induced by segmental chromosomes in maize is associated with severe phenotypic syndromes including reduced stature, tassel alterations and knot transplacement (Makarevitch et al. 2008), and, where gene expression was examined, it was also found to be affected. Besides, the copy number of genes on individual chromosome can be multiplied by aneuploidization. Specifically, phenotypes are regulated quantitatively, and multiplying a single allele can contribute to a phenotypic alternation. Thus, aneuploidization can facilitate mapping of quantitative trait loci. The effects of aneuploidy on genomic structure and epigenetic changes have been documented (Mittelsten Scheid et al. 1996; Papp et al. 1996; Matzke et al. 2003; Huettel et al. 2008). Aneuploidy-associated structural rearrangement was triggered by chromosomal imbalance, and epigenetic alteration also occurred in specific genotypes.

Although recent investigations have referred to aneuploidy and its genetic variation (Henry et al. 2005), as well as the effects of aneuploidy on genome structure and expression (Huettel et al. 2008), so far there have been few studies on aneuploidy transgeneration and its underlying mechanism in polyploid Arabidopsis in comparison with wealth of information available on the role of polyploidization in plant speciation. Using asynaptic gene *asy1* (Caryl et al. 2000), the present study aims to characterize aneuploidy transmission and its cytogenetic role in polyploid Arabidopsis. In practice, we hope our work will provide a useful tool for plant breeding through utilizing or avoiding aneuploidy.

## Materials and methods

### Plant materials and growth conditions

Diploid wild-type and heterozygous *asy1 Arabidopsis thaliana* Columbia ecotype (Col) were obtained from the Arabidopsis Biological Resources Center (ABRC). All plants used were grown in a growth chamber provided with compound light systems of white and fluorescent lamps at 22°C with a 16/8 h light/dark alternating photoperiod.

### Plant tetraploidization and flow cytometric analysis

Tetraploid *A. thaliana* (wild-type and heterozygous *asy1*) were generated as follows: diploid Arabidopsis seeds were surface sterilized with 13%(w/v) bleach solution (sodium hypochlorite), sown on 1/2 MS agar medium (Murashige and Skoog 1962) supplemented with 0.5% sucrose, stratified for 4 days at 4°C in the dark to relieve seed dormancy, then moved to 16/8 h light/dark, and grown for 2 weeks. Seedlings on the plate were submerged for 2 h in 0.1% (w/v) colchicine solution in darkness. The treated seedlings were washed with copious amounts of fresh tap water to remove the residual chemical solution, and then gently transplanted into moist soil. Seeds were collected from individual plants and plumpish candidates were selected under a stereomicroscope and sown on moist soil. Nuclear genome content of these plants was estimated by flow cytometry. Briefly, at the rosette stage, a single leaf was collected and chopped in 250 µl ice-cold nuclei extraction buffer with sharp razor blade in a round Petri dish sitting on a bed of ice until a fine suspension was obtained. After incubating on ice for 30 s to 1 min, 750 µl ice-cold Cy-Stain (Partec) solution was added to stain nuclei with fluorescent dye, and the suspension was filtered through 50 µm nylon mesh and incubated for 1 min, then analyzed in a flow cytometer with UV light (wavelength $\lambda = 420$ nm) for ploidy determination. The ploidy level of unknown samples was compared with internal standards determined by chromosome counting of known diploid and tetraploid plants generated under the same growth conditions.

### Genomic DNA extraction and genotyping

Fresh young leaves of individual plants were collected and quickly frozen in liquid nitrogen. Genomic DNA was extracted with Nucleon PhytoPure Genomic DNA Extraction Kits (GE Healthcare) according to the manufacturer's instructions. Tetraploid *asy1* plants were selected from among a large selfed population of heterozygous *asy1*; genotyping was achieved by PCR reactions with the insertional T-DNA primer pairs kan +/kan− and wild-type ASY1-geno-F/ASY1-geno-R: kan + (5′-TTTTGTCAAGA CCGACCTGTCC-3′), kan− (5′-ATGCTCTTCGTCCAG ATCATCC-3′), ASY1-geno-F (5′-CTCCATTTCGTATT AGCTGT CG-3) and ASY1-geno-R (5′-CTAGTCTA-CAAGTCGAAATGAGTC-3). PCR reactions were performed using standard procedures: denaturing at 94°C for 30 s; annealing at 55°C for 30 s; elongation at 72°C for 30 s to 1 min; 35 cycles in total.

### Cytological analysis

Chromosomes were prepared as described previously by Ross et al. (1997) and Armstrong et al. (2002) with minor modifications. Briefly, whole inflorescences with flower buds of appropriate sizes of 0.5–2.0 mm in length were collected, and fixed in freshly prepared ethanol/acetic acid (3:1) overnight at room temperature. Fixed inflorescences were then rinsed in distilled water (3 × 5 min) and citrate buffer (10 mM sodium citrate, pH 4.5; 3 × 5 min). Subsequently, the flower buds were digested with mixed enzymes in 0.3% (w/v) pectolyase (Sigma), cellulase (Sigma) and cytohelicase (Sepracor) in citrate buffer at 37°C for 3 h. After digestion, the flower buds were transferred to citrate buffer and stored at 4°C until use. Individual flower buds were transferred onto clean slides under a dissecting microscope and chopped to form a homogeneous mixture with addition of a drop (5–7 µl) of 60% acetic acid until transparent. Slides were placed on a hot plate (55°C) for 10 s, a clean glass cover was applied and gently squashed, and the slides were then quickly frozen and the cover removed with a blade. The prepared slides were air-dried and finally stained with 5 µg/ml DAPI (4′, 6′-diamidino-2-phenylindole) solution.

### Germination test and chromosome count

To test their viability, aneuploid seeds collected from selfed and crossed tetraploid plants were surface sterilized with 13% (w/v) bleach solution, and sown on solid 1/2 MS agar medium. Meanwhile, root-tips of 3-day-old seedlings were pretreated for 1 h in ethanol/acetic acid (3:1) fixative at room temperature and then rinsed with citrate buffer solution (10 mM, pH 4.5; 3 × 5 min), and then incubated in an enzyme mixture including 0.3% (w/v) pectolyase (Sigma), 0.3% (w/v) cytohelicase (Sepracor), and 0.3% (w/v) cellulase (Sigma) in citrate buffer for 30 min at 37°C. After enzymatic treatment, the root-tips were transferred to 45% acetic acid for 5–10 min until the root-tips turned transparent. Chromosome squash was prepared as previously described by Armstrong et al. (2002) and stained with 10 µg/ml DAPI solution.

## Results

### Colchicine-induced tetraploidization

Different methodologies can be used to generate autotetraploid Arabidopsis from diploid progenitors. Polyploidization occurs during plant callus culture in vitro. Although the ploidy levels range between 2 and 15 × (10–75 chromosomes) in Arabidopsis primary tissue culture, the frequency of polyploidy cells is nevertheless still affected by culture time (Fras and Maluszynska 2004). Thus direct polyploidization by colchicine treatment was preferred as a simpler and faster procedure. In addition, chromosome behavior was checked after colchicine-induced autotetraploidization in Arabidopsis wild-type background (Weiss and Maluszynska 2000; Santos et al. 2003), which provides an additional reference for analyzing autotetraploid *asy1*. In the procedure, diploid heterozygous *asy1* were submerged in a 1% colchicine solution for 2 h in darkness, and then transferred to soil. The plumpish selfed seeds collected were sown and, at the rosette stage, the genomic contents of individual plants were determined by flow cytometric analysis (Fig. 1a–d). Counting of the chromosomes of the young flower buds at the mitotic prophase confirmed the results of flow cytometric analysis (Fig. 1e). The genotypes of tetraploid candidates were determined by PCR with primer pairs corresponding to wild-type and the T-DNA insertion referred to above.

### Tetraploid *asy1* and its fertility

To analyze aneuploidy propagated by tetraploid *asy1*, the segregating population was chosen from the selfed tetraploid heterozygous *asy1* on the basis of Mendel's law of segregation. Of the tetraploid candidates, 3 out of 113 were identified as tetraploid *asy1*, and 4 out of 15 diploid candidates as diploid *asy1*, which fitted well with expected segregation ratios ($P$ value = 0.01, 1:36 and 1:4, respectively). In most phenotypic features, tetraploids resemble diploids in all aspects of their vegetative growth and development in Arabidopsis (Fig. 2); however, owing to doubled genome content, tetraploids have an elongated growth period and increased organ size. Besides, tetraploid seeds appear relatively plumpish and larger than diploids. Conversely, tetraploids have reduced fertility compared to diploids, indicating the likely disrupted viability of pollen and/or embryo sac after tetraploidization. Likewise, diploid and tetraploid *asy1* show considerably reduced fertility and much shortened siliques compared with wild-type (Fig. 3). Nevertheless, the tetraploid *asy1* exhibits fertility levels similar to that of diploid *asy1* in the mutant, in contrast to the wild-type background (Fig. 4). This intriguing observation leads us to hypothesise that polyploids potentially tolerate more mutation than diploids via aneuploidy formation with respect to fertility.

### Male meiotic observation in tetraploid *asy1*

Cytological analysis of diploid *asy1* has been described during development of pollen mother cells (Ross et al. 1997) and embryo-sac cells (Armstrong et al. 2002). However, less information is available about meiotic

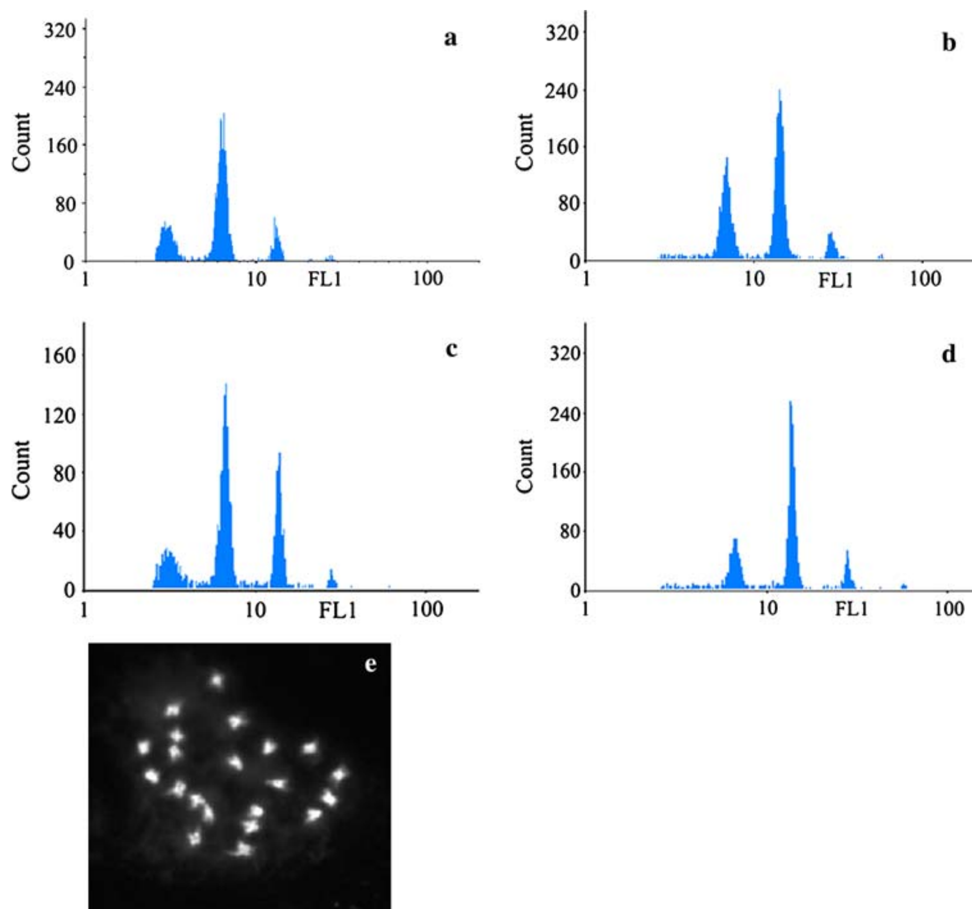


**Fig. 1** Flow cytometric analysis for tetraploidy after colchicine treatment (**a–d**) and chromosome counts (**e**). In the cytometric analysis, the first gain peak of genomic contents at the tetraploid-level shifts two-fold behind that of the diploid level. **a**, **b** Controls in diploid and tetraploid 2 × Col and 4 × Col; **c**, **d** the assayed diploid and heterozygous *asy1* tetraploid. **e** Chromosome counting of tetraploid *asy1* at prophase of mitosis (20 chromosomes, each chromosome contains two sister chromatids)

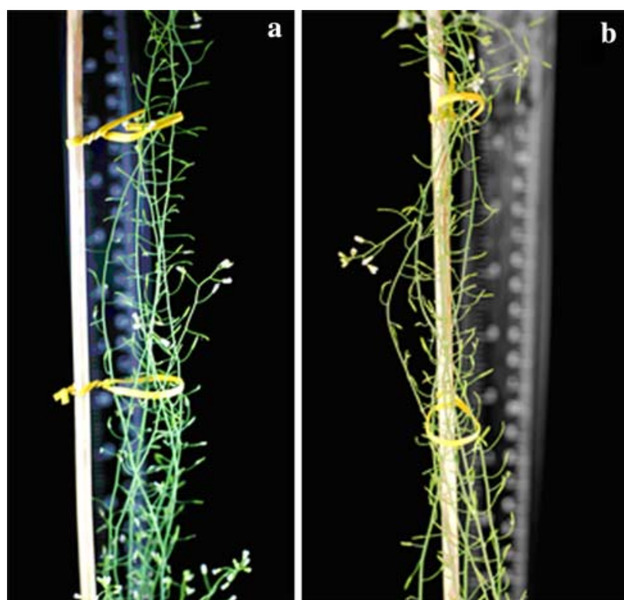


**Fig. 2** Phenotype of diploid and tetraploid *asy1*. **a** 2 × *asy1*, **b** 4 × *asy1*

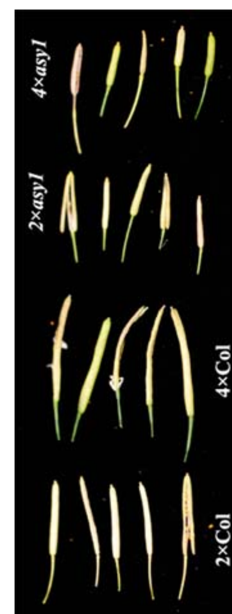


**Fig. 3** Fruits of diploid and tetraploid Col and *asy1*

events of this mutation after direct tetraploidization. Furthermore, although descriptions of meiosis in tetraploid Arabidopsis are supported by data from fluorescence in situ hybridization (Weiss and Maluszynska 2000; Santos et al. 2003), the double-folded chromosome complements, as well as the tiny configuration of individual chromosomes render meiotic analysis of autotetraploids intractable

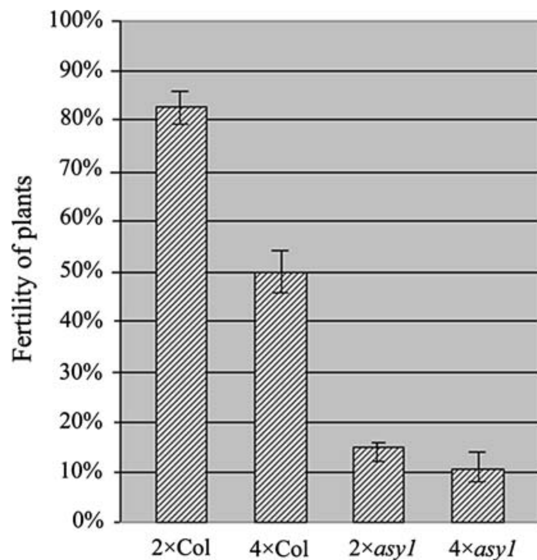


**Fig. 4** Reduced fertility in diploid and tetraploid *asy1*

and elusive. Nevertheless, in view of the importance of autopolyploidization, the understanding of meiosis in autotetraploids seems absolutely necessary for polyploidy research. Thus, the typical meiotic stages in tetraploid *asy1* are elaborated in parallel with those of the wild-type.

In autotetraploid wild-type Arabidopsis, at the onset of meiosis, the indistinguishable homologous chromosomes (chromatins) gradually condense and initiate synapsis from zygotene to pachytene during prophase I (Fig. 5a); at late prophase I, homologous chromosomes align and become distinguishable with bright DAPI-stained (peri-) centromeres after completing condensation (Fig. 5b); at metaphase I, homologous chromosomes behave as quadrivalents (Fig. 5c) and/or bivalents (Fig. 5d) with several entangled chiasmata; at the transition from metaphase I to anaphase I, homologous chromosomes separate equally in opposite directions (Fig. 5e); from metaphase II to anaphase II, coherent sister chromatids separate and are pulled apart gradually by the centromere-associated spindles (Fig. 5f). In contrast, in tetraploid *asy1*, homologous chromosomes could partially synapse at early prophase I (Fig. 5g); in succession, the respective chromosomes (with two associated sister-chromatids) appear identifiable, but homologous chromosomes fail to align together (Fig. 5h); afterwards, the majority of chromosomes form univalents (Fig. 5i) or exhibit residual bivalents at metaphase I (Fig. 5j); such univalents are often then retained behind as lagged chromosomes at the first cell division (Fig. 5k). Subsequently, in meiosis II, the lagged chromosomes cannot simutaneously separate into four defined nuclei, leading to indefinite loss of chromosomes (Fig. 5l). Normal chromosome behavior was observed in tetraploid wild-type, whereas in tetraploid *asy1*, chromosome behavior was distorted despite the partial synapsis and bivalent formation, which is probably attributable to the second yeast HOP1 homologue, ASY2 (Caryl et al. 2000). Therefore, male meiosis in tetraploid *asy1* differs from that of tetraploid wild-type in aspects of meiotic chromosome (chromatin) behavior such as chromatin synapsis, and alignment and segregation, which thus probably produces the odd numbers of chromosome complements in the male reproductive gametes.

Female meiosis in different plant species has been observed in recent years. Havekes et al. (1997) found that, in wild-type tomato, the number of chiasmate chromosome arms in female meiosis was slightly higher than in male meiosis. In the completely asynaptic mutant *as6*, chromosome pairing and chiasma formation were virtually absent in both sexes, but in the partially asynaptic mutant *asb*, a higher number of chiasmate chromosome arms in female meiosis than in male meiosis was observed. Although similar chromosome behaviors were obtained in male and female meiosis in wild-type Arabidopsis (Ross et al. 1996; Armstrong and Jones 2001), in the asynaptic mutant *asy1*, the complete failure of bivalent formation reflected the more severe defects in female meiosis than male meiosis (Sanchez Moran et al. 2001; Armstrong and Jones 2001). This result might indicate a product of gametes with different chromosome numbers after male and female meioses in Arabidopsis *asy1* mutants.

## Analysis of aneuploidy transmission in tetraploid *asy1*

The diploid *asy1* exhibits disrupted synapsis and failure of homologous pairing, engendering mostly univalent formation, causing severely improper segregation of homologous chromosomes during meiosis (Caryl et al. 2000; Armstrong et al. 2002). Fertility observation of diploid *asy1* shows a reduced seed set. The seeds of selfed tetraploid *asy1* display varying sizes, and appear shrunken or plumpish (Fig. 6b); approximately 55.1% of seeds are shrunken, and roughly 58% of examined seeds fail to germinate. In contrast, the seeds of tetraploid wild-type are mostly stuffed and exhibit a uniform size (Fig. 6a), as well as a higher germination ratio (approximately 90%). Similarly, backcrossed tetraploid *asy1* × a wild-type pollinator resembles the status of selfed tetraploid *asy1* with regards to seed development and germination. However, an unexpected outcome was that seed development and viability were significantly improved by crossing tetraploid *asy1* (as pollen donor) with wild-type (Table 1). Other independent interploidy crosses of diploids and tetraploids show that the viability of triploid seeds depends mostly on the ploidy level of maternal plant (data not shown). That is, a triploid embryo could be developed in both cross directions, whereas the aborted endosperm was frequently examined when the interploidy cross was conducted with a diploid as

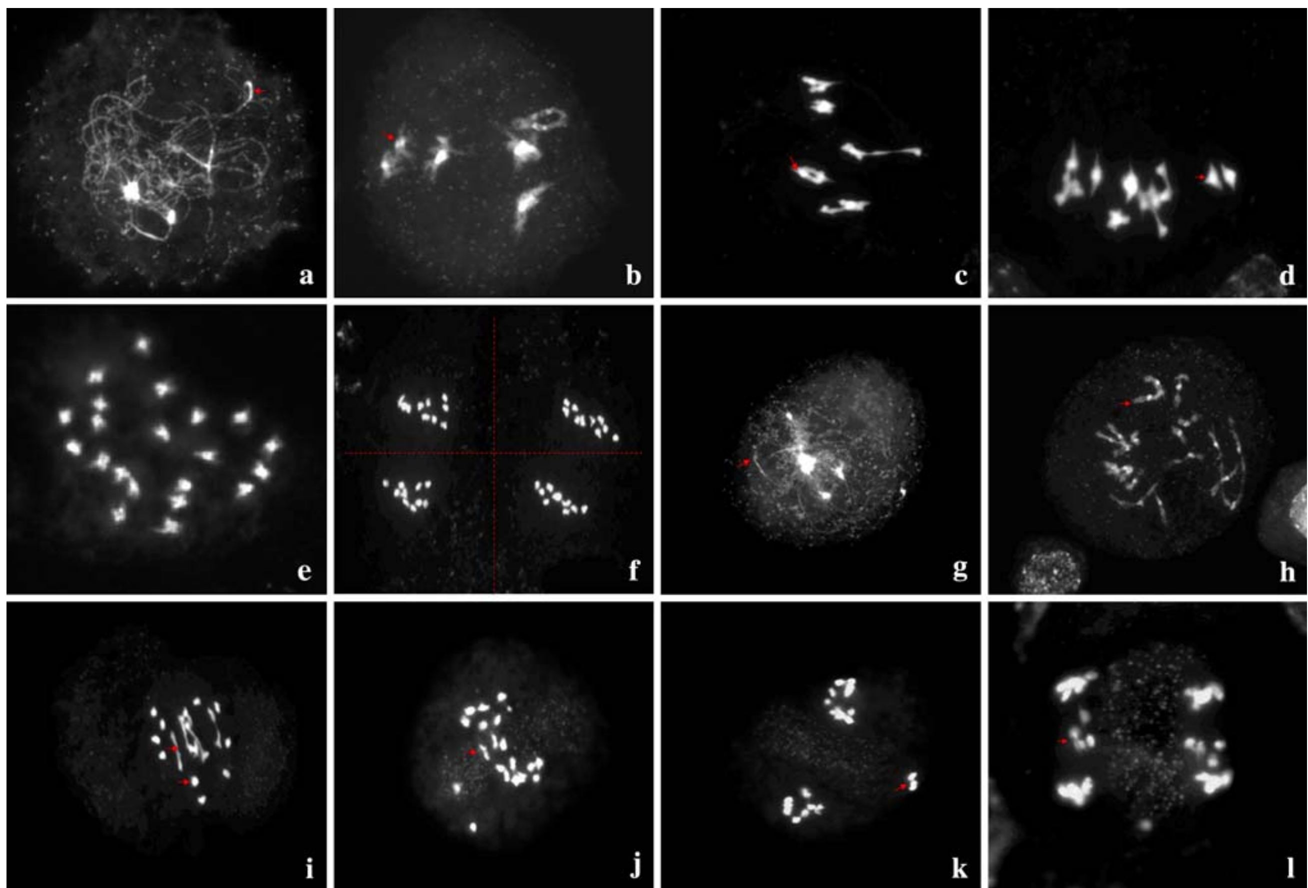


**Fig. 5** Progressive meiotic stages of autotetraploid Arabidopsis. Tetraploid wild-type (**a**–**f**) and *asy1* mutant (**g**–**l**). In wild type at early prophase I (**a**), the linear chromatin is completely synapsed (*arrow* bright-stained linear synapsed chromatin); **b** condensed chromosomes align in late prophase I (*arrow* paired bivalents); **c–d** paired homologous chromosomes show quadrivalents or bivalents (*arrows*); **e** at anaphase I, homologues separate equally to opposite poles; **f** at telophase II, four nuclei with equal numbers of chromosomes eventually form. In contrast, in tetraploid *asy1* (**g**) linear chromatin appears with partial synapsis at prophase I (*arrow* bright-stained linear synapsed part); **h** homologous chromosomes fail to align and exhibit as univalents in late prophase I (*arrow* single asynaptic univalent); **i–j** at metaphase I, the homologous chromosomes show mainly univalents or residual bivalents (*arrows*); **k** at anaphase I, the homologous chromosomes separate into two poles with retarded chromosomes (*arrows* three lagged chromosomes); **l** sister chromosomes separate with retarded chromosomes at the transition from metaphase II to anaphase II (*arrows* four lagged chromosomes)

the maternal plant. This result confirmed the differential parental effects on seed development (Scott et al. 1998).

The viable seeds of the selfed tetraploid *asy1* were dissected by chromosome counting of root-tips. The data (Table 2) show that about 80% of seeds analysed have reduced chromosome numbers (< 20), and at least 25% of analyzed progeny show that the chromosome loss can probably amount to a set of haploid genome; about 7% of observed seeds have increased chromosome complements (> 20). In comparison, the data for the tetraploid wild-type show that nearly 90% of seeds (31:35) possess a normal set of chromosomes in spite of the occasional occurrence of chromosome gain or loss (1–2:35), which can probably be attributed to the unique formation of multivalents such as trivalents plus univalents (Santos et al. 2003) leading to the missegregation of the paired chromosomes of such configurations. These results indicate that asynapsis-induced loss of chromosomes determines aneuploidy formation in tetraploid *asy1*. Moreover, the severe loss of individual chromosomes hints at a possible stochastic event during meiosis.

To identify the source of aneuploidy (hyperploidy and hypoploidy) during gametogenesis, we crossed tetraploid *asy1* with wild-type reciprocally. Chromosome counting indicated that about 45% of the backcrossed seeds analysed have an increased or reduced number of chromosomes when tetraploid *asy1* was the pollen recipients, but only 15% of observed seeds show a gain of chromosomes. In comparison, about 70% of seeds examined display loss of chromosomes when tetraploid *asy1* was chosen as the pollen donor, but approximately 12% of backcrossed seeds show the euploid chromosome number provided that

**Fig. 6** Seeds of different phenotypes. **a** Shrunken or plumpish selfing 4 × Col (control); **b** 4 × Col × 4 × asy1; **c** selfing 4 × asy1, **d** 4 × asy1 × 4 × Col

**Table 1** Seed development and germination

| | Materials | Seed phenotype | | | $\chi^2$ | Germinated seedlings | | | $\chi^2$ |
|---|---|---|---|---|---|---|---|---|---|
| | | Shrunk | Total | Shrunk (%) | | Nonviable | Total | Nonviable (%) | |
| Selfings | 4 × Col | 3 | 178 | 1.69 | – | 21 | 213 | 9.86 | – |
| | 4 × asy1 | 124 | 225 | 55.1 | 75.1* | 74 | 127 | 58.3 | 49.1* |
| Crosses | 4 × asy1 × 4 × Col | 108 | 240 | 45.0 | 61.9* | 69 | 106 | 66.7 | 54.1* |
| | 4 × Col × 4 × asy1 | 14 | 149 | 9.3 | 8.77[a] | 13 | 102 | 12.7 | 0.47[a] |

* Highly significant, $P < 0.00$ l

[a] Not significant

**Table 2** Chromosome analysis of progeny from tetraploid wild-type and the *asy1* mutant

| | Materials | Distribution of chromosome number (*n*) of root-tips analyzed (%) | | | | | Total analyzed |
|---|---|---|---|---|---|---|---|
| | | $5 < n < 10$ | $10 < n < 15$ | $15 < n < 20$ | $n = 20$ | $n > 20$ | |
| Selfings | 4 × Col | 0 (0) | 1 (2.85) | 2 (5.71) | 31 (88.6) | 1 (2.85) | 35 |
| | 4 × asy1 | 5 (7.1) | 14 (19.7) | 38 (53.5) | 9 (12.7) | 5 (7.1) | 71 |
| Crosses | 4 × asy1 × 4×Col | 1 (2.3) | 5 (11.6) | 13 (30.2) | 5 (11.6) | 19 (44.2) | 43 |
| | 4 × Col × 4×asy1 | 3 (9.1) | 11 (33.3) | 10 (30.3) | 4 (12.1) | 5 (15.2) | 33 |

Numbers in parentheses denote the corresponding percentage

tetraploid *asy1* was selected as pollen donors or recipients. As shown in Table 2, these results prove that hyperploids transmit mainly via female gametogenesis, and hypoploids have a duoparental transmission during the reproductive life cycle but a paternal pathway is preferred in Arabidopsis.

## Discussion

The development of Arabidopsis as a plant model for genetic studies is attributed to its small size, short life cycle, its selfing mode of propagation, and the availability of its genomic sequence (Goodman et al. 1995). However,

regarding a model for analysis of polyploidy in flowering plants, little is known about ploidy transmission on the basis of autopolyploid Arabidopsis. In this study, we sought to characterize the pathway of aneuploidy transmission in polyploid Arabidopsis.

Differential gametic transmission affects inheritance of rust resistance in allohexaploid wheat (Luig 1960). And the transmission of aneuploid gametes is influenced by the competitiveness of pollen tube growth and fertilization after microsporogenesis and megasporogenesis (Boyd et al. 1970). The induced tetraploid of *Lolium perenne* shows decreasing frequency of aneuploids with higher numbers of chromosomes in advanced generations, and hyperploids are recovered mainly through female transmission (Ahloowalia 1971). However, the transmission of $n + 1$ gametes in cabbage trisomics proved that the transmission of extra chromosomes in different trisomics varied in both male and female gametes, and it has been speculated that there is an equal chance of extra-chromosome transmission during gametogenesis (Zhang et al. 2008). Analysis of aneuploid progenies from the asynaptic amphidiploid *Scilla scilloides* (AABB) clarified that there was no preferential chromosome transmission between genome A and B, and that viable pollen grains might determine aneuploid production (Uchino and Tanaka 1995). Our results indicate that the transmission of aneuploids in autotetraploid Arabidopsis occurs in both male and female gametes, with increased hyperploid transmission arising mainly in female (embryosac) cells. Of the two autotetraploid *Festuca pratensis*, *Lolium multiflorum* produces more hyperploids, but *Lolium perenne* shows equal yields in hypoploids and hyperploids (Klinga 1986). Euploids were superior to aneuploids and hyperploids were superior to hypoploids in most developmental features' furthermore, aneuploidy had more pronounced effects on reproductive development (Klinga 1986). In contrast, aneuploids in tetraploid ryegrass were morphologically indistinguishable from eu-tetraploids (Ahloowalia 1971). Our results show that tetraploid *asy1* aneuploids resemble eu-tetraploids with respect to several morphologies including reduced fertility.

In animals, including humans, aneuploidy can cause miscarriages and mental retardation (Parry et al. 2002; Duesberg 2007). In four different mice trisomic lines, proliferation and metabolic properties were altered by the presence of an additional chromosomes, as well as immortalization (Williams et al. 2008). Likewise, the development of endosperm was arrested by interploidy hybridization, suggesting a potential determinant of disproportionate contributions of paternal/maternal chromosome complements in Arabidopsis (Scott et al. 1998). Alternatively, imprinted genes such as *MEDEA* have been proven to influence endosperm development (Vinkenoog et al. 2003). In addition, the locus *SENSITIVE TO DOSAGE IMBALANCE* (*SDI*) could tolerate more aneuploidy by buffering the effects of dosage imbalance in triploid-derived recombinant inbred lines (Henry et al. 2007). Dilkes et al. (2008) clarified that the viability of interploidy-crossed seeds was regulated by maternally expressed *WRKY* transcriptional factor *TTG2* by sporophytically affecting endosperm cellularization. In the study, we prove that viable aneuploid gametes with varying chromosome content affect the development of seeds after fertilization, and that the disproportionate ratio of parental chromosome complements may contribute to aberration of seed development.

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