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Apomeiotic pollen mother cell development in the apomictic *Boechera* species

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Abstract

Pollen mother cell (PMC) development in the apomictic *Boechera* species *B. holboellii*, *B. gunnisoniana* and *B. divaricarpa* were investigated by various cytological methods. In prophase I, in triploid species *B. holboellii* and *B. gunnisoniana* the individual chromosomes condensed into long strands within the nucleus. Then, in metaphase I, each PMC formed a restitutional nucleus thereby bypassing the rest of the first meiotic division. This is interpreted as representing apomeiosis. Subsequently, the restitution nuclei underwent a single cytokinesis as evidenced by the production of dyads. The cells within each dyad were separated by a callose wall. Most of the PMC in *B. holboellii* and *B. gunnisoniana* produced dyads, but a small proportion generated conspicuous tetrads. In contrast, diploid apomict *B. divaricarpa* produced only tetrads by simultaneous cytokinesis.

Additional key word: apomixis, endosperm, male gametes.

Introduction

In most angiosperm species genetically diverse offspring are produced by sexual reproduction. Central to this process is the generation of female and male gametes within specialized haploid gametophytes. The female gametophyte is the embryo sac that contains two gametes, the egg and central cell. The male gametophyte is the pollen grain that contains a large vegetative and two sperm cells. Seed development in angiosperms involves a unique process called "double fertilization" where one sperm fertilizes the egg cell, giving rise to an embryo, and the second sperm from the same pollen tube fuses with the two central cell nuclei to produce the endosperm. Therefore, a seed consists of a 2n embryo and 3n endosperm which have genomic ratios of 1maternal: 1paternal and 2m:1p, respectively. Any deviation from this ratio, in either direction (more maternal or more paternal genomes) has serious effects on seed development, and usually leads to seed abortion (Haig and Westoby 1989, Lin 1984). Apomixis is an exception to this mode of reproduction where embryo formation occurs without fertilization of the egg. Three components are common in apomixis: generation of a cell capable of forming an embryo without prior meiosis (apomeiosis); the spontaneous, fertilization-independent development of the embryo (parthenogenesis); and the capacity to either produce endosperm autonomously or to use an endosperm derived from fertilization (Bicknell and Koltunow 2004). Apomixis occurs naturally among many angiosperm families (Carman 1997). Female meiosis is absent or modified to provide unreduced female gametes and the progeny of apomicts are consequently genetically identical to the mother plant. Apomictic *Boechera* species are attractive models to study the molecular biology of apomixis. They belong to the *Brassicaceae* and are therefore close relatives of *Arabidopsis thaliana* for which there are many molecular genetic tools available. They have been reported as facultative apomicts in which both sexual reproduction and apomixis occur together at both the diploid and triploid levels (Naumova *et al.* 2001). In addition, triploid populations of *B. holboellii* and *B. gunnisoniana*, in common with many apomicts, require fertilization to generate an endosperm (Böcher 1951, Naumova *et al.* 2001, Schranz *et al.* 2006). Flow cytometric analyses of mature seeds from triploid

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Abbreviations: DAPI - 4'-6-diamidino-2-phenylindole; FAA - formaldehyde + ethanol + acetic acid; FCSS - flow cytometric seed screen; m - maternal; p - paternal; PMC- pollen mother cell.
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B. holboellii populations showed that only unreduced female and male gametes participate in viable seed development (Naumova *et al.* 2001). However, *B. divaricarpa* is an interspecific hybrid between *B. stricta* and *B. holboellii* (Koch *et al.* 2003). *B. divaricarpa* individuals that were previously reported as diploid and triploid apomicts are produced a mixture of reduced and unreduced gamete production (Schranz *et al.* 2005, 2006).

The majority of apomicts are pseudogamous – that is the asexual embryo is supported by a sexual endosperm. However, in many apomicts such as diplosporous and aposporous *Poa* species, aposporous *Paspalum* spp., *Hypericum patalum* and *H. perfaratum*, *Potentilla argentea* and *Ranunculus auricomus* male meiosis is reductive (Matzk *et al.* 2000). As a result, fertilization of the two unreduced polar nuclei with a sperm result in a pentaploid endosperm with a 4m:1p ratio. As a 2m:1p ratio is usually essential for normal endosperm development and the production of viable seed, this so called 'endosperm problem' is circumvented by modified pathways of female gametogenesis or fertilization (Vinkenoog *et al.* 2003). For example, in apomictic *Pennisetum* species, as well as other panicoid apomicts, a 3n primary endosperm nucleus with a 2m:1p genomic ratio is formed by fertilization of a single polar nucleus, within a modified 4-nucleate embryo sac, by a reduced sperm (Ozias-Akins 2003). In *Ranunculus auricornus* both reduced sperm fertilize the unreduced polar nuclei, resulting in a 4m:2p ratio in the endosperm (Rutishauser 1954). Pseudogamy was also reported in number of species in *Boechera* including *B. holboellii* and *B. gunnisoniana* (Naumova *et al.* 2001, Taskin *et al.* 2004). The present study set out to determine how unreduced male gametes are produced in *Boechera* spp. by analyzing pollen mother cell (PMC) development. In the triploid apomicts we found that the unreduced male gametes are produced by apomeiosis followed by essentially normal cytokinesis involving two microspore nuclei. Therefore, in these species seed abortion is likely avoided by the creation of a 2m:1p endosperm ratio through fertilization of two unreduced polar nuclei (2n:3m) with an unreduced sperm (2n:3p). In contrast, reduced male gametes in diploid apomict *B. divaricarpa* are produced by meiosis followed by normal cytokinesis involving tetrad nuclei. Consequently, a reduced sperm produce a pentaploid endosperm with a 4m:1p ratio which is usually disruptive.

Materials and methods

Plants: In this study we used triploid *Boechera holboellii* (Hornemann) A. Löve & D. Löve and *B. gunnisoniana* (Rollins) W.A. Weber and diploid *B. divaricarpa* (A. Nelson) A. Löve & D. Löve plants. Triploid *B. holboellii* and *B. gunnisoniana* seeds were originally collected from North America and obtained from Dr. Bitty Roy (University of Oregon). Diploid apomict *B. divaricarpa* (ES:9) seeds were obtained from Dr. Eric Schranz (Schranz *et al.* 2005, Schranz *et al.* 2006). Seeds were germinated according to Schranz *et al.* (2005). Plants were grown on a peat:*Perlite* mix (1:4) for 28 d in a growth chamber under a 16-h photoperiod with irradiance of 350 μmol m^{-2} s^{-1} and temperature of 21 °C. Plants were then vernalized at 5 - 10 °C for 6 weeks and transferred back to the growth chamber (Schranz *et al.* 2005). The plants started to flower 4 weeks following transfer.

Anthers fixation, and staining procedures: The whole inflorescences were fixed in the FAA (40 % formaldehyde + 70 % ethanol + 98 % acetic acid in the ratio 3:7:1) solution for 24 h and stored in 70 % ethanol at 4 °C. Bud lengths were measured from the base of the pedicel to the tip of the outermost sepal. Anthers and pistils were dissected from the buds and their length measured from tip to tip.

The anthers fixed in FAA solution were washed with fresh ice-cold ethanol + acetic acid (3:1). Then the samples were washed with citrate buffer. Then, the samples were transferred to 0.3 cm^3 of pectolytic enzyme mixture (1 % cellulose and pectolyase in citrate buffer) for 40 min at 37 °C. After that the enzyme mixture was changed to ice-cold citrate buffer to stop digestion. The anthers were transferred to a clean slide and dissected with fine needle to make a suspension of cells. A small drop of 60 % acetic acid was added to the cells. The samples were spread on the slide by adding 0.1 cm^3 ice-cold ethanol + acetic acid (3:1) then air dried for a few minutes. The samples were stained with DAPI (10 μg dm^{-3}) in *Vectashield* mounting medium and studied under a *Nikon* (Bath, UK) *Eclipse 90i* microscope. For apomeiotic studies, the anthers previously fixed in FAA solution were dissected with fine needles and prepared by squashing in orcein or aceto-carmine. The callose tissue around the PMC was detected by aniline blue staining (Spielman *et al.* 1997). Mature pollen was incubated in a DAPI in *Vectashield* mounting medium (Spielman *et al.* 1997). The pollen grains sizes were measured using a *Olympus* (Canakkale, Turkey) CX31 microscope.

Flow cytometry: Seeds selected from 10 progenies from triploid *Boechera* species population growing in the greenhouse were used to analyze ploidy level of the embryo and endosperm by flow cytometry. Seeds were collected from mature siliques of open pollinated individuals. One to 20 bulked samples of 50 seeds were chopped with razor blade in DAPI staining buffer filtered, and store on ice. A ploidy analyser (*Partec*, Germany) was used for measurements (Krahulcová and Suda 2006, Matzk *et al.* 2000, 2001).

Results and discussion

Boechera species are perennial plants with the simple *Brassica*-type flowers arranged in whorls where the age of a bud is related to its distance from the centre of the whorl (Scott *et al.* 1991). The basic chromosome number of *B. holboellii* is 7, and polyploidy (typically 3x) and B chromosomes are common (Sharbel *et al.* 2005). In this work, we used triploid *B. holboellii* and *B. gunnisoniana* accessions (Roy 1995) that were previously reported as a facultative diplosporous (*Taraxacum* type) apomicts (Naumova *et al.* 2001, Taskin *et al.* 2004) and diploid apomict *B. divaricarpa* (Schranz *et al.* 2005). Flow cytometric analysis of mature seeds from the triploid species showed that pseudogamy is predominant and that only the unreduced female and male gametes contribute to mature seed (Naumova *et al.* 2001, Taskin *et al.* 2004). We set out to use a combination of cytological techniques and measurements to determine the mechanism by which unreduced male gametes are produced in these species.

Anther length and PMC development in *B. holboellii*: In order to characterize the cytological details of PMC development, we first established the relationship between anther length and the developmental stage of the PMC. Individual buds and dissected anthers were obtained from 5 different *B. holboellii* plants and their lengths measured. The results showed a strong relationship between bud and anther length as described previously (Scott *et al.* 1991). Anther length reached a maximum of approximately 1.5 - 2 mm, while bud length continued to increase. Thus, the cytological details of PMC development were analyzed according to anther length. The results revealed that there are six different

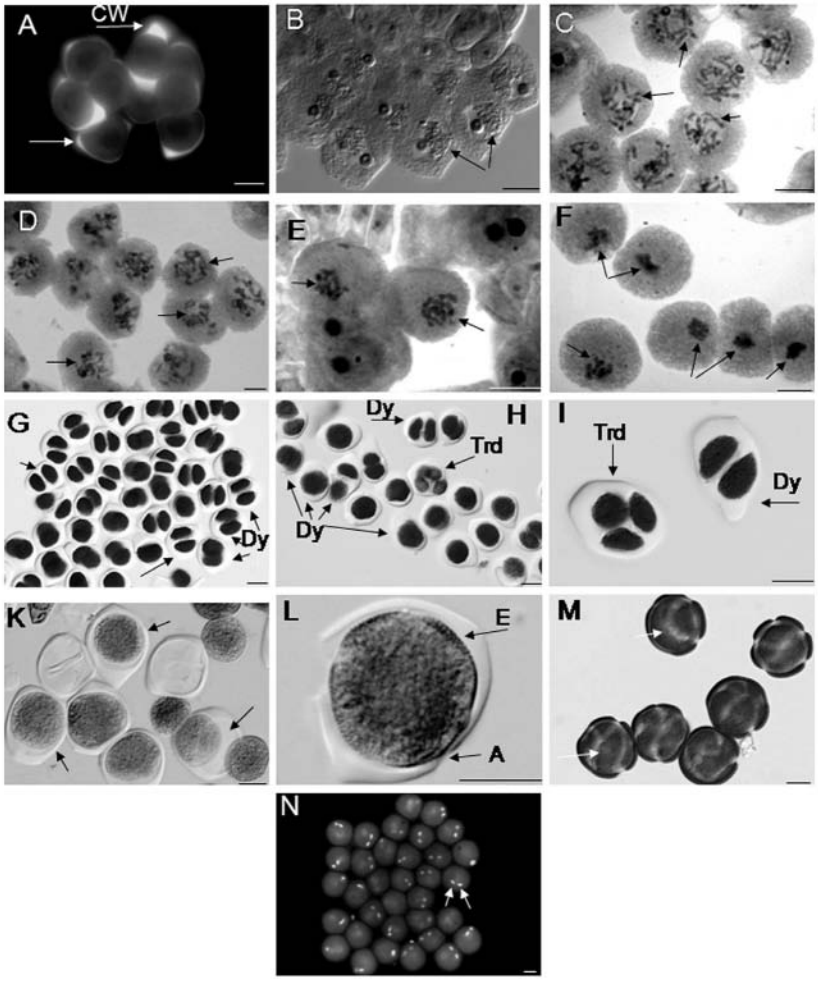

Fig. 1 The male gametophyte development in triploid *B. holboellii*. *A* - PMCs surrounded by callose walls (CW) stained with aniline blue at anther length 0.5 mm; *B* - prophase I shows condensed chromosomes (*arrows*); *C* - bivalents and trivalents (*arrows*); *D* - nuclear membrane began to disappear (*arrows*); *E* - homolog chromosomes condensed at equatorial plate at metaphase I (*arrows*); *F* - restitutional nucleus; *G* - dyads (Dy); *H* to *I* - dyad and tetrad (Trd) of cells; *K* - dissolution of dyad callose walls (*arrows*); *L* - exine (E) layers and germinal apertures (A) formation; *M* - young microspores contained single nucleus (*arrow*). *N* - mature pollen grains with 2 sperm cells (*arrows*).

development stages of PMCs that can be easily associated with anther length. In angiosperms the PMC differentiates from archeosporial cells within the anther and begins to form a callose wall just prior to the onset of meiosis. The appearance of callose provides a useful marker for the beginning of meiosis. Callose wall synthesis usually continues during meiosis and, when meiosis is complete, it separates the meiotic products (Scott *et al*. 2004).

In order to analyze meiosis in *B. holboellii* anthers, callose wall deposition was investigated by staining with aniline blue and aceto-carmine. The PMCs surrounded by a thick callose wall were recognized when the anther size reached approximately 0.5 - 0.6 mm (Fig. 1*A*). The various meiotic stages including prophase I, metaphase I and anaphase I were also observed at the same stage. In prophase I, when the nucleolus and nuclear membrane were still intact, the individual chromosomes had begun to condense into long strands within the nucleus (Fig. 1*B*). Subsequently, the homologous chromosomes appeared and became completely paired as evidenced by the presence of bivalents and trivalents (Fig. 1*C*) (Böcher 1951). The nucleolus and the nuclear membrane then began to disappear (Fig. 1*D*). During the following stages, the homologous chromosomes began to organize on the equatorial plate. Therefore, this stage is recognized as metaphase I (Fig. 1*E*). At this stage, each PMC formed a restitutional nucleus as evidenced by the condensed chromosomes failed to undergo anaphase I and telophase I to separate into two groups. This process is also known in other apomictic species as apomeiosis, circumvention of meiosis (Grimanelli *et al.* 2001, Noyes 2005) (Fig. 1*F*). Subsequently, at an anther length of about 0.7 mm, the restitution nuclei underwent a single mitosis, which we interpret as meisois II, followed by cytokinesis as evidenced by the production of a dyad of cells encased in a callose wall (Fig. 1*G*). However, in this work, we also observed a few tetrads with a frequency of 2 % of cells encased in a callose wall (Fig. 1*H*,*I*). Since the microspore nuclei act as foci for microtubule arrays within the cytoplasm that guide the deposition of the callose cross walls (Spielman *et al.* 1997), a spore with two nuclei may simply organize a single cross wall (as in monocots after meiosis 1), while a spore with 4 nuclei will organize 4 intersecting cross walls. Hence dyads are formed because meiosis I was truncated in *B. holboellii*. Following cytokinesis, the individual microspores of the dyad initiated pollen wall development as evidenced by the appearance of distinct exine layers and germinal apertures just prior to microspores release (Fig. 1*K*,*L*). When the young microspores were released by dissolution of the dyad callose walls each contained a single nucleus (Fig. 1*M*). The mitotic divisions within the pollen grains completed when the anther length reached about 1.0 - 1.1 mm. Therefore, the mature pollen grain of *B. holboellii* is composed of a vegetative cell and 2 sperm cells (Fig. 1*N*). This study revealed that *B. holboellii* follows a modified pathway from other species within the *Brassicaceae* to produce pollen grains. The tetrads that were observed in this study suggest that reductional divisions were also present in *B. holboellii* male gametophyte development (Fig. 1*H*,*I*). This suggested *B. holboellii* produces a mixed population of reduced and unreduced pollen grains. It was previously established that there is a strong correlation between pollen size and ploidy level of the gamete (Sharbel *et al.* 2005). We therefore tested for presence of a mixed pollen population by measuring pollen size in *B. holboellii*. This showed that three classes of pollen grains were produced in *B. holboellii*. While the most abundant pollen grains (73 %, $n = 3461$) have diameters of 22.5 - 25.0 ± 1.2 μm, there is also a class of smaller pollen grains (20 %, $n = 3461$) with an average diameter of 18.7 ± 1.5 μm and a class of larger grains (7 %, $n = 3461$) with a diameter of 29.5 ± 2.6 μm (Fig. 1*M*). The smaller pollen grains are likely to be the product of a reductional division whilst the irregularly shaped grains are probably degraded pollen in *B. holboellii*. Pollen size measurement reported for *Boechera* (Böcher 1951, Schranz *et al.* 2006, Sharbel *et al.* 2005) documented pollen sizes of *B. holboellii* individuals in various ploidy levels and revealed great variability. In *B. holboellii*, diploid individuals produce pollen of relatively uniform size, while triploids and individuals with B chromosomes produce both small degraded pollen and larger sized pollen with normal appearance (Sharbel *et al.* 2005).

We also determined seed abortion frequency in order to analyze whether the small percentage of pollen in *B. holboellii* could create disruptive 4m:1p ratios in endosperm. The average number of seeds per siliques was found as 40 ($n = 604$). However, 40 % of seeds were relatively large and plump while 60 % were smaller and shriveled that were aborted in *B. holboellii*.

We also used flow cytometric seed screen (FCSS) to determine the reproduction pathways in *B. holboellii*. The seed samples of triploid *B. holboellii* yielded 2C, 4C and 6C peaks, most likely representing an asexual 2C embryo developed from unreduced egg cell without fertilization (2m:0p), and a 6C sexual endosperm originated by fertilization of the two unreduced polar nucleus by an unreduced sperm (4m:2p). 4C peak most likely represent endopolyploidization in embryo. The FCSS analyses of *B. holboellii* revealed that *B. holboellii* seeds reproduce with pseudogamy (while embryo formation took place autonomously, endosperm developed after fertilization with unreduced male gametes). It was previously reported that some plants within the population of *B. holboellii* cv. Colorado produced seeds by autonomous apomixis (Naumova *et al.* 2001). However, we did not observe it in the accession used in this work.

PMC development in *B. gunnisoniana*: PMC development in triploid apomict *B. gunnisoniana* also followed a modified pathway to produce pollen grains. PMCs surrounded by a thick callose wall were first recognized when the anther length reached about 0.5 mm (Fig. 2*A*). Subsequently, various meiotic stages, including early prophase I and metaphase I, were present. In early pro-

phase I, chromosomes were highly extended and condensed into long threads with one large and numerous smaller sections brightly stained with DAPI (Fig. 2*B*). The brightly fluorescing sections may represent heterochromatin blocks. We observed little or no pairing between the chromosomes at pachytene (Fig. 2*B*). This situation persisted at metaphase I, where many chromosomes remained as univalents (Fig. 2*C*). At later stages, meiosis I was failed, and chromosomes migrated to the opposite poles of the PMC with 21 equal numbers each (Fig. 2*D*). In some samples, the abnormalities of chromosome segregation resulted in the formation of micronuclei (Fig. 2*E*). Then, the chromosome groups decondensed (Fig. 2*F*) and, at an anther length of about 0.7 mm, a dyad of cells encased in a callose wall was formed by cytokinesis (Fig. 2*G*). Although high frequencies of dyad cells (up to 90 %) were produced in *B. gunnisoniana*, we also observed a few tetrads (8 %). The irregular chromosome segregation produced triads probably with unequal chromosome numbers (Fig. 2*H*). Normal meiosis that would have to be present for the account of tetrad was not observed.

The seed samples of triploid *B. gunnisoniana* were previously analyzed by FCSS (Taskin *et al.* 2004). *B. gunnisoniana* seed samples also yielded 2C, 4C and 6C peaks, but in one sample 2C and 5C peaks were also obtained, most likely representing a hybrid 5C endosperm developed from 2 unreduced polar nuclei fertilized by reduced sperm (4m:1p). The FCSS analyses of *B. gunnisoniana* revealed that *B. gunnisoniana* seeds reproduce with pseudogamy (while embryo formation took place autonomously, endosperm developed after fertilization with reduced or unreduced male gametes) (Taskin *et al.* 2004).

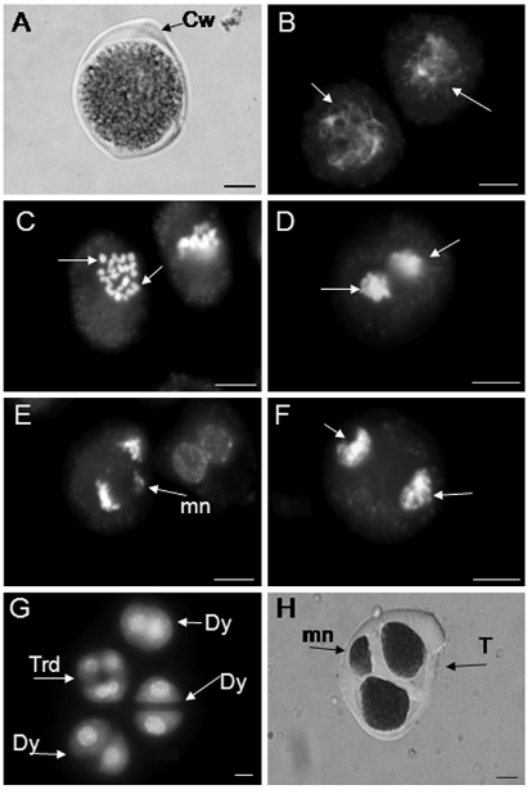

Fig. 2. The male gametophyte development in triploid *B. gunnisoniana*. *A* - PMCs surrounded by a thick callose wall (CW); *B* - early prophase I shows condensed chromosomes (*arrows*); *C* - metaphase I complement with 21 univalents (*arrows*); *D* - meiosis I failed, chromosomes (*arrow*) migrated to the opposite poles of the PMC with 21 equal numbers each; *E* - the abnormalities of the chromosome segregation results micronuclei (mn) formation; *F* - decondensed chromosomes (*arrows*); *G* - dyad (Dy) and tetrad (Trd) of cells; *H* - the irregular chromosome segregation produced triads (T).

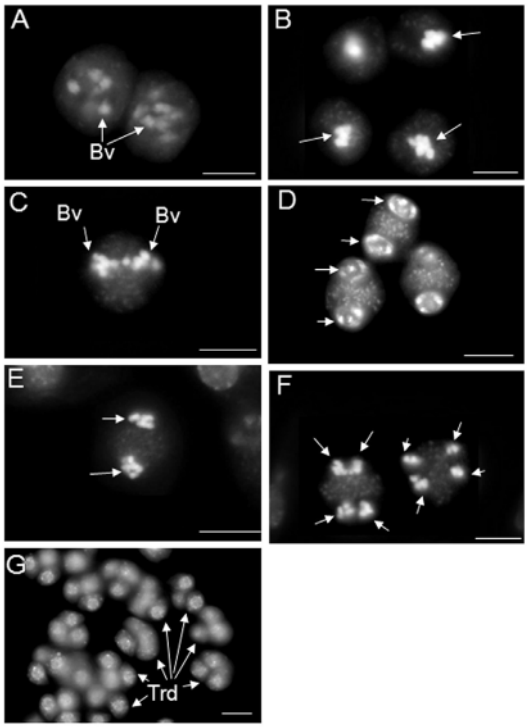

Fig. 3. The male gametophyte development in diploid *B. divaricarpa*. *A* - Diplotene nuclei containing 7 bivalents (*arrow*); *B* - metaphase I complement with seven bivalents (*arrows*) aligned at the equator of the cell; *C* - anaphase I, the two sets of half bivalents (Bv, *arrows*) migrated to the poles; *D* - partially decondensed chromosome groups (*arrow*) at telephase I; *E* - metaphase II complements (*arrow*). *F* - the chromosomes (*arrows*) separating at anaphase II; *G* - tetrad (Trd) cells.

PMC development in *B. divaricarpa*: *B. divaricarpa* is an interspecific hybrid between *B. stricta* and *B. holboellii* (Koch *et al.* 2003). The diploid and triploid apomict *B. divaricarpa* lineages were reported previously (Schranz *et al.* 2005). In order to analyze cytological details of PMC development, we used a diploid apomict *B. divaricarpa* line (Schranz *et al.* 2005). PMC surrounded by a thick callose wall were recorded when the anther size reached about 0.5 - 0.6 mm. In prophase I, we only observed diplotene nuclei containing 7 bivalents

linked by chiasmata (Fig. 3*A*). When the anther reached about 0.7 mm in length, metapahase I was observed, with seven bivalents aligned at the equator of the cell and showed that meiosis is fully synaptic (Fig. 3*B*) (Schranz *et al.* 2005). The two sets of half bivalents migrated to the poles in anaphase I (Fig. 3*C*). Subsequently, (at telophase I), the chromosome groups partially decondensed before the second meiotic division commenced (Fig. 3*D*). Significantly, at the completion of the meiosis I, the cytokinesis that in *B. holboellii* and *B. gunnisoniana* produces a dyad cells encased in a callose wall, occurred without the formation of callose cross walls in *B. divaricarpa*. The chromosomes reappeared at metaphase II (Fig. 3*E*) and started to separate at anaphase II (Fig. 3*F*). Cytokinesis then produced a tetrad of nuclei, each containing a reduced chromosome number (Fig. 3*G*).

The behaviour of male gametogenesis in apomict *B. divaricarpa* was previously analysed. Pollen size measurements and microsatellite analyses were consistent with normal male meiosis (Koch *et al.* 2003, Schranz *et al.* 2006). However, direct observations on male meiosis stages have not previously been reported. Our results suggest that *B. divaricarpa* produces reduced pollen grains. The average number of seeds per siliques was also determined for *B. divaricarpa* as 62 (n = 438). However, 70 % of seeds were relatively large and plump while 30 % were smaller and shriveled that were aborted in *B. divaricarpa*.

The breeding systems of *Boechera* species were investigated previously (Böcher 1951, Matzk *et al.* 2000, Naumova *et al.* 2001, Roy 1995, Roy and Rieseberg 1989, Schranz *et al.* 2005, 2006, Sharbel *et al.* 2004, 2005, Taskin *et al.* 2004). Among these, both reduced and unreduced male gamete formation were reported in *B. holboellii* (Böcher 1951, Naumova *et al.* 2001, Schranz *et al.* 2005, Schranz *et al.* 2006). Böcher (1951) explained apomeiotic PMC development in great detail; the PMC development proceeds either by the formation of restitution nuclei or contraction nucleus in both triploid and diploid accessions of *B. holboellii* and resulted in unreduced dyad or monad pollen formation. In this work, we determined when and how unreduced male gametes are produced in triploid accessions of *B. holboellii* and *B. gunnisoniana*. Callose deposition around PMC indicated the onset of meiotic events. However, in metaphase I each PMC formed a restitutional nucleus and bypassed the rest of the first meiotic division (apomeiosis) in triploid *Boechera* species. Subsequently, PMC with a restitution nuclei underwent a single cytokinesis as evidenced by the production of dyads. The cells within each dyad were separated by a callose wall. Although most of the PMC produced dyads (up 98 % in *B. holboellii*), we also observed a few tetrads. In most eudicots including *Arabidopsis*, male cytokinesis is simultaneous, that is callose wall synthesis follows the completion of meiosis II and results directly in the production of a tetrad of microspores (Scott *et al.* 2004). Consequently, dyads are not normally observed. In contrast, in most monocotyledons cytokinesis is successive. Two rounds of callose cross wall synthesis occur, resulting in dyads following meiosis I, and tetrads following meiosis II. Therefore, we infer that dyads within triploid apomictic *Boechera* species produce unreduced sperm. This is in accord with flow cytometric analyses of mature seeds from various *B. holboellii* and *B. gunnisoniana* populations that showed only unreduced female and male gametes participate in viable seed development (Naumova *et al.* 2001, Taskin *et al.* 2004). In this work, we found that *B. holboellii* and *B. gunnisoniana* show an altered male gametophyte development: the unreduced gametes are produced by apomeiosis at a high frequency. Therefore, in triploid apomictic *Boechera* species, the 2m:1p ratio is apparently achieved by fertilization of two unreduced polar nuclei (2n:3m) with an unreduced sperm (2n:3p) to yield 6m:3p endosperm. Although, high levels of set seed were previously reported for *B. holboellii* (Naumova *et al.* 2001) we found that 60 % of seed were aborted. The small percentage of pollen could account for a proportion of this abortion through the creation of disruptive 4m:1p ratios in endosperm. We also consider that tetrads observed in triploid apomictic *Boechera* species may provide reduced sperms that is potentially fertilize unreduced egg to yield triploids, which is the most common ploidy level in *Boechera* (Böcher 1951). However, in diploid apomictic *B. divaricarpa* line, seven bivalents were observed in metaphase I and, at the end of the meiosis I, a dyad cells occurred without formation of callose cross walls. Subsequently, meiosis II proceeds normally and a tetrad of nuclei encased in a callose wall are produced by a single cytokinesis. Therefore, we infer that tetrads in diploid apomict *B. divaricarpa*, produce reduced sperm. Although, diploid apomictic hybrid species *B. divaricarpa* (ES: 9) were reported to produce a mixture of reduced and nonreduced gametes (Schranz *et al.* 2006), according to our results, meiosis in this plant is regular. Therefore, fertilization of the two unreduced polar nuclei with a reduced sperm results in a pentaploid endosperm with a 4m:1p ratio which is usually disruptive. Also, seed set analysis in *B. divaricarpa* showed that only 30 % of seeds are aborted. However, the seed samples of diploid *B. divaricarpa* (ES9) were recently analyzed by FCSS (Kantama 2007). According to the analyses *B. divaricarpa* (ES9) seed samples yielded 2C and 6C. Thus, these results most likely representing an asexual 2C embryo developed from unreduced egg cell without fertilization (2m:0p), and a 6C sexual endosperm originated by fertilization of the two unreduced polar nucleus by two reduced sperm (4m:2p). This implies that in contrast to the triploid *Boechera* apomicts, *B. divaricarpa* circumvents the endosperm problem by one of the mechanisms discussed previously. These results may also indicate facultative apomixis in *B. divaricarpa*. Further work is required to distinguish between these alternatives.

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