Meiosis of autotetraploid *Osmunda banksiiifolia* produced by induced apospory and the DNA contents of spores produced

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**ABSTRACT:** Autotetraploid *Osmunda banksiiifolia* (2n=4x=88) was produced by induced apospory for the first time. The sizes of guard cells and mature spores showed the differences between the autotetraploid and the diploid sporophytes (p<0.01). The spermatozoids of 2x antheridia were also larger than those of x antheridia (p<0.01). The meiotic chromosome configuration of the autotetraploid sporophyte showed univalent, bivalent and multivalent chromosomes at metaphase I and lagging chromosomes were frequently observed at both anaphase I and anaphase II. Among 30 spores one showed to be unreduced. From the 4x gametophyte 8x sporophytes could not be produced in *O. banksiiifolia*.

**KEYWORDS:** Apospory, Autotetraploid, Meiosis, *Osmunda banksiiifolia*

Apospory in which gametophytic tissue takes place from the sporophyte without the intervention of spores is well known phenomenon in ferns. It only occurs sporadically but it can be induced in ferns (Walker 1979). Since aposporously induced prothallium has the same chromosome number as the donor sporophyte, the sporophyte produced sexually from such prothallium is autopolyplid. Induced apospory was reported in many ferns (Raghavan 1989) and in some of autoploid sporophytes the meiosis was observed. Autotetraploid *Osmunda regalis* (Manton 1950) and autotetraploid *Osmunda lancea* (Kawakami et al. 2012) showed the presence of numerous multivalents and autoploid *Asplenium ruta-muraria* subsp. *Dolomiticum* (Bouharmont 1972) and autoploid *Pteris viitata* (Palta and Mehara 1983) showed a great preponderance of bivalents with only a few multivalent present. The number of multivalents was not constant even in the same plant and the exact numbers were varying from cell to cell. On the other hand, tetraploid ferns in nature, for example, *Asplenium ritoense* (2n=4x=144) (Kawakami 1970), *Thelypteris ducerctive-pinnata* (2n=4x=120) (Kurizono 1987), and *Lepisorus thunbergianus* (2n=4x=100) (Takei 1974; Mitui et al. 1987) showed a manner like allopoloids with n=72 ½ (Mitui 1968), n=60 ½ (Mitui 1968; Hirabayashi 1969; Masuyama 1979) and n=50 ½ (Mitui 1968), respectively. No multivalents were observed in their spore mother cells. For the study of chromosome pairing and multivalent chromosome formation in polyploid plants, artificially produced autopoloids are very important and useful materials. Much more materials are required and the information of meiosis is necessary. Since we produced autotetraploid *Osmunda banksiiifolia* (2n=4x=88) for the first time, the chromosome behavior at meiosis was observed. Furthermore, the sizes of guard cells and mature spores between 2x and 4x sporophytes and the sizes of spermatozoids between x and 2x antheridia were compared, and then the relative DNA contents of gametophytes derived from spores of the tetraploid sporophyte were investigated by using flow-cytometry.

**MATERIALS AND METHODS**

Sporophytes of *Osmunda banksiiifolia* (Pr.) Kuhn collected from a sporophyte cultivated in Nagoya Botanical Gardens in Japan were used for axenic culture. Surface-sterilized sporophytes of *O. banksiiifolia* were sown on 1/4 strength of Murashige and Skoog (MS) (1962) medium supplemented with 0.75% sucrose and 0.8% agar. After one year culture, sporophytes raised from gametophytes were used. For apospory, cutting fronds were put on the surface of 1/2 strength of MS medium supplemented with 2% sucrose and 0.9% agar and cultured for two months. Gametophytes raised from the margin of the frond were transferred onto 1/2 strength of MS medium supplemented with 2% sucrose and 0.9% agar and cultured for five years. Meiotic chromosomes were observed by fixing sporangia with 3:1 ethanol-aceitic acid for 30 min at 5°C and squashing them in 2% aceto-orcein solution. For the observation of mitotic chromosomes, root tips were harvested and pretreated in 0.002 M 8-hydroxyquinoline for 3 h at room temperature, fixed and hydrolyzed in the mixture of 1 N HCl and 45%...
acetic acid for 1 min at 60°C, and then stained with 2% aceto-orcein solution. The DNA contents of nuclei in fronds were estimated by flow-cytometry using a Partec Ploidy Analyzer PA (Partec Münster, Germany) (Kawakami et al. 2003).

**RESULTS**

Gametophytic tissue was raised from the cutting frond after two-month culture and sporophytes were produced from gametophytes developed from the frond after one year. The sporophyte cultivating in pot for several years was almost normal in appearance and hardly distinguishable from the normal diploid sporophyte, however, the pinnas were a little curly (Fig. 1). The DNA content of the sporophyte produced showed the double of the donor sporophyte and the mitotic chromosome was 2n=88 (Fig. 2).
Fig. 3. The comparative sizes of guard cells of the donor diploid sporophyte (a) and the artificially produced tetraploid sporophyte (b) in *O. banksiiifolia*. Bars=25 μm

Fig. 4. The comparative sizes of spores produced of the donor diploid sporophyte (a) and the artificially produced tetraploid sporophyte (b) in *O. banksiiifolia*. Bars=50 μm

Fig. 5. The comparative sizes of spermatozoids of the normal haploid (a) and the aposporous diploid (b) gametophytes in *O. banksiiifolia*. Bars=10 μm
Guard cells (Fig. 3), mature spores (Fig. 4) and spermatozoids (Fig. 5) between the donor diploid and the tetraploid sporophytes were compared. The mean size of 50 guard cells, 50 spores and 50 spermatozoids (Table 1) obtained from these sporophytes were compared.

Table 1. The mean size of guard cells, spores and spermatozoids obtained

<table>
<thead>
<tr>
<th></th>
<th>Diploid</th>
<th>Tetraploid</th>
<th>Significant difference $\chi^2$</th>
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<tbody>
<tr>
<td>Guard cell</td>
<td>60µm</td>
<td>75µm</td>
<td>$P^{***} &lt; 0.01$</td>
</tr>
<tr>
<td>Spore</td>
<td>61µm</td>
<td>83µm</td>
<td>$P^{***} &lt; 0.01$</td>
</tr>
<tr>
<td>Spermatozoid</td>
<td>14µm</td>
<td>17µm</td>
<td>$P^{***} &lt; 0.01$</td>
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Fig. 6. Meiosis of the tetraploid *O. banksiiifolia* with 2n=88. a: Meiotic chromosomes with bivalent chromosomes and multivalent chromosomes at metaphase I, b: Meiotic chromosomes with univalent, bivalent and multivalent chromosomes at metaphase I, c: Chromosome separation with lagging chromosomes at anaphase I, d: Chromosome separation with lagging chromosomes at anaphase II, e: Tetrads spores, f: Dyad spores. Bars=10µm (a, b, c, d). Bars =50µm (e, f).
was very significant (p<0.01) by Chi-squared test. The guard cells, mature spores and spermatozoids obtained from gametophytes in the tetraploid sporophyte were larger than those of the donor diploid sporophyte.

The meiotic chromosomes of the tetraploid *O. banksiifolia* (2n=88) showed bivalents and quadrivalents in some spore mother cells at metaphase I (Fig. 6a), however, in most of the mother cells, a few or several univalents with bivalents and multivalents were observed (Fig. 6b) and lagging chromosomes were frequently observed at both anaphase I (Fig. 6c) and anaphase II (Fig. 6d). Spores produced were mostly tetrads (Fig. 6e) and among them a few dyads were observed (Fig. 6f).

The relative DNA contents of nuclei in gametophytes germinated from spores were investigated. Of 30 gametophytes, 29 had 2x genomes and one had 4x genomes (Fig. 7). From the 4x gametophyte, sporophytes with octoploid genomes were not produced within 5-year culture.

**DISCUSSION**

Since base chromosome number of *Osmunda* is x=22 (Lovis 1977; Takamiya 1996), the artificially produced sporophytes with 2n=88 are autotetraploids. Autotetraploid *O. banksiifolia* (2n=4x=88) was reported here for the first time. The result that sizes of guard cells of polyploid *O. banksiifolia* were larger than those of the donor plant is coincident with the results of *O. regalis* (Manton 1950), *Pteris vittata* (Palta and Mehra 1983) and *Pteridium aquilinum* (Takahashi 1962). The result that sizes of spermatozoids of the 2x gametophyte were larger than those of the x gametophyte is coincident with the results of *O. regalis* (Manton 1950) and *Pteridium aquilinum* (Takahashi 1962). It was shown that polyploidy in *O. banksiifolia* is associated with increase of guard cells, spores and spermatozoids (p< 0.01).

The meiotic chromosomes of artificially produced autotetraploid *Asplenium ruta-muraria* showed a great preponderance of bivalents with a few multivalent (Bouharmont 1972), and those of autotetraploid *O. regalis* showed numerous multivalents (Manton 1950). In the present case of autotetraploid *O. banksiifolia*, though many quadrivalents were observed as shown in *O. regalis*, a few or several univalents addition to bivalents and multivalents were observed frequently. These results might indicate that quadrivalent chromosome formation by four homologous chromosomes at meiosis is unstable in artificially produced autotetraploid ferns and the exact number of quadrivalent chromosomes might be varying from cell to cell. The presence of univalent chromosomes produced by the disjoining of four homologous chromosomes makes meiosis irregular, and also makes the spore fertility extremely decreasing. On the other hand, tetraploids maintained in nature consistently showing only bivalents at meiosis might uniformly set good spores. It is therefore considered that autotetraploid ferns in nature might have a few or some quadrivalent chromosomes first, however, to make the meiosis regular, the
suppression system of multivalent chromosome formation might be obtained in the process of evolution as indicated by Walker (1984).

It is said that induced apospory in a normal sexual species is not repeated (Walker 1979). In the present study, from the 4x gametophyte octoploid sporophytes could not be produced. The study why gametes with activity of fertilization are not produced in 4x gametophytes is necessary.

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LITERATURE CITED