MEIOTIC CHROMOSOME BEHAVIOUR OF APOGAMOUSLY PRODUCED HAPLOID SPOROPHYTES IN *WOODWARDIA ORIENTALIS* AND *W. PROLIFERA*

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ABSTRACT

Sporogenesis of dihaploid *Woodwardia orientalis* with 2n=68 and haploid *W. prolifera* with 2n=34 produced by induced apogamy was investigated. The meiotic chromosome behaviour in spore mother cells of dihaploid *W. orientalis* showed many bivalent chromosomes, while that of haploid *W. prolifera* showed 34 univalent chromosomes at metaphase I. These observations support a basic chromosome number of x=34 for *Woodwardia*, with *W. orientalis* being an autotetraploid (2n=4x=136) and *W. prolifera* a diploid (2n=2x=68). Guard cell sizes of both induced haploid sporophytes were smaller than those their parental sporophytes ($t<0.01$).

INTRODUCTION

Following the development of a method to induce apogamy in gametophytes by sterile culture on a nutrient medium containing sugar, apogamous sporophyte formation in eight species has been induced (Whittier & Steeves, 1960; 1962), and apogamous sporophyte formation in 15 fern species has been induced by using sugar and hormones (Kawakami et al., 1995; 1996; 1997). These apogamously produced plants have half the chromosome number of the parental sporophyte – haploids being derived from a diploid species, or dihaploids from a tetraploid.

Polyploidy and high basic chromosome number are characteristic cytological features in ferns (Walker, 1979). The apogamously produced haploid plants from tetraploids are useful material for cytological study to uncover the origins of the tetraploid sporophytes through observation of meiotic chromosome behaviour in spore mother cells. Bivalent chromosomes support an autotetraploid origin, whereas mostly univalent chromosomes support an allotetraploid origin. Such observations have been reported in several induced apomict ferns (Manton, 1950; Manton & Walker, 1954; Bouhamont, 1972; Palta & Mehra, 1983; Kawakami et al., 1995; 1996).

The chromosome number of *Woodwardia orientalis* Sw. was determined to be n=68 (Mitui, 1967; 1968; Tsai & Shieh, 1983) and 2n=136 (Takamiya et al., 1992). The chromosome number of *W. prolifera* Hook. et Arn. was determined to be n=34 (Mitui, 1967; 1968; Tsai & Shieh, 1983) and 2n=68 (Takamiya et al., 1992). *Woodwardia orientalis* with 2n=136 has been recognised to be the only tetraploid in the genus *Woodwardia* (Takamiya et al., 1992); *W. prolifera* is diploid with 2n=68 (Takamiya et al., 1992), but was at one time treated as a subspecies of *W. orientalis* (*W. orientalis* var. *formosana* Rosent.) because of the very close external morphological resemblances with *W. orientalis* (Iwatsuki, 1992), but it has been proposed that *W. orientalis* (2n=4x=136) might be autotetraploid derived from *W. prolifera* (2n=68) as a result of chromosome doubling (Mitui, 1968). However, karyomorphological studies support the view that *W. orientalis* is an allotetraploid, derived by chromosome doubling from a hybrid of *W. prolifera* and an unknown diploid (Takamiya et al., 1992).

To help clarify the origin of *W. orientalis*, meiotic behaviour in spore mother cells of induced apomicts from *W. orientalis* and *W. prolifera* have been studied, and the relative DNA contents and guard cell sizes compared the apomicts and the parental sporophytes.

MATERIALS AND METHODS

Woodwardia orientalis was collected from Ishinomaki, Toyohashi City, Aichi Pref. and *W. prolifera* was a gift from the Higashiyama Botanical Gardens, Nagoya City, Aichi pref. The induced apomicts from *W. orientalis* and *W. prolifera* were developed by Kawakami et al. (1997). They have been cultivated for more than 10 years. Sporangia were fixed in 3:1 ethanol-acetic acid for 30 min at 5°C and squashed in 2% aceto-orcein solution. DNA content of nuclei in fronds was estimated by flow-cytometry using a Partec Ploidy Analyzer PA (Partec Münster, Germany) (Kawakami et al., 2003).

RESULTS

The fronds of the apomicts were smaller than those of the parental sporophytes, but otherwise were similar in morphology (Figure 1). The sprouting fronds were tinged in red in both in the haploid and the parental plant of *W. prolifera* while those of *W. orientalis* were green.

Comparison of guard cell size is shown in Figure 2. The mean size of 100 guard cells was 54.0 μm in normal *W. orientalis* and 40.2 μm in the corresponding dihaploid, 45.5 μm in normal *W. prolifera* and 31.7 μm in the haploid. Guard cells of apogamously produced sporophytes were significantly smaller in *W. prolifera* and in *W. orientalis* ($t<0.01$). Chromosome counts were confirmed for the parental plants of *W. orientalis* (68 bivalents, 2n=136) and *W. prolifera* (34 bivalents, 2n=68) (Figure 3).

Sporogenesis of dihaploid *W. orientalis* (2n=68) showed (Figure 4) many bivalents and some univalents at metaphase I (Figure 4B). Rarely 34 bivalents were observed (Figure 4C). Lagging chromosomes were seen frequently at anaphase I. Two nuclei were formed in the first division (Figure 4D) and tetrad spores with some abnormal spores were produced after the second division (Figure 4E). In most sporangia young spores were observed (Figure 4F), but at a later stage spores collected from fronds were mostly sterile. Eight out of approximately 1000 spores showed an ability to germinate.

Sporogenesis of haploid *W. prolifera* (Figure 5) showed only 34 univalents at metaphase I (Figure 5B). Occasionally two nuclei of different sizes (Figure 5C) or three nuclei (Figure 5D) were observed. A few tetrads with irregular shapes were found (Figure 5E), but no spores developed in the sporangia (Figure 5F).

DNA content of nuclei is given in Figure 6. Dihaploid *W. orientalis* showed approximately half that of the parental tetraploid (Figure 6A) and haploid *W. prolifera* showed approximately the half of that of the diploid (Figure 6B).

DISCUSSION

The relationship between guard cell size and polyploidy has been demonstrated in ferns (e.g. Takahashi, 1962; Kawakami et al., 2007; Kawakami et al., 2017). In the present study the size of guard cells shows a significant reduction in both induced apogamous plants.

For *Woodwardia orientalis* both an autotetraploid origin from *W. prolifera* (Mitui, 1968), and an allotetraploid origin from *W. orientalis* and another diploid taxon, based on karyomorphology (Takamiya et al.,1992), have been proposed. The observations of many (up to 34) bivalents in the spore mother cells of the apogamously produced diploid sporophyte in the present study supports the view that *W. orientalis* is autotetraploid.

For polyploid sporophyte formation in ferns it is considered that unreduced spores play very important role in nature (Gastony 1986). Autotetraploid *W. orientalis* (2n=136), therefore, might be produced by fertilization of 2x gametes derived from unreduced spores produced in the diploid *W. orientalis* (2n=68). Since it is known that unreduced spores are produced in haploid, triploid and hybrid sporophytes in which homologous chromosome pairing is failed at meiosis, in the case of diploid *W. orientalis*, unreduced

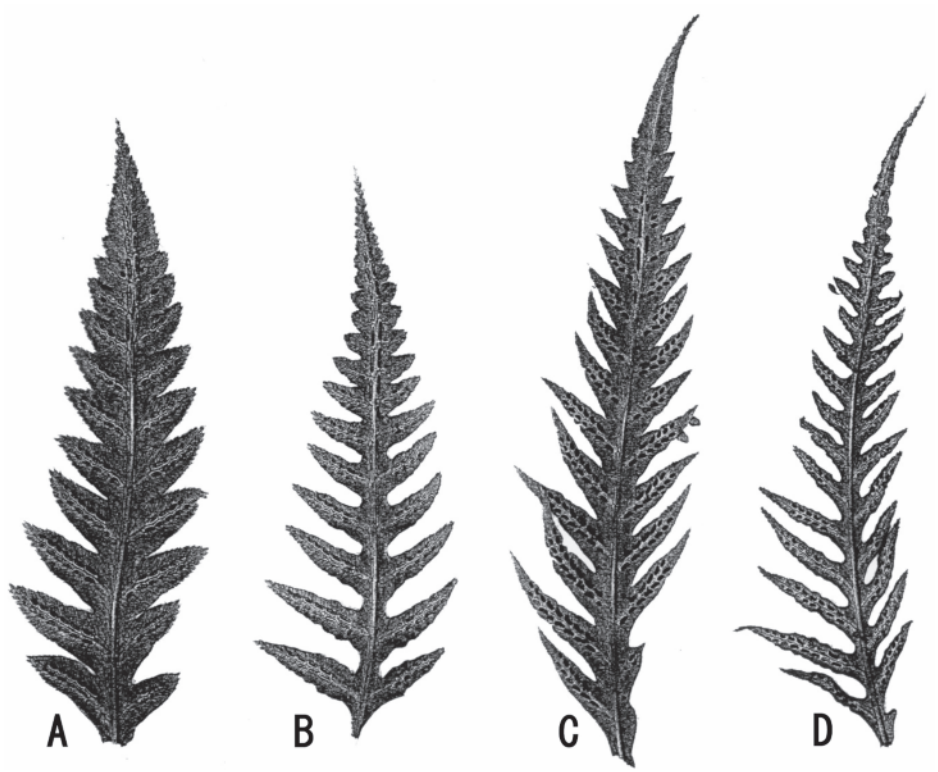

Figure 1. Fronds of *Woodwardia orientalis* and *W. prolifera*. A: Tetraploid *W. orientalis* (2n=136); B: Dihaploid *W. orientalis* (2n=68); C: Diploid *W. prolifera* (2n=68); D: Haploid *W. prolifera* (2n=34).

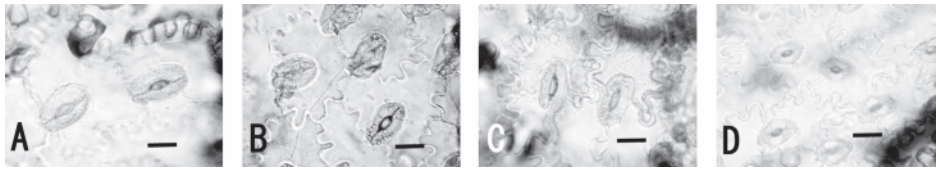

Figure 2. Guard cells of *Woodwardia orientalis* and *W. prolifera*. A: Tetraploid *W. orientalis* (2n=136); B: Dihaploid *W. orientalis* (2n=68); C: Diploid *W. prolifera* (2n=68); D: Haploid *W. prolifera* (2n=34). Scale bars; 25 μm.

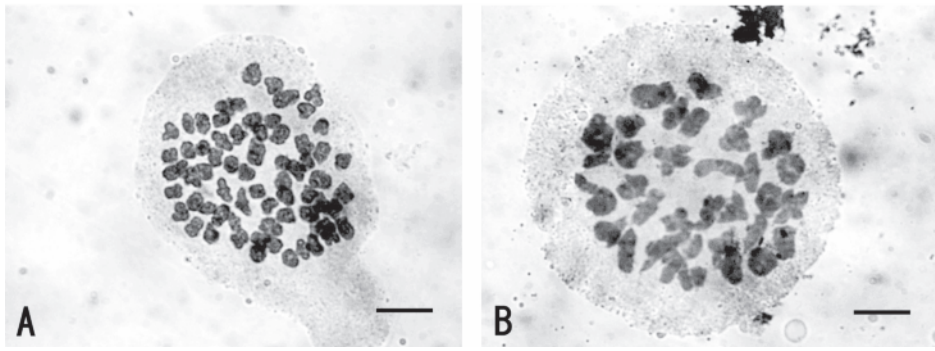

Figure 3. Meiotic chromosomes in *Woodwardia orientalis* and *W. prolifera*. A: Tetraploid *W. orientalis* showing 68 bivalents; B: Diploid *W. prolifera* showing 34 bivalents. Scale bars; 10 μm.

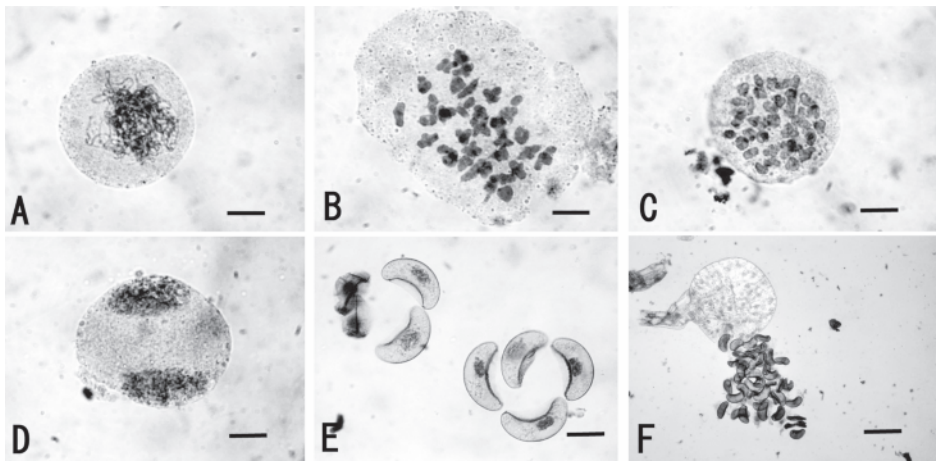

Figure 4. Sporogenesis in dihaploid *Woodwardia orientalis* (2n=68). A: prophase; B: meiotic chromosomes with many bivalents and a few univalents; C: meiotic chromosomes with 34 bivalents; D: two nuclei formed by the first division; E: tetrads with normal and abnormal spores; F: spores from one sporangium. Scale bars: A,B,C,D: 10 μm; E: 25 μm; F: 100 μm.

spores might be produced in diploid *W. orientalis* with some chromosome variation that was occurred accidentally by some means. This conflicts with the evidence based on karyomorphology (Takamiya et al., 1992), that gives a karyotype of 2n=136=8m+24sm+104(st+t), but, on morphology, does not have four homologous chromosome sets. However, differentiation within the karyotype does not appear to have been sufficient to prevent the ability of full pairing (34 bivalents) in the dihaploid. The evidence presented here does support an autotetraploid origin of *W. orientalis*, but does not clarify whether the parental diploid of *W. orientalis* was *W. prolifera*.

The evidence that 34 univalents were observed at metaphase I in haploid *W. prolifera* confirms the basic chromosome number of *Woodwardia* as x=34.

In our previous studies, meiotic chromosome behaviour of haploid sporophytes

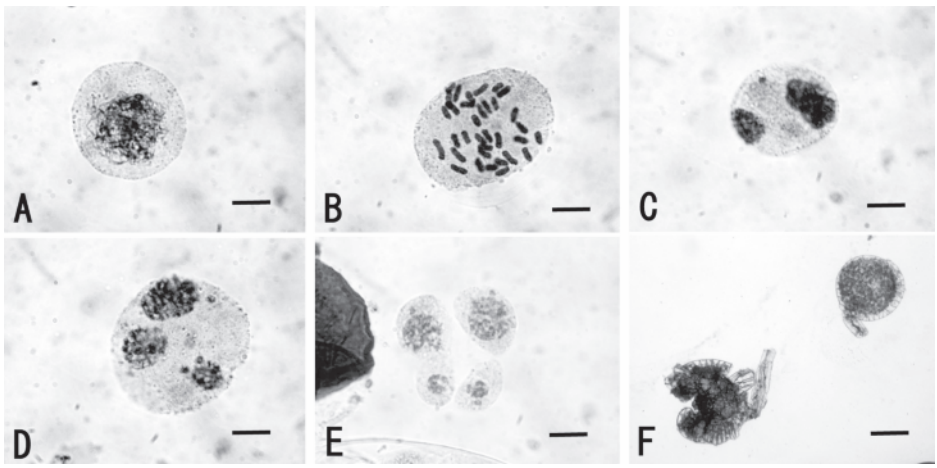

Figure 5. Sporogenesis in haploid *W. prolifera* (2n=34). A: prophase; B: meiotic chromosomes with only 34 univalents; C: two nuclei of different sizes; D: three nuclei of different sizes; E: a tetrad with irregularly sized spores; F: sporangia with no spores. Scale bars: A,B,C,D,E: 10 μm; F: 100 μm.

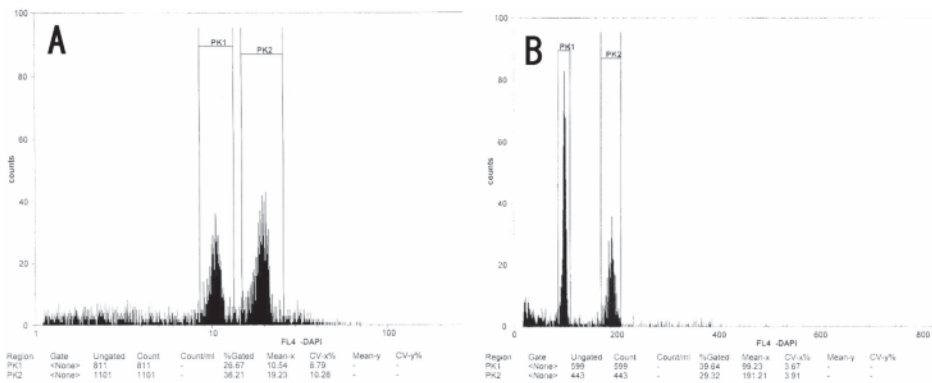

Figure 6. Relative DNA contents of nuclei of sporophytes. A: *Woodwardia orientalis* with 2n=68 (PK1) and with 2n=136 (PK2). B: *Woodwardia prolifera* with 2n=34 (PK1) and with 2n=68 (PK2).

produced by induced apogamy has been observed in four fern species: dihaploid *Pteris dispar* Kze. (2n=2x=58) with many bivalents (maximum 29) produced fertile reduced spores (Kawakami et al., 1996); dihaploid *Pteris multifida* Poir (2n=2x=58) showed a few bivalent chromosomes, and dihaploid *Pteris semipinnata* L. (2n=2x=58) showed only 58 univalent chromosomes but neither produced fertile reduced spores (Kawakami et al., 1995; Kawakami et al., 1996). The few fertile spores produced in dihaploid *W. orientalis* in the present study might be reduced spores, if the 34 bivalents at metaphase I can occasionally give balanced segregation, or by restitution before or during meiosis. Haploid *W. prolifera* with 34 univalents at metaphase I did not produce any fertile spores. Another induced haploid, *Osmunda japonica* (2n=x=22) with 22 univalents produced many fertile unreduced spores, presumably as a result of restitution (Kawakami et al., 2003).

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