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New Cytotypes of Four Japanese Ferns of Athyriaceae and Dryopteridaceae

Kiyotaka Hori[1*] and Noriaki Murakami[2]

[1]*The Kochi Prefectural Makino Botanical Garden 4200-6 Godaisan, Kochi 781-8125, Japan*

[2]*Makino Herbarium, Tokyo Metropolitan University, 1-1 Minami-osawa, Hachioji, Tokyo 192-0397, Japan*

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In this study a tetraploid sexual cytotype (2n = 160) of *Athyrium christensenianum* and tetraploid apogamous cytotypes (2n = 164) of *Dryopteris erythrosora*, *D. kinokuniensis*, and *D. nipponensis* have been reported for the first time from Japan.

Keywords: *Athyrium*, apogamous, chromosome, *Dryopteris*, fern, sexual

INTRODUCTION

In the sexual lineages of ferns, meiosis produces 64 haploid spores per sporangium after 4-times mitotic divisions of spore mother cells, each of which contains half of the parental chromosome numbers (Manton, 1950). In contrast, apogamous ferns produce 32 spores per sporangium by following one of the two alternative spore-generating pathways to yield chromosomally unreduced diplospores (Grusz, 2016): premeiotic endomitosis (Döpp, 1932; Manton, 1950) or meiotic first division restitution (Braithwaite, 1964). Most apogamous ferns produce their spores by following the former pathway (Manton, 1950).

In Japan, the chromosome numbers and reproductive modes of ferns have been studied well, covering over 74% taxa to date (Nakato and Ebihara, 2018). However, the information on chromosome numbers remains limited, necessitating the examination of previous studies wherein the cases with different cytotypes were included for the same species (Takamiya, 1996). Accordingly, this study focused on one *Athyrium* species and three *Dryopteris* species.

Regarding *Athyrium*, we focused on *A. christensenianum* (Koidz.) Seriz. The origin of *A. christensenianum* has been considered a hybrid between the diploid sexual *A. crenulatoserrulatum* Makino and tetraploid sexual *A. decurrentialatum* (Hook.) Copel. (Kurita, 1964; Hirabayashi, 1970a; Park and Kato, 2003). Furthermore, *A. christensenianum* is considered an incipient apomict owing to its ability of producing sporophytes from its gametophytes without fertilization (Park and Kato, 2003). However, Kurita (1964) and Hirabayashi (1970a) reported 40 bivalents and 40 univalents during meiosis in *A. christensenianum*. This type of meiosis cannot produce triploid spores, although it can produce diploid and haploid spores. Furthermore, no such cytotypes of *A. christensenianum* have been reported in the field as yet.

Regarding *Dryopteris*, we focused on the *D. erythrosora* complex (Hori et al., 2018a). In Japan, most members of the *D. erythrosora* complex have been reported to be triploid apogamous, except for *D. caudipinna* Nakai (diploid sexual; Hirabayashi, 1970b), *D. koidzumiana* Tagawa (diploid sexual; Mitui, 1967, 1968; Hirabayashi, 1969), and *D. kinkiensis* Koidz. ex Tagawa (tetraploid sexual; Hirabayashi, 1967). In addition, tetraploid apogamous *D. purpurella* Tagawa (Kurita, 1966) and tetraploid (unknown reproductive mode) *D. formosana* (Christ) C.Chr. (Nakato, 1987) have been reported from Japan.

To the best of our knowledge, this study is the first to provide information on the chromosome numbers of Japanese ferns through observation of the mitotic chromosome numbers of

* Corresponding author, email: khori@makino.or.jp

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A. christensenianum, *D. erythrosora* (D.C. Eaton) Kuntze, *D. kinokuniensis* Sa. Kurata, and *D. nipponensis* Koidz.

MATERIAL AND METHODS

Living individuals of *A. christensenianum*, *D. erythrosora*, *D. kinokuniensis*, and *D. nipponensis* were obtained from Japan (see Supplementary material). The live plants and voucher specimens of these species are stored in the Herbarium of the Kochi Prefectural Makino Botanical Garden (MBK) or Makino Herbarium (MAK). For the observation of mitotic chromosomes, root tips were pretreated in 0.004 M 8-hydroxyquinoline for 7 h at 17°C–20°C. Following fixation in an ethanol and acetic acid (3 : 1) solution for 15–30 min, root tips were hydrolyzed in 1 N HCl and 45% acetic acid (1:1) for 10 min at 60°C; then, they were stained and squashed in 2% aceto-orcein solution (Hori et al., 2018b). To estimate the reproductive mode of each sample or herbarium specimen, the spore numbers of each sporangium were counted. At least five sporangia with normal spores per specimen were studied. If the spore number of a certain sporangia of a plant was counted to be 64, the plant was considered to have reproduced sexually. In contrast, if the spore number of a certain sporangia was counted to be 32, the corresponding plant was considered to have reproduced apogamously (Manton, 1950).

RESULTS AND DISCUSSION

Previous studies implied the existence of a diploid cytotype of *A. christensenianum* (Kurita, 1964; Hirabayashi, 1970a; Park and Kato, 2003). Unexpectedly, the mitotic metaphase chromosome number observed in an individual of *A. christensenianum* from Kochi Pref. (*Hori 2974*) was 2n = 160 (Fig. 1a) with 64 spores per sporangium. The basic chromosome number of *Athyrium* was x = 40; accordingly, the corresponding sample was determined to be a tetraploid sexual cytotype. The origin of *A. christensenianum* was considered a hybrid between the diploid sexual *A. crenulatoserrulatum* and tetraploid sexual *A. decurrentialatum*. However, the existence of the tetraploid sexual cytotype of *A. christensenianum* suggested that the origin of *A. christensenianum* is more complicated.

The mitotic metaphase chromosome number observed in the materials of *D. erythrosora*, *D. kinokuniensis*, and *D. nipponensis* were 2n = 164 (Figs. 1b–d) with 32 spores per sporangium. The basic chromosome number of

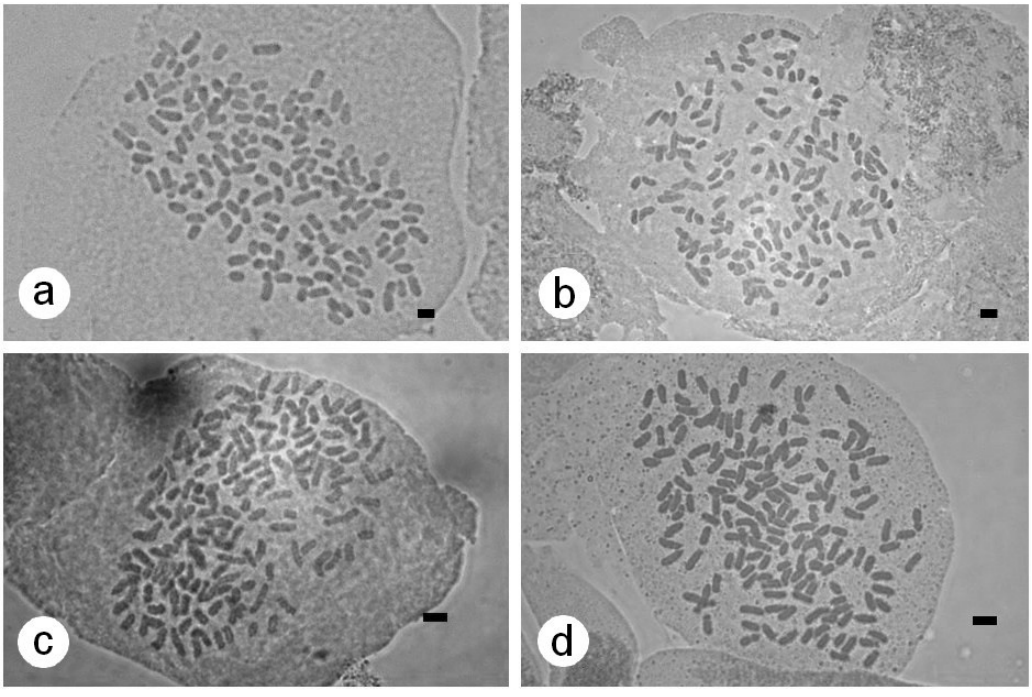

Fig. 1. Mitotic metaphase chromosomes of the (**a**) tetraploid sexual (2n = 160) *Athyrium christensenianum*, (**b**) tetraploid apogamous (2n = 164) *Dryopteris erythrosora*, (**c**) *D. kinokuniensis*, and (**d**) *D. nipponensis*. Scale bar = 4 μm.

Dryopteris is x = 41; accordingly, these samples were determined to be of the tetraploid apogamous type. The discovery of tetraploid apogamous cytotypes in this study suggests the existence of more tetraploid apogamous cytotypes in the *D. erythrosora* complex.

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