

NEW CYTOTYPES OF FOUR JAPANESE FERNS OF ATHYRIACEAE AND DRYOPTERIDACEAE

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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. crenuloserrulatum* 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.Ch. (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

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

number of a certain sporangia was counted to be 32, the corresponding plant was considered to have reproduced apogamously (Manton, 1950).

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

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. crenuloserrulatum* 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

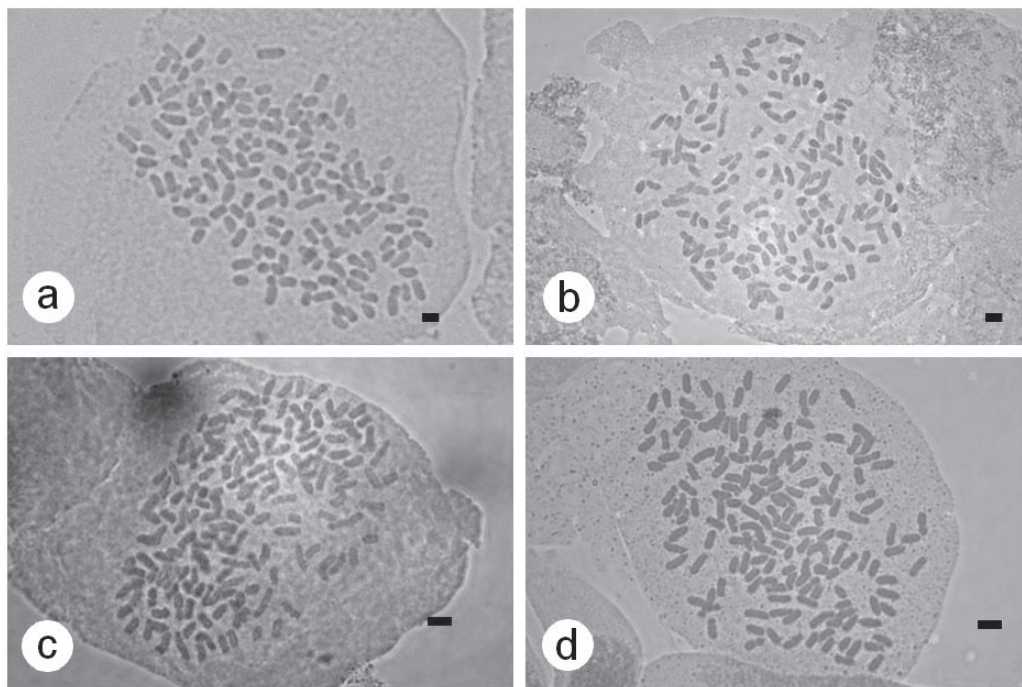


Fig. 1. Mitotic metaphase chromosomes of the (a) tetraploid sexual ($2n = 160$) *Athyrium christensenianum*, (b) tetraploid apogamous ($2n = 164$) *Dryopteris erythrosora*, (c) *D. kinokuniensis*, and (d) *D. nipponensis*. Scale bar = 4 μ m.

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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REFERENCES

- BRAITHWAITE AF. 1964. A new type of apogamy in ferns. *New Phytologist* 63: 293–305.
- DÖPP W. 1932. Die apogamie bei *Aspidium remotum* Al. Br. *Planta* 17: 87–152.
- GRUSZ AL. 2016. A current perspective on apomixis in ferns. *Journal of Systematics and Evolution* 54: 656–665.
- HIRABAYASHI H. 1967. Chromosome numbers in Japanese species of *Dryopteris* (2). *Journal of Japanese Botany* 42: 44–48.
- HIRABAYASHI H. 1969. Chromosome numbers in Japanese species of *Dryopteris* (3). *Journal of Japanese Botany* 44: 85–95.
- HIRABAYASHI H. 1970a. Chromosome numbers in several species of Aspidiaceae (2). *Journal of Japanese Botany* 45: 45–52.
- HIRABAYASHI H. 1970b. Chromosome numbers in Japanese species of *Dryopteris* (4). *Journal of Japanese Botany* 45: 11–19.
- HORI K, ZHOU XL, SHAO W, YAN YH, EBHARA A, WANG RX, ISHIKAWA H, WATANO Y, and MURAKAMI N. 2018a. Hybridization of the *Dryopteris erythrosora* complex (Dryopteridaceae) in Japan and adjacent areas. *Hikobia* 17: 299–313.
- HORI K, YAN YH, INOUE Y, and MURAKAMI N. 2018b. Evidence for maternal ability in hybridization of apogamous fern species: *Dryopteris tsushimense* K. Hori & N. Murak. and *D. subtsushimense* K. Hori et N. Murak. (Dryopteridaceae), new two tetraploid apogamous species which is of hybrid origin from Tsushima Island, Japan. *Acta Phytotaxonomica et Geobotanica* 69: 171–188.
- KURITA S. 1964. Cytotaxonomy of *Cornopteris*. *The Botanical Magazine, Tokyo* 77: 131–138.
- KURITA S. 1966. Chromosome numbers of some Japanese ferns (6). *Journal of Japanese Botany* 41: 173–180.
- MANTON I. 1950. *Problems of cytology and evolution in the Pteridophyta*. Cambridge University Press, 04 May 1951, 316 pp., Cambridge.
- MITUI K. 1967. Chromosome studies on Japanese ferns (3). *Journal of Japanese Botany* 42: 105–110.
- MITUI K. 1968. Chromosomes and speciation in ferns. *Science Reports of Tokyo Kyoiku Daigaku Section B* 13: 285–333.
- NAKATO N. 1987. Notes on chromosomes of Japanese pteridophytes (1). *Journal of Japanese Botany* 62: 261–267.
- NAKATO N, and EBHARA A. 2018. Chromosome numbers of eleven ferns in Japan. (Athriaceae, Dryopteridaceae and Tectariaceae). *Bulletin of the National Museum of Nature and Science Series B Botany* 44: 23–30.
- PARK CH, and KATO M. 2003. Apomixis in the interspecific triploid hybrid fern *Cornopteris christensenianum* (Woodsiaceae). *Journal of Plant Research* 116: 93–103.
- TAKAMIYA M. 1996. *Index to chromosomes of Japanese Pteridophyta (1910–1996)*. Japan Pteridological Society, Tokyo.