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A new sexual diploid of *Dryopteris erythrosora* complex (*Dryopteridaceae*) from Oki Islands, Japan

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Abstract A new sexual diploid of *D. erythrosora* complex was discovered from the Oki Islands in Japan. Spores of this plant were observed and the meiotic chromosome number was confirmed to be n=41II(2x). This diploid plant differs from the two other endemic sexual diploid species in the *D. erythrosora* complex, *D. caudipinna* and *D. koizumiana*, in having pale-green indusia. This newly reported diploid plant will help inform the origin and diversity of agamosporous species within the *D. erythrosora* complex in Japan.

Keywords: *Dryopteris*, indusia, agamosporous, sexual diploid, Oki Islands

Dryopteris erythrosora (Eat.) O. Kuntze is a representative triploid agamosporous fern of Japanese *Dryopteris*. The *Erythrosora* group consists of seventeen species, and 2 forms in Japan (Hirabayashi, 1974). With the exception of two sexual diploid species, *D. caudipinna* Nakai, and *D. koizumiana* Tagawa, one sexual tetraploid *D. kinkiensis* Koiz. ex Tagawa, and one agamosporous tetraploid *D. purpurella* Tagawa, all of the species in this group are agamosporous triploids (Hirabayashi, 1974; Takamiya, 1996). Most agamosporous triploid species are distributed widely throughout Honshu, Shikoku and Kyushu in Japan and are extremely variable in morphological and genetic characteristics. It is considered that most agamosporous triploids or tetraploids originated from some sexual diploid species. These polyploids had characteristics that allowed them to grow well in man-made habitats, and are thought to have spread quickly in Japan as a result (Iwatsuki, 1992). Despite this proposed evolutionary scenario, only two sexual diploid species have been so far reported in this group in Japan. One of these diploids is *D. koizumiana*, an endemic to the Ryukyu Islands, Kagoshima, in the south of Japan. The other diploid is *D. caudipinna*, which is distributed in Honshu, but occurs only in very limited local areas, such as the Izu Islands in Kanagawa prefecture (Iwatsuki, 1992); Tsushima Island, Tochigi, Ibaraki and Shimane prefectures (Nakaike, 1992, Lin,unpublished). *Dryopteris caudipinna* has been considered to be an ancestral form of the *D. erythrosora* complex (Iwatsuki, 1992). However, it is not sufficient to elucidate the extreme polymorphism and diversity of the species in Honshu, Kyushu, Shikoku by this one endemic sexual diploid species alone. As such, the origin and mechanism of the formation of diversity mechanism in this agamosporous group remains problematic.

In this study, we report a new sexual diploid form of *D. erythrosora* from Oki Islands, Shimane prefecture. *D*.

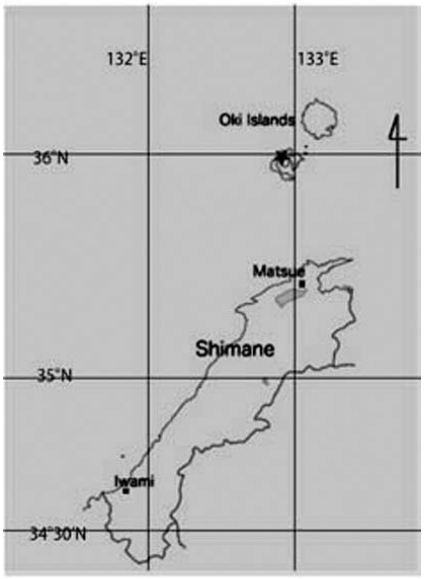

Figure 1. The map of Shimane prefecture. ★shown the locality of Nishinoshima-cho, Oki Islands.

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erythrosora is typically quite variable, but is generally recognized as a triploid agamosporous species. The discovery of a sexual diploid plant within this species is quite interesting, and may offer information for resolving questions about the origin of the diversity in the *D. erythrosora* complex.

Materials and Methods

One individual of *D. erythrosora* with pale-green indusia was found during our field investigations of *D. caudipinna* in Nisinoshima, Oki Islands (Fig. 1). This plant was growing among a mixed population of *D. erythrosora* (apo. 3x) and *D. caudipinna* (sex. 2x) (Lin, Unpublished) in Japanese cedar (*Cryptomeria japonica* D.Don) forest. The mature fronds were used for morphological observation. The voucher specimen (Lin et al. 05052724, Nishinoshima-cho, Oki Islands, Shimane prefecture, 2005, May, 27) is deposited in the Department of Biological Science, Faculty of Life and Environmental Science, Shimane University.

Figure 2. The sexual diploid form of *Dryopteris. erythrosora* complex from Oki Islands. **A.** A frond. **B.** Pinnae with sori. **C.** A part of a pinna, shown the pale-green indusia and bullate scales of pinna rachis. **D.** A part of frond. (Arrow shown the shorter basal basiscopic pinnules of lowest pinnae). **E.** The stipe with deep brown-blackish scales.

Fresh leaf material was fixed in acetic acid-alcohol (1 : 3) solution, store at 5-10℃, and sporangia were stained with 2% acetic acid-orcein, and squashed by standard method (Lin et al. 1990) for meiosis observation.

Results and Discussion

Morphological aspects of the plant are noted below (Fig. 2). Stipes stramineous, with dense scales. Scales brown at pinna rachis, deep brown to blackish brown at stipes, or black at stipe base (Fig. 2E). Laminae oblong-ovate, about 40-50cm long, bipinnate, pinnae narrowly oblong, caudate at apex. The basal basiscopic pinnules of the lowest pinnae are shorter than others (Fig. 2D). All of these characters are within the range of variation of *D. erythrosora*, and no specific differences can be found in this plant from *D. erythrosora*. On the other hand, the sudden-narrowing and elongate upper part of laminae, as well as linear-lanceolate to linear pinnules are similar to *D. caudipinna* (Fig. 2B). However, this plant is different from both species in having stramineous stipes (not red or purplish), and pale green (non-red) indusia (Fig. 2C). A similar variant form with different colored indusia has been previously reported as *D. erythrosora* form. *viridisora* (Nakai ex H. Itô) H. Itô (Itô, 1939), but was reported cytologically to be an agamosporous triploid, with n=123 chromosomes (Hirabayashi, 1974).

The results of this study indicate that the individual from the Oki Islands is a normal sexual diploid, with sixteen spore mother cells having reductive division and 41 bivarent gametic chromosomes in sporogenesis (Fig. 3), resulting in normal 64 spores in each sporangium. We therefore conclude that this is a new sexual diploid form of *D. erythrosora* complex in Japan.

According to the morphological observations, this plant may be determined to be *D. erythrosora* form. *viridisora*, which is also a new distribution record in Oki Islands, Shimane prefecture. Furthermore, it is worthy to note that the plant was found growing together with *D. caudipinna* (sex. 2x) and *D. erythrosora* (apo. 3x), and morphologically closely resembles both of these two species. This

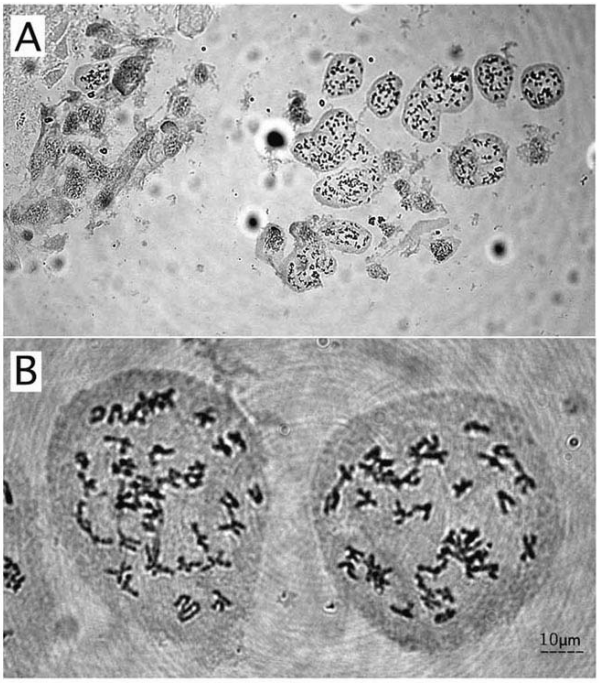

Figure 3. The sporogenesis of the sexual diploid form of *D. erythrosora* from Oki Islands **A.** Sixteen spore mother cells at metaphase I. **B.** Two spore mother cells shown the gametic chromosomes, n=41II.

plant may be interpreted as a variety of *D. caudipinna with* non-red indusia that arose from some genetic mutation, or it is a relict species, within the lineage of one of the ancestral diploid progenitor species of *D. erythrosora* complex. In either case, it is possible that this sexual diploid can inform research on the origin and diversity of agamosporous species, especially with those species having non-red indusia, such as, *D. nipponensis* (3x) Koidz. widely distributed in Japan including Oki Islands, as well as the agamosporous triploid of *D. erythrosora* form. *viridisora* in Kyusyu, Shikoku and Mie (Hirabayashi, 1974). Further research including field investigation of the mixed populations, additional samples using cytological, molecular, and morphological characters will help clarify additional details about the origin and diversity of the group.

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References

Hirabayashi Haruki (1974) Cytogeographic Studies on *Dryopteris* of Japan. Harashobo, Tokyo.

Itô Hiroshi (1936) Filices Japonensis .Bot. Mag. Tokyo 50 : 69

Iwatsuki Kunio (1992) Ferns and fern allies of Japan. Heibonsha Ltd., Publishers, Tokyo.

Lin Su-Juan, Kato Masahiro and Iwatsuki Kunio (1990) Sporogenesis, reproductive mode, and cytotaxonomy of some species of *Sphenomeris, Lindsaea,* and *Tapeinidium* (Lindsaeaceae). Amer. Fern J. 80 : 97-109.

Nagaike Toshiyuki (1992) New flora of Japan Pteridophyta. Shibondo, Tokyo. pp 400-463.

Sugimura Yoshinori (1997) A flora of pteridophyte in Shimane prefecture. Bulletin of the Hosizaki Green Foundation, No. 1 : 221-232 (in Japenese)

Takamiya Masayuki (1996) Index to chromosomes of Japanese Pteridophyta. Japanese Pteridological Society. Nippon Print Center, Tokyo. pp 18-28.