Chromosome Numbers of Eleven Ferns in Japan (Athyriaceae, Dryopteridaceae and Tectariaceae)

Narumi Nakato¹ and Atsushi Ebihara², *

¹ Narahashi 1–363, Higashiyamato-shi, Tokyo 207–0031, Japan
² Department of Botany, National Museum of Nature and Science, Amakubo 4–1–1, Tsukuba, Ibaraki 305–0005, Japan
*E-mail: ebihara@kahaku.go.jp

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Abstract Mitotic chromosome numbers of eleven leptosporangiate fern taxa (Athyriaceae, Dryopteridaceae and Tectariaceae) in Japan were counted and their reproductive modes were estimated by observing spore number per sporangium. Chromosome numbers were recorded for the first time accompanied by chromosome illustrations for five species (Deparia coreana, D. henryi, D. kiusiana, D. minamitanii and Tectaria phaeocaulis), two infraspecific taxa (Dryopteris sparsa var. ryukyuensis and Arachniodes yasu-inouei var. angustipinnula) and three putative interspecific hybrids (Anisocampium × saitoanum, Athyrium × bicolor and Arachniodes × mirabilis).

Key words: Anisocampium, Arachniodes, Athyrium, chromosome number, Deparia, Tectaria.

Japan is one of the best-studied areas for chromosome numbers of ferns and lycophytes (i.e. pteridophytes) in the world, with data available for 667 taxa (ca. 60% of the total pteridophyte flora; Nakato and Ebihara, 2016). This coverage increases to ca. 74% (538 taxa) if hybrid taxa are excluded. Furthermore, coverage reaches nearly 80% (368/463 taxa) for eupolypods, a large clade comprising ca. 6000 species including Aspleniaceae, Athyriaceae, Dryopteridaceae, Polypodiaceae and Thelypteridaceae (Schneider et al., 2004). Although base chromosome numbers of eupolypods are not highly variable and mostly range from $x=31$ to 41 (Smith et al., 2006), information drawn from chromosome observations such as ploidy, spore fertility and reproductive mode can be powerful clues for identifying biological entities in species complexes that have resulted from reticulate evolution (e.g. Ebihara et al., 2014). With the goal of contributing to the complete taxon sampling of chromosome observations for Japanese pteridophytes, we observed mitotic chromosomes and spores in eight species plus three interspecific hybrids of Japanese ferns that previously lacked published chromosome information.

Materials and Methods

Materials used for chromosome counting are listed in Table 1. All voucher specimens are deposited in TNS. Methods for counting mitotic chromosomes in root tips of living stocks followed those of Ebihara et al. (2014). Reproductive mode was determined by counting spore number per sporangium and by spore shape regularity in the voucher specimens or in cultivated stocks.

Results and Discussion

Athyriaceae

*Anisocampium × saitoanum* (Sugim.) M.Kato — $2n=120$ (3x, sterile) [Fig. 1]

A putative hybrid taxon between *Anisocampium niponicum* (Mett.) Y.C.Liu, W.L.Chiou et
Table 1. Plant material used in this study with chromosome counts

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Chromosome number</th>
<th>Reproductive mode</th>
<th>Voucher</th>
<th>Locality</th>
<th>Chromosome figure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Athyriaceae</td>
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<tr>
<td><em>Anisocampium</em> × <em>saitoanum</em></td>
<td>2n = 120, (3x, x = 40)</td>
<td>sterile</td>
<td>Nakato 2804</td>
<td>Fukuoka Pref., Kasugayu-ku, Sasaguri-machi, Mt. Wakasugi</td>
<td>Fig. 1</td>
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<tr>
<td>(Sugim.) M.Kato</td>
<td></td>
<td>(spore irregular)</td>
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<tr>
<td><em>Athyrium rupestre</em> Kodama</td>
<td>2n = 80, (2x, x = 40)</td>
<td>sexual</td>
<td>Nakato &amp; Ebihara 3420</td>
<td>Aomori Pref., Mutsu-shi, Mt. Kamafuse</td>
<td>Fig. 2</td>
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<tr>
<td></td>
<td></td>
<td>(64 s/s)</td>
<td>[TNS VS-1286239]</td>
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<tr>
<td><em>Athyrium × bicolor</em> Seriz.</td>
<td>2n = 200, (5x, x = 40)</td>
<td>sterile</td>
<td>Nakato 1675</td>
<td>Fukushima Pref., Yama-gun, Atsushikanomura</td>
<td>Fig. 3</td>
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<td></td>
<td></td>
<td>(spore irregular)</td>
<td>[TNS VS-1277150]</td>
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<tr>
<td><em>Deparia coreana</em> (H.Christ) M.Kato</td>
<td>2n = 160, (4x, x = 40)</td>
<td>sexual</td>
<td>AE 3547 (= Nakato 3178)</td>
<td>Aomori Pref., Hachinohe-shi, Shirahama</td>
<td>Fig. 4</td>
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<td>(64 s/s)</td>
<td>[TNS VS-1265113]</td>
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<tr>
<td><em>Deparia kiusiana</em> (Koidz.) M.Kato</td>
<td>2n = ca. 240, (6x, x = 40)</td>
<td>sexual</td>
<td>Nakato 2870</td>
<td>Aichi Pref., Kitashitara-gun, Shitara-cho (cultivated in Tsukuba Botanical Garden)</td>
<td>Fig. 5</td>
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<td></td>
<td></td>
<td>(64 s/s)</td>
<td>[TNS VS-1280185]</td>
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<tr>
<td><em>Deparia minamitani</em> Seriz.</td>
<td>2n = 240, (6x, x = 40)</td>
<td>sexual</td>
<td>Nakato 2871</td>
<td>Miyazaki Pref., Kobayashi-shi (cultivated in Tsukuba Botanical Garden)</td>
<td>Fig. 6</td>
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<td></td>
<td></td>
<td>(64 s/s)</td>
<td>(KS2007-285)</td>
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<td></td>
<td>[TNS VS-774852]</td>
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<tr>
<td><em>Deparia henryi</em> (Baker) M.Kato</td>
<td>2n = 120, (3x, x = 40)</td>
<td>apogamous</td>
<td>AE 3533</td>
<td>Osaka Pref., Takatsuki-shi, Kawakubo</td>
<td>Figs. 7, 8</td>
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<td></td>
<td></td>
<td>(32 s/s)</td>
<td>[TNS VS-1268624]</td>
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<td>Dryopteridaceae</td>
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<td><em>Dryopteris sparsa</em> (Buch.-Ham. ex D.Don) Kuntze var. ryukyuensis Seriz.</td>
<td>2n = 164, (4x, x = 41)</td>
<td>sexual</td>
<td>A. Ebihara, T. Oka &amp; T. Minamitani 3247</td>
<td>Miyazaki Pref., Miyazaki-shi, Tano, Miyazaki University Experimental Forest</td>
<td>Fig. 9</td>
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<td></td>
<td></td>
<td>(64 s/s)</td>
<td>[TNS VS-1176839]</td>
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<td><em>Arachniodes yasu-inouei</em> Sa.Kurata var. angustipinnula Seriz.</td>
<td>2n = 164, (4x, x = 41)</td>
<td>sexual</td>
<td>Nakato 3002</td>
<td>Miyazaki Pref., Kobayashi-shi, Nagakuino (cultivated by T. Minamitani)</td>
<td>Fig. 10</td>
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<td></td>
<td></td>
<td>(64 s/s *)</td>
<td>[TNS VS-1244729]</td>
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<tr>
<td><em>Arachniodes × mirabilis</em> Sa.Kurata</td>
<td>2n = 123, (3x, x = 41)</td>
<td>sterile</td>
<td>Nakato 3000</td>
<td>Miyazaki Pref., Ebino-shi, Iino (cultivated by T. Minamitani)</td>
<td>Fig. 11</td>
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<td></td>
<td></td>
<td>(sporangium empty)</td>
<td>[TNS VS-1247772]</td>
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<td>Tectariaceae</td>
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<td><em>Tectaria phaeocaulis</em> (Rosenst.) C.Chr.</td>
<td>2n = 80, (2x, x = 40)</td>
<td>sexual</td>
<td>AE 3563</td>
<td>Okinawa Pref., Kunigami-gun, Kunigami-son, Iyudake</td>
<td>Fig. 12</td>
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<td></td>
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<td>(64 s/s)</td>
<td>[TNS VS-1265102]</td>
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</table>

*1 Spore number counted on a herbarium specimen (TNS VS-1261195).
M. Kato and *A. sheareri* (Baker) Ching. The present result (triploid) can be explained by combining the ploidies of the putative parent species: diploid *A. niponicum* (*n* = 40 [Kurita, 1960; Mitui, 1968; Hirabayashi, 1970], *2n* = 80 [Tatuno and Okada, 1970]) and tetraploid *A. sheareri* (*n* = 80 [Kurita, 1976]). Although this is the first formal chromosome record for this taxon, the triploid individual with irregular meiosis that Kurita (1976) reported as *Athyrium* (*Anisocampium*) *sheareri* was almost certainly this hybrid (S. Kurita, personal communication). This hybrid sometimes occurs where *A. sheareri* is not found, but the individual we examined produced fully irregular spores and is not suggestive of apogamous reproduction. Further sampling is necessary to determine the rate of fertility of spores for this hybrid.

*Athyrium rupestre* Kodama — *2n* = 80 (2x, sexual) [Fig. 2]

Although the base chromosome number of *Athyrium* had been considered to be *x* = 40 (Kramer *et al.*, 1990), Nakato (1988) observed *x* = 39 in *A. nikoense* Makino, a member of the *A. yokoscense* group, and a subsequent careful study (Tsuji, 2006) confirmed that *A. yokoscense* (Franch. et Sav.) H.Christ and its close relatives (*A. kirishimaense* Tagawa and *A. tashiroi* Tagawa) have the base number *x* = 39 without
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exception. *Athyrium rupestre* is a closely allied species to *A. yokoscense* in appearance, but its inclusion in the *A. yokoscense* clade has not yet been supported by any molecular phylogenetic analyses (Adjie et al., 2008; Ebihara, 2011). Our present count confirms the base number $n = 40$ and $2n = 80$ by Mitui (1970, 1980), and it suggests that *A. rupestre* retains the plesiomorphic state of the base number.

*Athyrium × bicolor* Seriz. — $2n = 200$ (5x, sterile) [Fig. 3]

We here report the first chromosome count for the taxon. It is a putative hybrid between *A. vidalii* (Franch. et Sav.) Nakai and *A. neglectum* Seriz. subsp. neglectum, often collected in sympatry. Considering the present result (pentaploid) and the tetraploidy of *Athyrium vidalii* ($n = 80$ [Kurita, 1960; Mitui, 1968]), we propose that the other parental species *A. neglectum* subsp. neglectum is likely hexaploid.

*Deparia coreana* (H.Christ) M.Kato — $2n = 160$ (4x, sexual) [Fig. 4]

Shimura (1984) reported a haploid chromosome number $n = 80$ without any chromosome illustration or citation of a voucher specimen for this species. Our present result ($2n = 160$, tetraploid) is the first formal chromosome record for this species, and is congruent with Shimura’s report. Among the six species belonging to the Dryoatyhrium (DR) clade (Kuo et al., 2016) in Japan, this is the sole tetraploid species — the remaining members are include two diploid sexual species (*D. pterorachis* (H.Christ) M.Kato and *D. viridifrons* (Makino) M.Kato) and three triploid apogamous species (*D. okuboana* (Makino) M.Kato, *D. unifurcata* (Baker) M.Kato and *D. henryi* (Baker) M.Kato, see below) (Takamiya, 1996).

*Deparia kiusiana* (Koidz.) M.Kato — $2n = ca. 240$ (6x, sexual) [Fig. 5]

This is the first chromosome count for this species. Closely related *Deparia dimorphophylla* (Koidz.) M.Kato, formerly treated at the varietal rank under *D. kiusiana*, is also known as a hexaploid (Nakato, 1996). The *Athyriopsis* (AT) clade (Kuo et al., 2016) shows high species diversity in Japan (seven species), but its constituent taxa are exclusively polyploid (tetraploid and hexaploid [Takamiya, 1996; Aman and Serizawa, 2011]).

*Deparia minamitanii* Seriz. — $2n = 240$ (6x, sexual) [Fig. 6]

This is the first chromosome count for this species. This is a critically endangered species confined to just a few localities in Kyushu, and it has closely related species with unknown ploidy distributed in China (Kuo et al., 2016).

*Deparia henryi* (Baker) M.Kato — $2n = 120$ (3x, apogamous) [Figs. 7, 8]

This species was unrecognized in most of the florae of Japan (e.g. Iwatsuki et al., 1995) except for misapplication of the name to *D. okuboana* (Makino) M.Kato (e.g. Kurita, 1960). Mitsuta (2003) first noticed its occurrences in western Japan. Its laminar shape is similar to *D. coreana*, but the round-reniform soral shape is more similar to *D. okuboana*. Our present result suggests *D. henryi* is also distinct in its apogamous reproduction from sexually reproducing *D. coreana*. On the other hand, *D. okuboana* and *D. henryi* likely form a species complex largely composed of apogamous triploid individuals, and are awaiting global and comprehensive revision.

**Dryopteridaceae**

*Dryopteris sparsa* (Buch.-Ham. ex D.Don) Kunze var. *ryukyuensis* Seriz. — $2n = 164$ (4x, sexual) [Fig. 9]

The present result is the first chromosome count for this taxon. It was recognized by Serizawa (1971) as distinguishable from var. *sparsa* in having smaller fronds and growing on cliffs. Except for having cleft indusia at the mature stage, *D. hayatae* Tagawa is morphologically similar to this taxon, but is cytologically distinct, being a sexual diploid (Mitui, 1966, 1968, 1976; Kurita, 1967; Hirabayashi, 1969). It is notable that var. *sparsa* is cytologically variable (Dar-
naedi et al., 1989) amongst the populations in Japan, which include sexual tetraploid (Darnaedi and Iwatsuki, 1987; Darnaedi et al., 1989; Kurita, 1966; Hirabayashi, 1966, 1974), apogamous diploid (Darnaedi et al., 1989) and apogamous triploid (Darnaedi and Iwatsuki, 1987; Darnaedi et al., 1989; Hirabayashi, 1974) cytotypes.

Arachniodes yasu-inouei Sa.Kurata var. angustipinnula Seriz. — 2n = 164 (4x, sexual) [Fig. 10]

This is the first chromosome count for this taxon. It was initially collected in 1976, but thought to be extinct due to logging of the forest at the original site. Later it was described at the varietal rank under A. yasu-inouei (Serizawa, 2009), followed by rediscovery in November,
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2013 nearby the type locality. We observed chromosome numbers of the rediscovered stock collected and cultivated by T. Minamitani, and counted 64 spores per sporangium on a herbarium specimen. These results confirmed that its ploidy level and chromosome number are the same as those of *A. yasun-inouei* var. *yasun-inouei* (*n* = 82 [Shimura et al., 1982]).

*Arachniodes × mirabilis* Sa.Kurata — 2*n* = 123 (3x, sterile) [Fig. 11]

This is a putative hybrid between *A. amabilis* (Blume) Tindale var. *fimbriata* K.Iwats. (tetraploid, *n* = 82 [Mitui, 1966, 1968]) and *A. yoshinagae* (Makino) Ching (tetraploid, *n* = 82 [Kurita, 1966]; *n* = ca. 80 [Mitui, 1968]). Shimura (1983) reported chromosome number 2*n* = ca. 164, *n* = ca. 50I + ca. 64I without providing any chromosome illustration. Our count is the first formal chromosome record for this hybrid, but the ploidy level (triploid) is inconsistent with former record (tetraploid) by Shimura (1983), and suggests one of the parents is not tetraploid *A. amabilis* var. *fimbriata*, but rather another diploid taxon. One candidate diploid taxon is *A. amabilis* var. *amabilis*, which is reported as a sexual diploid (Shimura et al., 1982), but is so far undiscovered in Miyazaki Prefecture (Ebihara, 2017; T. Minamitani, pers. comm.).

*Tectariaceae*

*Tectaria phaeocaulis* (Rosenst.) C.Chr. — 2*n* = 80 (2x, sexual) [Fig. 12]

This is the first chromosome count for this species. *Tectaria* remains a poorly studied genus amongst Japanese ferns for chromosome numbers, that is, previously counts have been reported for only two of eight native species (i.e. *T. harlandii* (Hook.) C.M.Kuo [*n* = ca. 80, Mitui, 1976] and *T. subtriphyllla* (Hook. et Arn.) Copel. [*n* = 40, Mitui, 1976]).

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References


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