Chromosomal Studies of Ferns and Fern-allies in the
Republics of Fiji and Vanuatu, South Pacific I. Psilotaceae,
Ophioglossaceae, Marattiaceae and Schizaceae

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Abstract. Chromosome numbers of four ferns and a fern-ally native to Fiji and Vanuatu were examined. Chromosome numbers reported were \( n = 52 \) in Psilotum complanatum (Psilotaceae), \( n = \text{ca.} 240 \) in Ophioglossum petiolatum (Ophioglossaceae), \( 2n = 160 \) in Angiopteris evecta (Marattiaceae), \( 2n = 78 \) in Marattia smithii (Marattiaceae) and \( 2n = 60 \) in Lygodium reticulatum (Schizaceae). The chromosome numbers of Marattia smithii and Lygodium reticulatum is reported here for the first time, and those of the remaining three species were determined for the first time in Fijian and Vanuatuan plants. In Lygodium reticulatum, the karyomorphology of somatic chromosomes is described.

Key words: chromosome number, fern, fern-ally, Fiji, karyotype, Vanuatu

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During the period from December 1991 to January 1992, I had visited the republics of Fiji and Vanuatu as a member of the botanical expedition "Distribution and speciation of cryptogams in Fiji and adjacent areas, Oceania" headed by Dr. Z. Iwatsuki. I collected many living and fixed materials of ferns and fern-allies for cytological examination.

Fiji and Vanuatu are situated in the center of Melanesia. Several floral studies of their pteridophytes have been undertaken (see Braithwaite, 1975a; Brownlie, 1977), but, little or no cytological information on the ferns or fern-allies is available, except for detailed surveys of the Hymenophyllaceae and Timesipteris (Braithwaite, 1975b, 1986). Although my cytological findings are too limited to allow a discussion of the species groups in both countries, the chromosome numbers of most species in Melanesia are reported here for the first time. Chromosomal information for the ferns and fern-allies of Fiji and Vanuatu will contribute to our knowledge of the taxonomy of these groups.

Material and Methods

The taxa, collection sites, vouchers and chromosome numbers are listed in Table 1. Nomenclature follows Brownlie (1977). Vouchers are de-
posited in the herbarium of Kumamoto University (KUMA).

For observation of the meiotic chromosomes of *Psilotum* and *Ophioglossum*, young synangia and/or spikes were fixed in acetic alcohol (glacial acetic acid: 99% ethanol = 1:3) in the field for about one day then placed in 70% ethanol and brought to Japan. They were stored in a refrigerator until examination, and were re-fixed in acetic-alcohol for more than 1 hr just before preparation. Meiotic chromosomes were observed using the standard 2% aceto-orcein squashing method.

For culture, the stipules were cut from the trunks of *Angiopteris* and *Marattia* and whole plants were collected of *Lygodium*. These living materials were brought back to Japan and cultivated in pots in the greenhouse of the Botanical Garden, Faculty of Science, Kumamoto University.

For observation of the somatic chromosomes, the roots of cultivated plants were pretreated in 0.05% colchicine for 4 hrs (*Angiopteris* and *Marattia*) or 0.002M 8-hydroxyquinoline for 6 hr (*Lygodium*) at 20°C. They were then fixed in 45% acetic acid for 15–20 min at 4°C, macerated in 1N HCl for 20 sec at 60°C, and squashed in 2% aceto-orcein. Typification of interphase and prophase chromosomes follows Tanaka (1989); description of the metaphase karyotype follows Takamiya (1989).

**Results and Discussion**

1. *Psilotum complanatum* Sw. (Psilotaceae)
   
   At diakinesis and metaphase of the first meiotic division in two plants, 52 bivalent chromosomes were observed in both Fijian and Vanuatuan plants (Table 1, Figs. 1, 8). Subsequent meiotic stages were normal and the plants yielded many good spores. The gametic chromosome number *n* = 52 is the lowest in *Psilotum* (Brownsey and Lovis, 1987), thus the plants examined are diploids.

   The chromosome number of *P. complanatum* was previously established in a plant growing at Kew as *n* = 52–54 (Manton, 1950), and in plants collected in Papua New Guinea and Sarawak as *n* = 52 (Walker, 1984). The present count from two Melanesian plants confirms their reports. On the other hand, the occurrence of a tetraploid cytotype in Malaysia was mentioned by Bidin and Jaman (1983). The genus *Psilotum* includes only two species, *P. complanatum* and *P. nudum* (L.) P. Beauv. In the latter species, the tetraploid cytotype is more commonly reported than the diploid (Brownsey and Lovis, 1987), but in contrast, the tetraploid is reported rarely in *P. complanatum*.

2. *Ophioglossum petiolatum* Hook. (Ophioglossaceae)

   A meiotic chromosome number of *n* = ca. 240 was determined at diakinesis of the first meiotic division in a plant of *Ophioglossum petiolatum* (Table 1, Figs. 2, 9), and subsequent meiotic stages were normal and the plant yielded many good spores. Therefore, this Fijian plant is a tetraploid with *x* = 120. Previously, many polyploid series, from diploids with *n* = 120 to nonaploids with 2*n* = ca. 1100, were detected in *O. petiolatum* col-
TABLE 1. Taxa used in this study and their collection data and chromosome numbers.

<table>
<thead>
<tr>
<th>Species</th>
<th>Collection</th>
<th>Chromosome number</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Psilotaceae</em></td>
<td></td>
<td></td>
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<tr>
<td><em>Psilotum complanatum</em></td>
<td>FIJI. Viti Levu Isl., Namosi (west of Suva), 18°05'S, 178°10'E, 30m alt., Takamiya 9201.</td>
<td>n = 52</td>
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<td></td>
<td>VANUATU. Espiritu Santo Isl., Ipayato—Mt. Pic Santo, 15°32'S, 166°48'E, 800m alt., Takamiya 9748.</td>
<td>n = 52</td>
</tr>
<tr>
<td><em>Ophioglossaceae</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Ophioglossum petiolatum</em></td>
<td>FIJI. Taveuni Isl., De Voux Peak, 16°52'S, 179°55'W, 1150m alt., Takamiya 9284.</td>
<td>n = ca. 240</td>
</tr>
<tr>
<td><em>Marattiaceae</em></td>
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<td></td>
</tr>
<tr>
<td><em>Angiopteris evecta</em></td>
<td>FIJI. Taveuni Isl., Somosomo—De Voux Peak, 16°50'S, 179°55'W, 560m alt., Takamiya 9436.</td>
<td>2n = 160</td>
</tr>
<tr>
<td>(Forst.) Hoffm.</td>
<td>FIJI. Taveuni Isl., Somosomo—De Voux Peak, 16°50'S, 179°55'W, 100m alt., Takamiya 9410.</td>
<td>2n = 78</td>
</tr>
<tr>
<td><em>Marattia smithii</em></td>
<td>FIJI. Viti Levu Isl., Mt. Lomalagi, near Navai, 17°35'S, 178°00'E, 930m alt., Takamiya 9584.</td>
<td>2n = 78</td>
</tr>
<tr>
<td>Mett. ex Kuhn</td>
<td>VANUATU. Efate Isl., Mt. Macdonald, 17°35'S, 168°18'E, 400m alt., Takamiya 9645.</td>
<td>2n = 78</td>
</tr>
<tr>
<td><em>Angiopteris evecta</em></td>
<td>VANUATU. Espiritu Santo Isl., Ipayato—Mt. Pic Santo, 15°32'S, 166°48'E, 700m alt., Takamiya 9733; 600m alt., Takamiya 9744.</td>
<td>2n = 78</td>
</tr>
<tr>
<td><em>Schizaeaceae</em></td>
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<td></td>
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<tr>
<td><em>Lygodium reticulatum</em></td>
<td>FIJI. Viti Levu Isl., Mt. Nakorombamba, (west of Suva), 18°08'S, 178°23'E, 200m alt., Takamiya 9233.</td>
<td>2n = 60</td>
</tr>
<tr>
<td>Schkuhr</td>
<td>FIJI. Vanua Levu Isl., near Waisali, 16°40'S, 179°10'E 560m alt., Takamiya 9548.</td>
<td>2n = 60</td>
</tr>
<tr>
<td></td>
<td>VANUATU. Efate Isl., Mt. Macdonald, 17°35'S, 168°18'E, 360m alt., Takamiya 9645.</td>
<td>2n = 60</td>
</tr>
</tbody>
</table>

lected from India, Sri Lanka, China and Japan (see Khandelwal, 1990). However, there is no chromosomal report on *O. petiolatum* in the Southern Hemisphere, and the present tetraploid count of *n = 240* is the first for this species.

3. *Angiopteris evecta* (Forst.) Hoffm. (Marattiaceae)

A somatic chromosome number of *2n = 160* was determined in only one plant from Fiji, a tetraploid with *x = 40* (Table 1, Fig. 3). This species extends from India to Polynesia, thus there are many reports of chromosome numbers; diploids with *n = 40* or *2n = 80* reported from India and Malaysia (Mehra and Singh, 1955; Walker, 1981; Vasudeva and Bir, 1983; Singh and Roy, 1988), and tetraploids with *n = 80* or *2n = 160* from India and Sri Lanka (Manton and Sledge, 1954; Ninan, 1956; Irudayaraj and Manickam, 1987; Manickam and Irudayaraj, 1988). The tetraploid form of *A. evecta* is more common than the diploid form in south India, while the reverse is true in the north (Manickam and Irudayaraj, 1988). Further cytological investigations are needed to determine the cytogeographical
Figs. 1–7. Meiotic chromosomes at metaphase I (Figs. 1, 2), and somatic chromosomes at metaphase (Figs. 3, 4, 7), interphase (Fig. 5) and prophase (Fig. 6) of four ferns and a fern-ally from Fiji and Vanuatu. 1. *Psilotum complanatum* (*n* = 52, *Takamiya 9201*). 2. *Ophioglossum petiolatum* (*n* = ca. 240, *Takamiya 9284*). 3. *Angiopteris evecta* (*2n* = 160, *Takamiya 9436*). 4. *Marattia smithii* (*2n* = 78, *Takamiya 9733). 5–7. *Lygodium reticulatum* (*2n* = 60, *Takamiya 9233). Scale = 10 μm.
features of this species throughout its range.

4. *Marattia smithii* Mett. ex Kuhn (Marattiaceae)

   The chromosome number of this species is reported here for the first time. All five plants examined, two from Fiji and three from Vanuatu, displayed $2n = 78$ at the somatic metaphase (Table 1, Fig. 4), thus *M. smithii* is a diploid with $x = 39$.

   Two basic chromosome numbers were reported in the genus *Marattia*: *M. salicina* Smith collected from New Zealand has $n = 39$ with $x = 39$ (Brownlie, 1961); *M. alata* Swartz from Jamaica and *M. weinmannii* (Liebm. from Mexico are $n = 40$ and $2n = 80$, respectively (Walker, 1966; Smith and Mickel, 1977). In addition, *M. fraxinea* Smith displays two basic chromosome numbers, $n = 40$ (with $x = 40$) was established in a plant collected from South Africa (Walker in Manton, 1959) and $n = 78$ and $2n = 156$ (with $x = 39$) in one plant from India (Ninan, 1956; Irudayaraj and Manickam, 1987; Manickam and Irudayaraj, 1988). The basic chromosome number of $x = 40$ is common to every genus examined in Marattiaceae (see Walker, 1981, 1985). Records of $x = 39$ in *Marattia* are therefore considered to be secondarily derived from $x = 40$ (Walker, 1966). Although Camus (1990) recommended further investigation of the $x = 39$ count, the present finding of $2n = 78$ in *M. smithii* confirms the existence of $x = 39$ in the genus *Marattia*. Basic chromosome numbers in *Marattia* appear to be differentiated cytogeographically between $x = 39$ and $x = 40$; plants with $x = 39$ are distributed in India, Vanuatu, Fiji and New Zealand, while $x = 40$ are in Mexico, Jamaica and South Africa.

5. *Lygodium reticulatum* Schkuhr (Schizaeaceae)

   No cytological information was previously available for this species. The somatic chromosome number of $2n = 60$ was determined in three individuals (Table 1, Figs. 6–7, 10, 11). Three aneuploidic basic chromosome numbers, $x = 28$, 29 and 30, have been reported in *Lygodium* (Roy and Manton, 1965). *Lygodium reticulatum* is a diploid with $x = 30$. Of the three plants examined, one from Fiji (*Takamiya 9233*) and one from Vanuatu (*Takamiya 9645*) were analyzed for their detailed karyomorphology. There was no marked difference in morphology between them at interphase, prophase and metaphase:

   The chromosomes at interphase had numerous small chromomeric granules and lightly stained fibrous threads scattered uniformly throughout the nucleus without any distinctive condensed bodies (Fig. 5). At prophase, all chromosomes concentrated homogeneously along their axis and early and late concentrated segments were not distinguishable from each other (Fig. 6). The morphology of the chromosomes at interphase is categorized as diffuse type, and at prophase as continuous type.

   At metaphase, the chromosomes decrease gradually in length from the longest subterminal centromeric chromosome of $4.5 \mu m$ to the shortest submedian of $2.1 \mu m$ (Figs. 10, 11). The variation in chromosome length is thus assigned to the gradual karyotype. The mean chromosome length was $3.16 \mu m$. Four chromosomes (Nos. 49, 50, 53, 54) were median cen-
Figs. 8 and 9. Explanatory drawings of Figs. 1 and 2, respectively. Scale = 10μm.

Figs. 10 and 11. Somatic metaphase chromosomes of *Lygodium reticulatum* (2n = 60, Takamiya 9233). 10. Explanatory drawing of Fig. 7. 11. Serial arrangement of metaphase chromosomes in Fig. 10. Scale = 5μm.
tromeric chromosomes (m), 12 (Nos. 5, 6, 17, 18, 25, 26, 39, 40, 55, 56, 59, 60) submedian (sm), and the remaining 44 are subterminal or terminal (st + t). The karyotype is formulated as 2n = 60 = 4m + 12sm + 44(st + t). A subterminal (No. 13) and a submedian (No. 18) chromosomes had satellites.

In *Lygodium*, the somatic metaphase karyotypes were reported only for two species, *L. micans* Sturm with 2n = 58 and *L. venustum* Swartz with 2n = 116 (Walker, 1985). Judging from the karyograms illustrated in Walker (1985), the karyotype formula of *L. micans* is 2n = 58 = 0m + 6sm + 52(st + t); *L. venustum* is 2n = 116 = 6m + 6sm + 104(st + t). Both species have a basic chromosome number of x = 29. Comparing his report with the present observations of x = 30, the chromosome complement of x = 30 includes many more median and submedian chromosomes than in the x = 29 plants. To determine the karyotypic relationships among aneuploidal basic chromosome numbers of x = 28, 29 and 30 in *Lygodium*, further research is needed.

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References


& Tomorrow's Printers & Publishers, New Delhi.

摘 要

高宮正之：南太平洋フィジー及びパナマ共和国産シダ植物の染色体研究 I. マツバラン科，ハナヤスリ科，リュウピンタイ科，フサシダ科

1991年から1992年にかけて、文部省国際学術調査「フィジー諸国とその周辺地域における植物の分布と分化」、代表者岩井善之助（当時広島大学理学部教授、現在植物研究所岡崎分室）の一員として参加。フィジー及びパナマ共和国産のシダ植物を、現地で固定あるいは生植物を持ち帰り、染色体研究を行った。本地域のシダ植物の染色体の報告は、コケシノ科とイヌシカクサとなる属を除いてこれまでなかった。本論文は、研究論文の第1報として5種のシダ植物について報告した。マツバラン科のPsilotum complanatumは、減数分裂中期で52個の二価染色体を形成し四倍体だった。ハナヤスリ科のOphioglossum petiolatum（コヒロハナヤスリ）は、同じく約240個の二価染色体を形成し四倍体だった。本種の四倍体は初めての報告である。リュウピンタイ科のAngiopteris evectaは、体細胞中
期で $2n = 160$ で四倍体だった。リュウビンタイ科の Marattia smithii は $2n = 78$ の二倍体、Lygodium reticulatum は $2n = 60$ の二倍体であり、この2種の染色体数は初報告である。L. reticulatum については、体細胞分裂各期の核形状も記載した。
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