Karyomorphological Studies on *Woodwardia* sensu lato of Japan

**Masayuki Takamiya, Kaori Osato**
**and Kanji Ono**

Department of Biological Science, Faculty of Science, Kumamoto University, Kurokami 2-39-1, Kumamoto, 860 Japan

Karyomorphological comparisons were made of five species of Japanese *Woodwardia*. There were no marked differences at interphase and prophase among the five species. *Woodwardia japonica*, *W. prolifera*, and *W. unigemmata* were diploid with 2n = 68 and the formulas of their metaphase karyotypes uniformly 4m(median centromeric chromosomes) + 12sm(submedian) + 52(st+t)(subterminal and terminal). *Woodwardia orientalis* was tetraploid with 2n = 136 and 8m + 24sm + 104(st+t), and the ratio of each chromosomal type to total complement was identical to that of three diploid species. These four species had several characteristics in common: $x = 34$, the longest chromosome of sm, and a mean chromosome length over 3.0 $\mu$m. Although *Woodwardia orientalis* showed some similarity to *W. prolifera*, it seems to be an allotetraploid which originated by chromosome doubling of a hybrid of *W. prolifera* and a diploid species as yet karyomorphologically unknown. *Woodwardia kempii* was tetraploid with 2n = 124 and 8m + 24sm + 92(st+t), and differed from the others in having $x = 31$, the longest chromosomes of t, and a mean chromosome length under 3.0 $\mu$m. This species has been classified as an independent genus, *Chieniopteris*, and our karyomorphological study supports this treatment.

**Key words:** Chieniopteris — Chromosome — Cytotaxonomy — Karyomorphology — Polyploidy — Woodwardia

*Woodwardia sensu lato* is a genus of Blechnaceae which comprises 12 or 20 species occurring mostly in the temperate and subtropical regions of the northern hemisphere (Copeland, 1947; Chiu, 1974). This genus has sometimes been classified as four separate genera: *Anchistea*, *Chieniopteris*, *Lorinseria*, and *Woodwardia sensu strict* (Ching, 1964; Pichi Sermolli, 1977). In Japan, six species of *Woodwardia s.l.*, *W. harlandii* Hook., *W. japonica* (L.f.) Sin., *W. kempii* Copel., *W. orientalis* Sw., *W. prolifera* Hook. et Arn. (= *W. orientalis* var. *formosana* Ros.), and *W. unigemmata* (Makino) Nakai, are known (Nakaike, 1982). Two of these, *W. harlandii* and *W. kempii*, belong to *Chieniopteris*, and the other four to *Woodwardia s.s.* (Ching, 1964).

Previously chromosome numbers have been determined for nine species of *Woodwardia s.l.* (Löve et al., 1977; Wang et al., 1984) and two distinct cytological features have been recognized. First, there are two basic chromosome numbers in this genus: *W. areolata* (L.) Moore and *W. virginica* (L.) T. Sm. have $x = 35$ while the other species $x = 34$. From a taxonomical viewpoint, species with $x = 34$ are considered as
belonging to *Woodwardia* s.s., but *W. areolata* is removed to the monotypic genus *Lorinseria*, and *W. virginica* to another monotypic genus, *Anchistea*. This cytological evidence supports the idea that *Woodwardia* s.l. consists of several natural groups. No data have yet been published on the cytological features of *Chieniopteris*.

Second, although polyploidy is one of the outstanding features of homosporous pteridophytes (Walker, 1984), this is a rare event in *Woodwardia*. *W. orientalis* has been recognized to be tetraploid with \( n = 68 \), and the remaining eight species diploid. Recently, Weng and Qiu (1988) discovered a new chromosome number, \( n = 17 \), in Chinese *W. japonica*. This discovery is noteworthy because (1) \( n = 17 \) is the lowest chromosome number observed to date in Blechnaceae, in which \( n = 28 \) had been regarded as the minimum chromosome number, so that large chromosome numbers of this family probably originated from \( n = 17 \) by polyploidy and aneuploidy; and (2) the ploidy level of all species of *Woodwardia* s.l. ought to be reconsidered, since plants with \( n = 34 \) or 35, which had been regarded as diploid, should be thought of as tetraploid.

Karyomorphological studies have provided much information on the relationships of plant species. However, there have been only a few reports on the karyomorphology of pteridophytes. In Blechnaceae, Walker (1985) detected the karyotypes of six taxa of *Blechnum* and *Salpichlaena volubilis* (Kaulf.) J. Sm. from Jamaica and Trinidad. No reports have focused on the karyotypes of *Woodwardia* s.l., as far as we know.

The objective of the present work is (1) to re-examine the chromosome numbers of *Woodwardia* s.l. in many plants collected from various localities and hopefully to detect the cytotype of \( 2n = 34 \); (2) to determine the chromosome number of *W. kempii* which belongs to *Chieniopteris*; and (3) to investigate the karyomorphological features of *Woodwardia* s.l. and to clarify the cytological relationships of species including the polyploidal nature of *W. orientalis*.

**Materials and Methods**

Karyomorphological studies were conducted on five Japanese species. Plants were collected mainly from Kumamoto Prefecture and cultivated at Kumamoto University. The locations of the collection sites are listed in Table 1. Voucher specimens are deposited in the herbarium of Kumamoto University (KUMA).

For the observations of somatic chromosomes, growing root tips were pretreated in 0.002 M 8-hydroxyquinoline for 5 hr at about 20°C. They were then fixed in 45% acetic acid for 20 min at 5°C and macerated in 1N HCl for 30 sec at 60°C, and stained with 2% aceto-orcein and squashed.