A Karyomorphological Study of Five Species and One Variety of *Cycas**

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Cycas, the Cycadaceae, is distributed in the tropical to subtropical regions in Asia, Africa and Australia and consists of eight species (Schuster 1932). Since *Cycas* is considered the most primitive genus among the cycads (Stevenson 1990), it could be a key genus for the study of cycad phylogeny to justify and clarify the genetic and species relationships among the cycad members.

Chromosomal, phylogenetic and evolutionary relationships of general cycad species other than *Cycas* species have been previously well-studied by Marchant (1968) and Moretti (1990). Chromosome numbers of three species of *Cycas* have been reported by Sax and Beal (1934) and Selvaraj (1980). Chromosomal phylogeny of *Cycas*, however, are poorly characterized in most standard references, especially with respect to karyomorphological details.

Materials and methods

The materials studied are shown in Table 1. Taxonomical treatment of *Cycas* followed Schuster (1932). *Cycas circinalis* L., *C. rumphii* Miq., *C. revoluta* Thunberg var. *revoluta* and *C. revoluta* var. *taiwaniana* (Carruth.) Schuster were cultivated in the greenhouse of Laboratory of Plant Chromosome and Gene Stock, Faculty of Science, Hiroshima University. *Cycas media* R. Brown var. *basaltica* (Gardner) Schuster was cultivated in the greenhouse of Satake Corporation and *C. siamensis* Miq. was cultivated in the greenhouse of Hiroshima Botanical Garden.

Young leaflets of the five species and one variety of *Cycas* were harvested and chopped into ca. 2 mm diameter, and were pretreated in 2 mM 8-hydroxyquinoline at 4°C for 8 hr. Then, they were fixed in acetic ethanol (1:3) at 4°C for 24 hr and were stored in 70% ethanol at –20°C. After they were soaked in distilled water and then 45% acetic acid for 10 min, they were macerated in 45% acetic acid at 60°C for 5 min. Some small-pieces of leaflets of *C. revoluta* var. *revoluta* were macerated in enzymatic mixture of 2% cellulase Onozuka RS (Yakult) and 1% pectolyase Y-23 (Seishin) in distilled water (w/v) at 36°C for one hour. Effects of the maceration in 45% acetic acid and the enzymatic solution on chromosome length and arm ratio were examined by *t*-test ($p < 0.05$). Since all of the chromosomes showed no significant differences in mean arm ratio and chromosome length, 45% acetic acid and the enzymatic solution were used alternatively for maceration of *Cycas* leaflets. The macerated pieces of leaflets were placed on glass-slides, and were stained in 1% aceto-orcein at room temperature for 4 hr. The preparations were made with 1% aceto-orcein by the squash method.

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Table 1. Comparison of chromosomes in five species and one variety of *Cycas*

Species	Interphase chromosome	Mitotic prophase chromosome	Karyotype			
			m	sm	st	t
Sect. Lemuricae						
Subsect. Pandemicae						
<i>C. circinalis</i>	Complex chromocenter type	Interstitial type	6	2	2	12
<i>C. rumphii</i>	Complex chromocenter type	Interstitial type	4	3	3	12
Subsect. Endemicae						
<i>C. media</i> var. <i>basaltica</i>	Complex chromocenter type	Interstitial type	5	5	0	12
Sect. Indo-sinenses						
<i>C. siamensis</i>	Complex chromocenter type	Interstitial type	4	6	0	12
Sect. Asiorientales						
<i>C. revoluta</i> var. <i>revoluta</i>	Complex chromocenter type	Interstitial type	4	6	0	12
<i>C. revoluta</i> var. <i>taiwaniana</i>	Complex chromocenter type	Interstitial type	2	8	0	12

m = median-centromeric chromosome. sm = submedian-centromeric chromosome. st = subterminal-centromeric chromosome. t = terminal-centromeric chromosome.

Chromosomes at early interphase isolated by mean of two nuclei placed side by side and the contour of those cells supposed immediacy after a somatic division, mitotic prophase defined by chromosomes remaining oblong-shape were observed. Chromosome numbers counted and lengths of long and short arms were measured at mitotic metaphase defined by chromosomes separated from each other.

Morphological classification of chromosomes at interphase and mitotic prophase followed Tanaka (1977). The chromosomes of each complement at mitotic metaphase were aligned in order of chromosome length from the longest to the shortest chromosomes. At least three chromosome complements at mitotic metaphase were measured to obtain mean arm ratio calculated by long arm length/short arm length. Chromosomes were classified according to centromeric positions defined with mean arm ratios (Levan *et al.* 1964). Additionally, average of total length of the chromosomes of the complement in each of the five species and one variety of *Cycas* was calculated.

Results and discussion

Five species and one variety of *Cycas* showed commonly the complex chromocenter type of the chromosomes at early interphase. Partial to whole region of the nucleus of the six taxa at early interphase was darkly stained (Fig. 1A–F, Table 1). They also showed commonly the interstitial type of the chromosomes at mitotic prophase, which indicated darkly-stained heterochromatins to tend in some portions to form clusters (Fig. 2A–F, Table 1). Those heterochromatins could correspond to the chromocenters at interphase since their distribution patterns were similar to each other in every taxon. Thus, the taxa of *Cycas* studied displayed morphologically quite similar chromosomes at early interphase and mitotic prophase.

Five species and one variety of *Cycas* showed the chromosome number of $2n=22$ (Fig. 3). The chromosome numbers of *C. circinalis* and *C. rumphii* documented here verified those reported by Sax and Beal (1934), and those of *C. revoluta* var. *taiwaniana* verified those reported by Peng *et al.* (1986). In contrast, the present chromosome count of $2n=22$ in *C. revoluta* var. *revoluta* (Fig. 3E) verified that reported by Sax and Beal (1934), but differed from the chromosome count of $2n=24$ reported by Ishikawa (1916) and from the chromosome counts of $2n=20$, 22 and 24 reported by Selvaraj (1980).

The karyotypes of five species and one variety of *Cycas* based on the data of mean arm ratios were tabulated in Table 2. They exhibited commonly 12 terminal-centromeric chromo-

Fig. 1. The chromocenter type of the chromosomes at early interphase in five species and one variety of *Cycas*. A. *C. circinalis*. B. *C. rumphii*. C. *C. media* var. *basaltica*. D. *C. siamensis*. E. *C. revoluta* var. *revoluta*. F. *C. revoluta* var. *taiwaniana*. Bar = 10 μ m.

osomes positioned according to chromosome length from the 5th (the longest terminal-centromeric) to the 16th (the shortest terminal-centromeric) chromosomes (Table 2). However, respective homologous chromosomes were not easily identified and paired (Table 2). The arm ratios of respective terminal-centromeric chromosome in 14 mitotic-metaphase complements of *C. revoluta* var. *revoluta* studied were unstable. The arm ratios of the 12 terminal-

Fig. 2. The interstitial type of the chromosomes at mitotic prophase in five species and one variety of *Cycas*. A. *C. circinalis*. B. *C. rumphii*. C. *C. media* var. *basaltica*. D. *C. siamensis*. E. *C. revoluta* var. *revoluta*. F. *C. revoluta* var. *taiwaniana*. Bar = 10 μ m.

centromeric chromosomes in *C. siamensis* were smaller than those of the other taxa (Table 2). These quantitative differences in arm ratio of terminal-centromeric chromosome could not be due to species specificity but be due to condensation of the long and the short arms of chromosome.

The four longest chromosomes in the complements at mitotic metaphase in the taxa were

Fig. 3. Karyotypes with $2n=22$ at mitotic metaphase in five species and one variety of *Cycas*.

A. *C. circinalis*. B. *C. rumphii*. C. *C. media* var. *basaltica*. D. *C. siamensis*. E. *C. revoluta* var. *revoluta*. F. *C. revoluta* var. *taiwaniana*. Bar = 10 μ m.

submedian-centromeric, excepting those in *C. circinalis* and *C. rumphii* were subterminal-centromeric with arm ratio values very close to 3.0, the highest extreme of the arm ratio range of the submedian-centromeric chromosome according to Levan *et al.* (1964). Selvaraj (1980) said that the pair of the longest chromosomes of *C. circinalis* had secondary constriction. However, the longest chromosomes of either *C. circinalis* or the other taxa studied here showed no secondary constriction. The seventeenth, the 18th, the 21st and the 22nd chromosomes in the complements at mitotic metaphase in all of the taxa studied were either median-centromeric or submedian-centromeric. Then, those median-centromeric chromosomes classified had always arm ratio value very close to 1.8, the lowest extreme of arm ratio range of the submedian-centromeric chromosome according to Levan *et al.* (1964).

The five species and one variety of *Cycas* studied showed the stable chromosome number

Table 2. Mean arm ratios of chromosomes in the complement at mitotic metaphase in five species and one variety of *Cycas*

Chromosome in order	CIR	RUM	BAS	SIA	REV	TAI
1st	3.2 (st)	3.4 (st)	3.0 (sm)	2.4 (sm)	2.9 (sm)	2.9 (sm)
2nd	3.0 (sm)	3.2 (st)	3.0 (sm)	2.5 (sm)	2.8 (sm)	3.0 (sm)
3rd	2.8 (sm)	3.2 (st)	2.7 (sm)	2.9 (sm)	2.9 (sm)	2.9 (sm)
4th	3.2 (st)	2.9 (sm)	2.9 (sm)	2.8 (sm)	2.9 (sm)	2.6 (sm)
5th	14.0 (t)	12.3 (t)	13.7 (t)	8.5 (t)	14.1 (t)	14.4 (t)
6th	13.5 (t)	12.2 (t)	12.0 (t)	9.6 (t)	13.6 (t)	14.0 (t)
7th	14.4 (t)	11.5 (t)	11.8 (t)	10.5 (t)	13.2 (t)	13.2 (t)
8th	12.6 (t)	12.1 (t)	10.9 (t)	8.2 (t)	14.0 (t)	13.3 (t)
9th	12.4 (t)	11.1 (t)	12.5 (t)	9.5 (t)	13.4 (t)	13.3 (t)
10th	12.3 (t)	10.6 (t)	11.4 (t)	9.0 (t)	12.6 (t)	12.4 (t)
11th	12.6 (t)	11.0 (t)	11.0 (t)	10.1 (t)	12.5 (t)	13.6 (t)
12th	11.5 (t)	11.0 (t)	10.9 (t)	8.3 (t)	12.7 (t)	13.6 (t)
13th	13.0 (t)	10.2 (t)	11.0 (t)	10.8 (t)	12.3 (t)	13.9 (t)
14th	10.8 (t)	10.7 (t)	10.6 (t)	8.5 (t)	12.2 (t)	12.7 (t)
15th	12.4 (t)	10.0 (t)	10.1 (t)	8.3 (t)	11.5 (t)	12.0 (t)
16th	13.3 (t)	8.6 (t)	10.5 (t)	7.2 (t)	10.7 (t)	10.1 (t)
17th	1.7 (m)	1.5 (m)	1.8 (sm)	1.6 (m)	1.8 (sm)	2.0 (sm)
18th	1.7 (m)	1.5 (m)	1.7 (m)	1.7 (m)	1.8 (sm)	2.0 (sm)
19th	1.0 (m)	1.1 (m)	1.2 (m)	1.5 (m)	1.2 (m)	1.1 (m)
20th	1.1 (m)	1.1 (m)	1.2 (m)	1.2 (m)	1.2 (m)	1.1 (m)
21st	1.5 (m)	2.0 (sm)	1.7 (m)	1.9 (sm)	1.6 (m)	1.8 (sm)
22nd	1.7 (m)	1.8 (sm)	1.6 (m)	2.1 (sm)	1.6 (m)	1.8 (sm)
Total chromosome length of complement	164.6	170.3	172.0	139.1	171.5	161.6

CIR=*C. circinalis*. RUM=*C. rumphii*. BAS=*C. media* var. *basaltica*. SIA=*C. siamensis*. REV=*C. revoluta* var. *revoluta*. TAI=*C. revoluta* var. *taiwaniana*. (m)=median-centromeric chromosome. (sm)=submedian-centromeric chromosome. (st)=subterminal-centromeric chromosome. (t)=terminal-centromeric chromosome. Although respective chromosome in order among six taxa had sometimes different chromosome type classified by the ranges of arm ratios proposed by Levan *et al.* (1964) from each other, their arm ratio values were approximation with each other.

of $2n=22$ and karyotype, that were quite similar to those of the other cycad-genera except for *Zamia* which had karyotype variability possibly caused by centromeric fusion and fission (Norstog 1980, Moretti and Sabato 1984, Moretti 1990). Thus, the present result supported Stevenson's hypothesis (1990) that *Cycas* was a monophyletic group by vegetative and reproductive morphological approaches. Although Schuster (1932) classified *Cycas* into three sections (Table 1), the present study showed no indication to follow those sections.

Summary

The chromosomes of five species and one variety of *Cycas* studied showed commonly the morphological characteristics of the complex chromocenter type at early interphase and the interstitial type at mitotic prophase. All of the taxa had the common chromosome number of $2n=22$ and very similar karyotypes to each other; *C. media* var. *basaltica* and *C. siamensis* showed the chromosome number of $2n=22$ for the first time.

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