A COMPARISON OF CHROMOSOME NUMBER AND KARYOTYPE IN SOMATIC CHROMOSOMES OF *STANGERIACEAE* (*CYCADALES*)

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Somatic chromosomes at mitotic metaphase of two species and two undescribed populations of *Bowenia*, and *Stangeria eriopus*, which were classified in *Stangeriaceae*, *Cycadales*, were compared using the standard aceto-orcein staining method. All *Bowenia* taxa showed a chromosome number of 2n = 18, while *S. eriopus* showed a chromosome number of 2n = 16. The chromosome number of 2n = 18 in *B.* 'Kuranda' is reported for the first time. The present karyotype analysis indicates that *B.* 'Kuranda' and another undescribed taxon, *B.* 'Tinaroo', are cytotaxonomically closer to *B. spectabilis* than *B. serrulata*, and that the karyotype of *Stangeria* is unlikely to have been derived from that of *Bowenia* by a simple chromosomal change such as centromeric fission and deletion.

Keywords. Chromosome, *Cycadales*, cytotaxonomy, *Stangeriaceae*.

Introduction

The family *Stangeriaceae* (*Cycadales*; Stevenson, 1992) consists of two genera: *Bowenia* Hook. ex J.D. Hook. and *Stangeria* (Kunze) Baillon. The genus *Bowenia* is endemic to Queensland, Australia, and includes two species: *B. serrulata* (W. Bull) Chamberlain and *B. spectabilis* Hook. ex J.D. Hook. The two species are distinguished by their leaflet margin and rootstock morphology (Jones, 1993). *Bowenia* 'Kuranda' and *B.* 'Tinaroo' are two other entities in this genus that have not yet been formally named and described (Wilson, unpublished). *Bowenia* 'Kuranda' and *B.* 'Tinaroo' are morphologically intermediate between *B. serrulata* and *B. spectabilis* (Jones, 1993; Wilson, unpublished), but are unlikely to be simple hybrids because the nearest populations of *B. serrulata* are over 700km away. The genus *Stangeria* is endemic to the east coast of South Africa and consists of only one species: *Stangeria eriopus* (Kunze) Baillon.

Although some cytotaxonomical studies of the family have been reported previously (Sax & Beal, 1934; Marchant, 1968; Moretti, 1990; Kokubugata *et al.*, 2000),

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differences in karyotypes seemed to vary with different cytotaxonomists, and there have been no cytotaxonomical investigations of *B.* 'Kuranda'. The present study thus aims to investigate their chromosome numbers and karyotypes using the aceto-orcein squash method, and to compare the karyotypes of all members of the family *Stangeriaceae*.

Materials and Methods

The taxonomic treatment follows Stevenson (1992). Plants of *Bowenia* investigated in the present study were collected in Queensland, Australia, and cultivated in the greenhouse of the Tsukuba Botanical Garden, National Science Museum, Tokyo (Table 1). A plant of *Stangeria eriopus* cultivated at the Royal Botanic Gardens, Sydney, was used for the present study. Voucher specimens were deposited in the National Herbarium of New South Wales, Royal Botanic Gardens, Sydney (NSW) and the National Science Museum, Tokyo (TNS).

Pretreatment, fixation, and storage of materials followed Kokubugata & Kondo (1998). Young leaflets were harvested and pretreated in 4mM 8-hydroxyquinoline at 4°C for 8h, then fixed in acetic ethanol (1:3) at 4°C for 24h, and transferred to and stored in 70% ethanol at −20°C. The stored leaflets were macerated in 45% acetic acid at 60°C for 3min before staining in 2% aceto-orcein at room temperature for 4h. They were then squashed in 45% acetic acid. Orcein-stained chromosomes were classified according to the position of centromeres defined by arm ratio (long arm length/short arm length; Levan *et al.*, 1964). Three mitotic metaphase plates per taxon were measured to obtain mean chromosome length and arm ratio. Chromosomes were then aligned with reference to the arm ratio in each complement for calculating mean chromosome length and arm ratio, and finally, aligned with reference to the chromosome length from the longest to the shortest for the idiogram presented here.

TABLE 1. *Stangeriaceae* species used in this study

Species	Origin	Accession no.
Bowenia serrulata	Queensland, Australia: Byfield	TBG122889
B. spectabilis	Queensland, Australia: Bellenden Kerr	TBG122893
B. 'Kuranda'	Queensland, Australia: Kuranda	TBG122898
B. 'Tinaroo'	Queensland, Australia: Black Mountain, Timber Reserve	TBG122895
Stangeria eriopus	Seed from Durban Botanic Gardens	RBGS903031

TBG, Tsukuba Botanical Garden, National Science Museum, Tokyo; RBGS, Royal Botanic Gardens, Sydney.

Results and Discussion

All *Bowenia* species investigated had a chromosome number of 2n = 18 (Fig. 1A–D), this being consistent with previous reports (Sax & Beal, 1934; Marchant, 1968; Moretti, 1990; Kokubugata *et al.*, 2000). On the other hand, *Stangeria eriopus* had a chromosome number of 2n = 16 (Fig. 1E), this also being consistent with previous reports (Sax & Beal, 1934; Marchant, 1968; Moretti, 1990).

Previous studies of *Cycadales* cytotaxonomy have been performed by Sax & Beal (1934), Marchant (1968), and Moretti (1990); unfortunately, however, they did not describe the chromosomal classification used in their studies. In the present study, we assume that 'chromosome with median centromere (m)' and 'chromosome with terminal centromere (t)' defined by Levan *et al.* (1964) correspond with 'median fiber' and 'terminal fiber' in Sax & Beal (1934), 'median' and 'terminal' in Marchant (1968), and 'M' and 'T' in Moretti (1990). We also assume that 'chromosome with submedian centromere (sm)' and 'chromosome with subterminal centromere (st)'

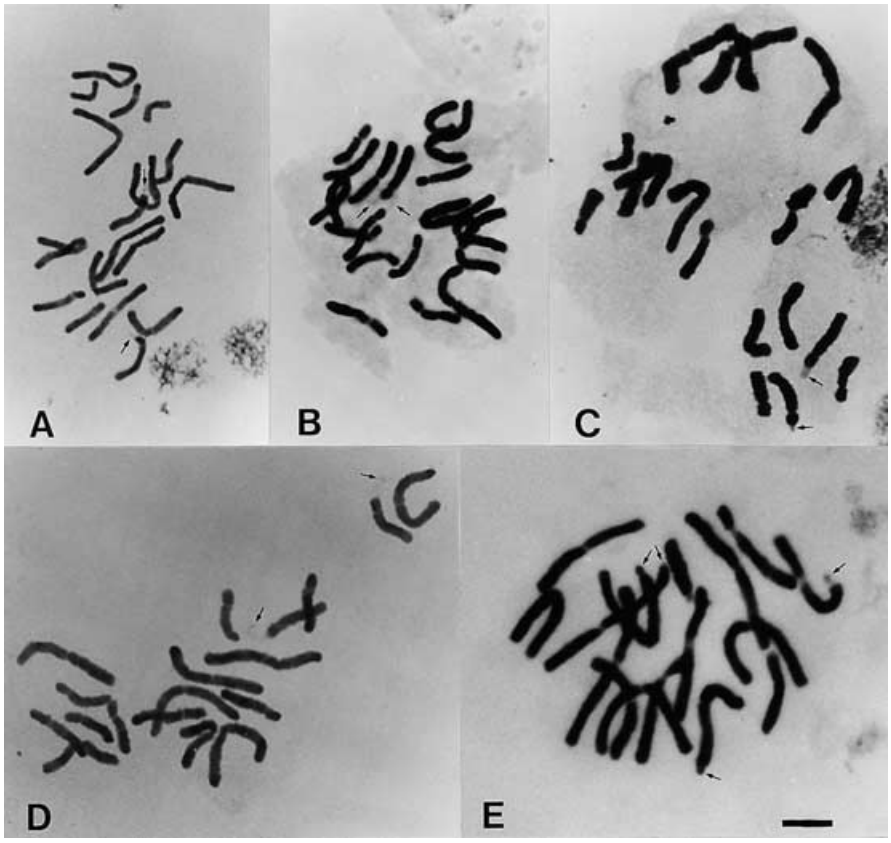

FIG. 1. Chromosomes of *Stangeriaceae* at mitotic metaphase stained with aceto-orcein: A, *Bowenia serrulata*; B, *B. spectabilis*; C, *B.* 'Kuranda'; D, *B.* 'Tinaroo'; E, *Stangeria eriopus.* Arrows indicate satellites. Scale bar = 10μm.

defined by Levan *et al.* (1964) correspond with 'sub-term. fiber' in Sax & Beal (1934), 'subterminal' in Marchant (1968), and 'S' or 'A' in Moretti (1990).

The karyotype of *B. serrulata* consists of 10m + 3sm + 5st (Fig. 2A, Table 2). This is very close to the karyotype reported by Kokubugata *et al.* (2000), but differs from that observed in the same species by Sax & Beal (1934), who reported 12m + 6st (or sm). The tenth chromosome was significantly shorter than the ninth one (Fig. 2A). There is a small possibility that the quantitative difference in chromosome length might be heteromorphic, and might correlate with the sex determination function of the chromosome. Unfortunately, the sex of the *B. serrulata* plant investigated could not be determined as it was a seedling. The karyotype of *B. spectabilis* consists of 8m + 6sm + 4st (Fig. 2B, Table 2). This is very close to the karyotype reported by Kokubugata *et al.* (2000), but differs from Moretti's (1990) report of 12m + 6st (or sm). The karyotype of *B.* 'Kuranda' consists of 8m + 6sm + 4st (Fig. 2C, Table 2).

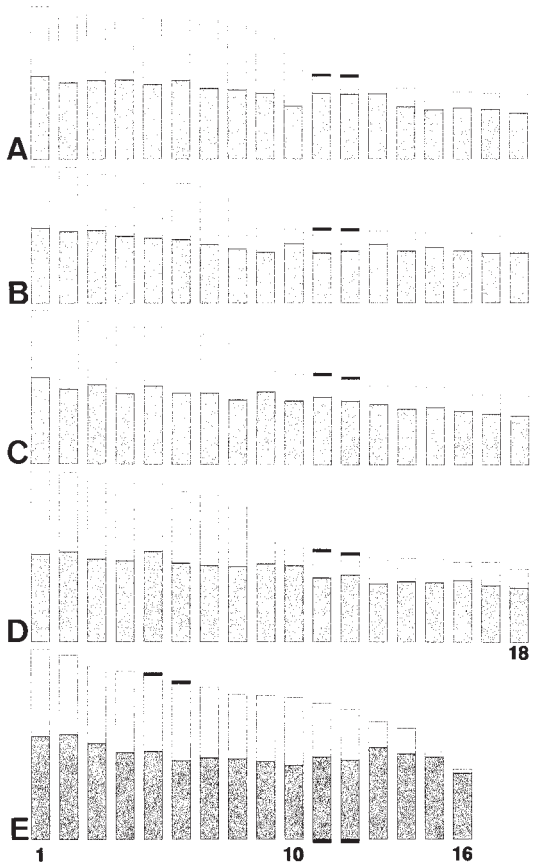

FIG. 2. Idiograms of *Stangeriaceae* based on means of three chromosome complements at mitotic metaphase: A, *Bowenia serrulata*; B, *B. spectabilis*; C, *B.* 'Kuranda'; D, *B.* 'Tinaroo'; E, *Stangeria eriopus.* Shaded areas: long arm; open areas: short arm; solid areas: satellites.

TABLE 2. Mean length and arm ratio of three chromosomes of five *Stangeriaceae* taxa

Chromosome no.	*Bowenia serrulata*		*B. spectabilis*		*B.* 'Kuranda'		*B.* 'Tinaroo'		*Stangeria eriopus*	
	L	R (F)	L	R (F)	L	R (F)	L	R (F)	L	R (F)
1st	26.6	1.2 (m)	23.7	1.2 (m)	27.3	1.3 (m)	29.3	1.0 (m)	30.3	1.2 (m)
2nd	25.8	1.1 (m)	23.6	1.1 (m)	25.6	1.1 (m)	29.1	1.1 (m)	29.4	1.3 (m)
3rd	25.2	1.2 (m)	22.4	1.3 (m)	25.5	1.2 (m)	27.7	1.1 (m)	27.6	1.2 (m)
4th	25.0	1.2 (m)	22.2	1.1 (m)	24.5	1.0 (m)	27.3	1.0 (m)	26.8	1.1 (m)
5th	24.6	1.1 (m)	20.9	1.2 (m)	24.5	1.3 (m)	27.2	1.3 (m)	26.1	1.2 (m)
6th	24.3	1.3 (m)	20.8	1.1 (m)	24.1	1.1 (m)	25.8	1.1 (m)	24.8	1.0 (m)
7th	24.2	1.0 (m)	20.3	1.0 (m)	23.2	1.2 (m)	25.2	1.1 (m)	24.3	1.2 (m)
8th	23.2	1.1 (m)	18.2	1.1 (m)	21.6	1.1 (m)	23.2	1.3 (m)	23.1	1.2 (m)
9th	22.4	1.1 (m)	13.0	2.1 (sm)	16.7	3.4 (st)	17.2	3.5 (st)	22.9	1.2 (m)
10th	18.5	1.0 (m)	12.9	4.0 (st)	16.1	2.4 (sm)	16.2	4.2 (st)	22.6	1.1 (m)
11th	14.4	4.0 (st)	12.6	2.2 (sm)	15.9	3.1 (st)	15.5	2.4 (m)	21.7	1.5 (m)
12th	14.3	3.8 (st)	12.5	2.6 (sm)	15.2	2.9 (sm)	14.9	3.4 (st)	20.7	1.6 (m)
13th	13.9	4.6 (st)	12.5	4.4 (st)	14.1	3.1 (st)	14.6	2.2 (sm)	18.7	3.6 (st)
14th	12.4	2.8 (sm)	12.2	2.8 (sm)	14.0	2.4 (sm)	14.4	2.6 (sm)	17.7	3.3 (st)
15th	11.8	2.7 (sm)	12.2	3.7 (st)	13.8	2.8 (sm)	14.3	2.5 (sm)	13.9	16.4 (t)
16th	11.7	3.2 (st)	12.1	2.9 (sm)	12.5	3.2 (st)	13.8	3.3 (st)	11.2	15.0 (t)
17th	11.5	3.1 (st)	11.6	2.9 (sm)	12.5	2.6 (sm)	13.8	2.4 (sm)	–	–
18th	11.3	2.4 (sm)	11.2	3.3 (st)	12.3	2.4 (sm)	12.6	2.8 (sm)	–	–

L, chromosome length (μm); R, arm ratio; F, chromosome form according to the position of centromeres defined by arm ratio of long arm length/short arm length (Levan *et al.*, 1964); m, chromosome with median centromere (1.0–1.7); sm, chromosome with submedian centromere (1.8–3.0); st, chromosome with subterminal centromere (3.1–7.0); t, chromosome with terminal centromere (7.1–∞).

The karyotype of *B.* 'Kuranda' is reported here for the first time. The karyotype of *B.* 'Tinaroo' consists of 8m + 6sm + 4st (Fig. 2D, Table 2). This is very close to the karyotype reported by Kokubugata *et al.* (2000).

Morphologically, *B. serrulata* has leaflets with toothed margins and a much-branched rootstock, while *B. spectabilis* has leaflets with entire margins and a sparingly branched rootstock (Jones, 1993). On the other hand, *B.* 'Kuranda' and *B.* 'Tinaroo' are morphologically intermediate in some respects between *B. serrulata* and *B. spectabilis*, i.e. they have toothed leaflets similar to those of *B. serrulata*, and sparingly branched rootstocks similar to those of *B. spectabilis* (Jones, 1993; Wilson, unpublished). A morphological difference between *B.* 'Kuranda' and *B.* 'Tinaroo' is that the leaflets of the former are larger and thinner than those of the latter (Wilson, unpublished). In the present investigation, although the chromosome number of all *Bowenia* material was found to be 2n = 18, the karyotype of *B.* 'Kuranda' and *B.* 'Tinaroo' was cytotaxonomically more similar to that of *B. spectabilis* (with eight chromosomes with median centromeres) than to that of *B. serrulata* (with ten chromosomes with median centromeres) (Fig. 2, Table 2). *Bowenia*

serrulata and *B. spectabilis* are too widely distributed for them to hybridize, and thus, according to Jones (1993), *B.* 'Tinaroo' is not a hybrid of the two *Bowenia* species. Norstog & Nicholls (1997) indicated that *B.* 'Tinaroo' is a variety of *B. spectabilis*, and the present results support the hypotheses of both Jones (1993) and Norstog & Nicholls (1997).

The karyotype of *S. eriopus* consists of 12m + 2st + 2t (Fig. 2E, Table 2). Two of twelve chromosomes with median centromeres showed an arm ratio very close to 1.8:1, the lowest extreme of the arm ratio range of submedian centromeres according to Levan *et al.* (1964), and they were aligned in the eleventh and twelfth positions with reference to mean chromosome length (Fig. 2E, Table 2). Sax & Beal (1934) reported the karyotype of *S. eriopus* to consist of 12m + 2st (or sm) + 2t, whereas Marchant (1968) and Moretti (1990) reported it to consist of 10m + 4st (or sm) + 2t. Although in the strict sense the karyotype of *S. eriopus* in the present study was closer to that reported by Sax & Beal (1968) than that reported by Marchant (1968) and Moretti (1990), it is likely that the eleventh and twelfth chromosomes of *S. eriopus* in the present observation correspond with two of four chromosomes with submedian (or subterminal) centromeres indicated by Marchant (1968) and Moretti (1990). Among *Cycadales*, the karyotype of *S. eriopus* was most similar to that of *Ceratozamia mexicana* Brong., which had a chromosome number of 2n = 16 and karyotype of 12m + 2st (or sm) + 2t (Sax & Beal, 1934; Marchant, 1968; Moretti, 1990; Kokubugata & Kondo, 1998).

A terminal satellite was located on the short arm of two chromosomes with subterminal centromeres (the eleventh and the twelfth) in all *Bowenia* (Fig. 1A–D, arrows), this verifying the previous report by Kokubugata *et al.* (2000). On the other hand, a satellite was located on the terminal region of the short arm of two chromosomes with median centromeres (the fifth and sixth) and on the terminal region of the long arm of two chromosomes with median centromeres (the eleventh and twelfth) in *S. eriopus* (Fig. 1E, arrows), this being very close to the observation reported by Marchant (1968).

In the karyotype evolution of *Cycadales*, Moretti (1990) hypothesized that *Stangeria* might have been derived from *Bowenia* by centromeric fission of two chromosomes with median centromeres, and loss of two chromosomes with submedian centromeres and two chromosomes with terminal centromeres; or by centromeric fission of two chromosomes with submedian centromeres, and loss of two chromosomes with median centromeres and the short arm of two chromosomes with submedian centromeres. If the chromosomal change hypothesized by Moretti (1990) had occurred in the evolution from *Bowenia* to *Stangeria*, two satellite chromosomes with submedian centromeres should appear in the chromosome complement of *Stangeria.* This is because the satellites on the two chromosomes of *Bowenia* are ribosomal DNA regions (Kokubugata *et al.*, 2000) and essential sites for the genome. Consequently it is expected that these two satellite chromosomes would not be deleted in the evolution from *Bowenia* to *Stangeria*. However, the present study shows satellites located on four chromosomes with median centromeres of *Stangeria*. Thus,

these results suggest that the progress of chromosomal evolution from *Bowenia* to *Stangeria* could not have occurred by simple centromeric fission and loss. Moretti (1990) commented that his scheme was just a 'working hypothesis', and that further investigation was necessary to analyse karyotype evolution in *Cycadales*. The present investigation showed some different karyotypes from previous reports (Sax & Beal, 1934; Marchant, 1968; Moretti, 1990), and there is a slight possibility that the differences might occur between different accessions of the same species. Fortunately, Marchant (1968) and Moretti (1990) described the sources of accessions examined and it might be necessary to investigate their morphological characters. Recently, chromosome banding techniques have been applied to somatic chromosomes in *Cycadales*, for example, using fluorescent *in situ* hybridization of ribosomal DNA probes (Kokubugata & Kondo, 1998; Kokubugata *et al.*, 2000). The application of this technique to the observation of chromosomes in *Stangeria* is essential for analyses of the chromosomal relationships within the family *Stangeriaceae* and to the other cycads.

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