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Stability and Variability of Chromosome Numbers in the Genus *Schoenoplectus* (Cyperaceae). I. *S. gemmifer*, *S. mucronatus* var. *mucronatus* and *S. triangulatus*

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Summary The chromosomes of the new species *Schoenoplectus gemmifer* and the allied *S. mucronatus* var. *mucronatus* and *S. triangulatus* were studied in culm-apex cells and root-tip cells. The mitotic metaphase chromosomes were small and dot-like. The chromosome number in culm-apex cells was stable and was observed to be $2n=74$ in *S. gemmifer*, $2n=38$ in *S. mucronatus*, and $2n=42$ in *S. triangulatus*, respectively. Thus, it was confirmed that the new species *S. gemmifer* was cytologically different from both *S. mucronatus* and *S. triangulatus*. Inter- and intra-individual variations in chromosome number, mixoploidy and aneuploidy, were found in the root-tip cells of the three species. Chromosome number ($2n$) varied from 68 to 74 in *S. gemmifer*, $2n=32$ to 39 in *S. mucronatus* and $2n=37$ to 44 in *S. triangulatus*, respectively. This phenomenon is very rare and this is the first report in the genus *Schoenoplectus*.

Key words Aneuploidy, Chromosome number, Cyperaceae, Mixoploidy, *Schoenoplectus*.

The genus *Schoenoplectus* (Rchb.) Palla is one of the segregated genera from the polymorphic and polyphyletic genus *Scirpus* L. s.l. and comprises about 50 species widely distributed in the world (Hayasaka and Ohashi 2000a). Recently, the taxonomy within *Schoenoplectus* was actively investigated by Hayasaka and Ohashi (2000a) and Smith and Hayasaka (2001, 2002). They recognized four sections, sect. Schoenoplectus, sect. Malacogeton (Ohwi) S. G. Smith and Hayasaka, sect. Actaeogeton (Rchb.) J. Raynal and sect. Supini (Cherm.) J. Raynal, in the genus *Schoenoplectus*. Moreover, the new taxon in the genus from Japan were reported by Arai and Miyamoto (1997), Hayasaka and Ohashi (2000b) and Kohno *et al.* (2001).

Schoenoplectus gemmifer C. Sato, T. Maeda and A. Uchino was recognized as a new species of sect. Actaeogeton from Japan based on morphological analyses and by comparison with its allies by Sato *et al.* (2004) and Maeda *et al.* (2004). To confirm the new species, we describe here the different chromosome numbers on *S. gemmifer* and its allies. Moreover, the mixoploidy, aneuploidy, intra- and inter-individual variation of chromosome numbers, is elucidated.

Materials and methods

Three plants of the genus *Schoenoplectus*, *S. gemmifer* C.Sato, T.Maeda and Uchino, *S. mucronatus* (L.) Palla var. *mucronatus* and *S. triangulatus* (Roxb.) Soják, were used for the chromosome analyses. Although *S. mucronatus* has 3 varieties with triquetrous culms, the typical variety, var. *mucronatus*, is different from var. *tataranus* and var. *ishizawae* in having no-winged angles of the culms (Kohno *et al.* 2001). Plants were collected from the western part of Japan (Table 1, Fig. 1) and cultivated in the experimental garden at the Faculty of Science, Kumamoto University.

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Table 1. Localities and numbers of material plants of *Schoenoplectus* for chromosome analysis

Species		Locality	No. of Plants	
S. gemmifer				
	Ng:	Nagare, Aso District, Kumamoto Pref.	1	
	Sm:	Senomoto, Aso District, Kumamoto Pref.	3	6
	Hb:	Hatabe, Aso District, Kumamoto Pref.	2	
S. mucronatus var. *mucronatus*				
	Hm:	Hitotsume, Yamaga City, Kumamoto Pref.	3	
	Sd:	Mt. Syodai-san, Tamana City, Kumamoto Pref.	1	6
	St:	Mt. Shira-take, Amakusa District, Kumamoto Pref.	2	
S. triangulatus				
	Sa:	Shimoarichi, Fukuyama City, Hiroshima Pref.	1	
	Td:	Mt. Tatsuda-yama, Kumamoto City, Kumamoto Pref.	2	7
	It:	Ishitobi, Minamata City, Kumamoto Pref.	4	

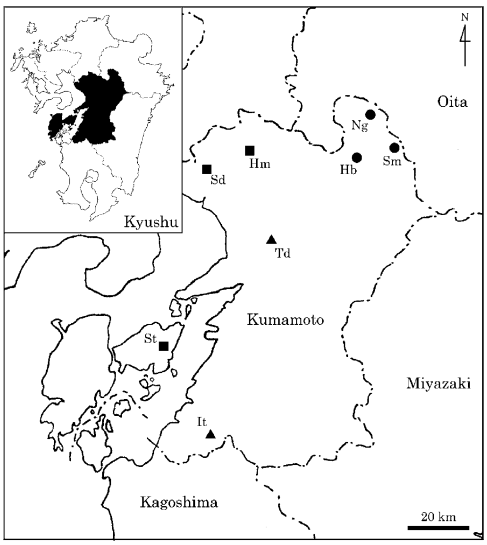


Fig. 1. Localities of material plants of *Schoenoplectus gemmifer* (●), *S. mucronatus* var. *mucronatus* (■) and *S. triangulatus* (▲) in Kyushu, Japan. The alphabetical codes correspond to the locality codes in Table 1.

For chromosome analysis, culm-apexes and root-tips were cut off from individuals and pretreated in 0.002 M aqueous solutions of 8-hydroxyquinoline for 5 h at room temperature before fixation with Carnoy's solution (3 : 1 mixture of absolute ethanol and glacial acetic acid) for over 2 h at 4°C. The culm-apexes were macerated in the enzyme solution consisting of 2% cellulase (Onozuka RS, Yakult Honsha Co., Ltd.) and 0.5% pectolyase (Y-23, Kikkoman Corporation), pH 4.2, at 37°C for 1 h and then centrifuged for 1 min at 10,000 rpm. The supernatant was discarded. The soft tissue mass was washed three times in distilled water by centrifuging for 5 min at 10,000 rpm, discarding the supernatant and resuspending in fresh distilled water. Washing was repeated twice with the refixative solution (3 : 1 mixture of methanol and acetic acid). The cell suspension was made by adding 100–200 μl of the refixative solution and resuspending softly by micropipeter. Ten to 20 μl of suspension was dropped onto slides washed with ethanol and air dried. For observation, slide preparations were stained with 2% aceto-orcein solution. After fixing with Carnoy's solution, the root-tips were macerated in the enzyme solution described above at 37°C for 50 min and washed sevral times in distilled water. They were then squashed in 2% aceto-orcein.

The chromosome count image of Fig. 2 was performed on a Macintosh computer using the public domain program, NIH Image (developed at the U.S. National Institutes of Health and available from the Internet by anonymous FTP from zippy.nimh.nih.gov or on floppy disk from the National Technical Information Service, Springfield, Virginia, part number PB95-500195GEI).

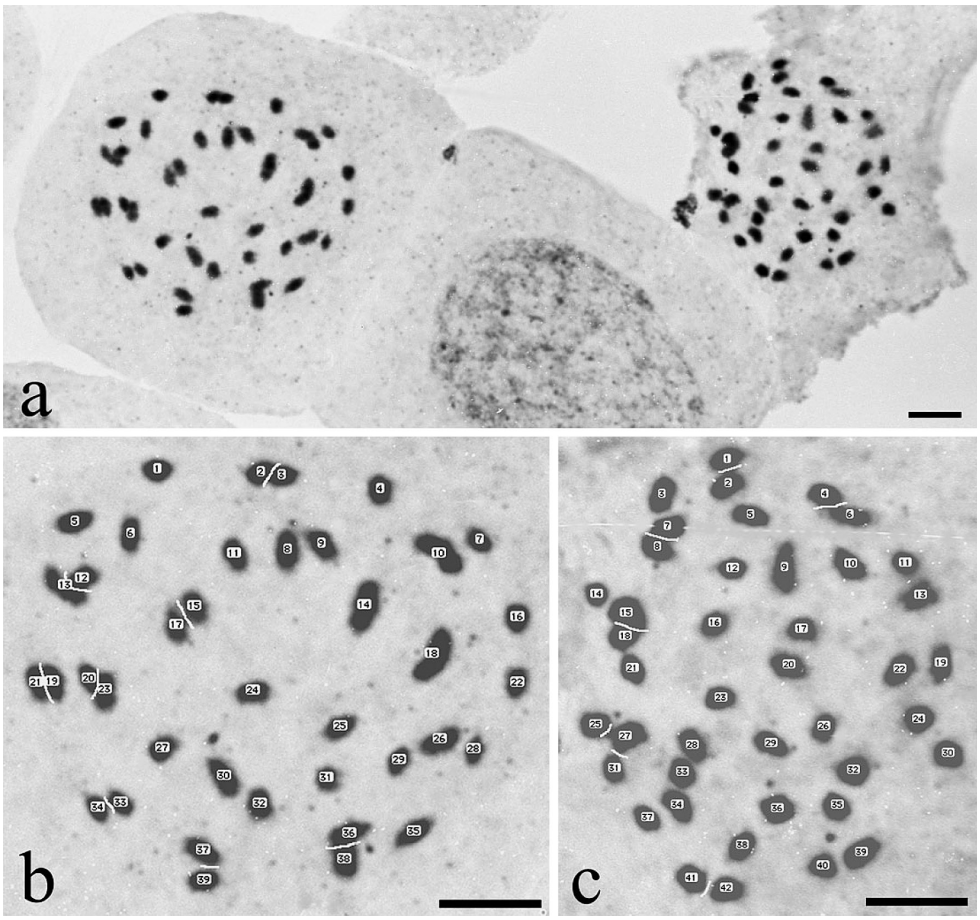


Fig. 2. Two cells with different chromosome numbers in a given root-tip of *S. triangulatus* (a). (b) and (c) were the results of chromosome count by NIH Image. Chromosome number in left cell was $2n=39$ (b) and that in right cell was $2n=42$ (c). Bar indicates 5 μm.

Results

In the 3 different species, the mitotic metaphase chromosomes were small and dotlike. Constrictions were not observed anywhere in any of the chromosomes (Fig. 2). Under the circumstances, karyotypes of the plants were not analyzed in detail. In the meristematic cells of the culm-apex, each species contained a specific chromosome number which was naturally stable in an individual. *S. gemmifer* had a chromosome number $2n=74$ in 3 culm-apexes from 3 individuals (Table 2) and *S. mucronatus* had $2n=38$ in 3 culm-apexes from 3 individuals (Table 3). In *S. triangulatus*, $2n=42$ was observed in 11 culm-apexes from 5 individuals (Table 4). Chromosome number, thus, was stable within individuals and within each species but differed between the 3 different species.

The intra- and inter-individual variations in chromosome number were identified in the meristematic cells of root-tips of all three species (Tables 2–4). Chromosome number was found to vary even within the same root-tip in respective species (Fig. 2). The results of *S. gemmifer* observations are shown in Table 2. Chromosome number varied among cells of an individual and between different individuals. In Root No. 1 of Ng-1, for example, chromosome numbers $2n=75$ and 76 were observed. In the same individual, 3 ($2n=73$, 75, 76) and 6 ($2n=70$, 72–76) different chromosome numbers were found in Root Nos. 2 and 5, respectively. This was true in all individuals observed.

Table 2. Frequency of cells with different chromosome numbers (2*n*) in *Schoenoplecctus gemmifer*

1. Culm-apex cell

Individual	Culm No.	Chromosome number (2*n*)									Total of cells
		68	69	70	71	72	73	74	75	76	
Sm-1	1							2			2
Hb-1	1							3			3
Hb-6	1							4			4
Total								9			9

2. Root-tip cell

Individual	Root No.	Chromosome number (2*n*)									Total of cells
		68	69	70	71	72	73	74	75	76	
Ng-1	1								1	2	3
	2						1		1	1	3
	3						1				1
	4					1					1
	5			1		1	1	1	1	4	9
	6							3		3	6
	Total			1		2	3	4	3	10	23
Sm-1	1					1	1	3	1		6
	2	1	1			2	1				5
	3		1		1		1	1			4
	Total	1	2		1	3	3	4	1		15
Sm-2	1					1	1	2	1	1	6
Sm-3	1					2	1				3
Hb-1	1									1	1
	2							1		2	3
	3					1		1			2
	Total					1		2		3	6
Hb-6	1					4	2	2			8
Grand total		1	2	1	1	13	10	14	5	14	61

As a result of intra-individual variation, chromosome number varied naturally among individuals. The range of variation was 2*n*=68 to 76 in 61 cells of 6 individuals from 3 localities. The most frequent chromosome numbers were 2*n*=74 and 76 and the next frequent was 2*n*=72. Intra-individual variation of chromosome number was also observed in *S. mucronatus* (Table 3). In Hm-1, for example, 4 types of cell with 2*n*=34 and 36 to 38 were found in Root No. 1 and 6 types of cells with 2*n*=32 and 34 to 38 in Root No. 2. Only one individual, Hm-5, showed a stable chromosome number of 2*n*=38. Naturally, inter-individual variation of chromosome number was observed. The

Table 3. Frequency of cells with different chromosome numbers (2*n*) in *Schoenoplectus mucronatus* var. *mucronatus*

1. Culm-apex cell

Individual	Culm No.	Chromosome number (2*n*)								Total of cells
		32	33	34	35	36	37	38	39	
Hm-1	1							3		3
Sd-2	1							6		6
St-6	1							4		4
Total								13		13

2. Root-tip cell

Individual	Root No.	Chromosome number (2*n*)								Total of cells
		32	33	34	35	36	37	38	39	
Hm-1	1			1		1	5	5		12
	2	1		1	2	3	3	3		13
	3							6		6
	4							6	1	7
	5						1	4		5
	6							2	1	3
	Total	1		2	2	4	9	26	2	46
Hm-2	1			1	3	4	1	3		12
	2				2	4	2	1	1	10
	Total			1	5	8	3	4	1	22
Hm-5	1							1		1
	2							3		3
	Total							4		4
Sd-2	1					1	1	3	1	6
	2						3	3		6
	3							1	1	2
	Total					1	4	7	2	14
St-3	1					2	3	6	1	12
	2			1	2	5	2			10
	3							1		1
	Total			1	2	7	5	7	1	23
St-6	1								1	1
	2						1	2		3
	Total						1	2	1	4
Grand total		1		4	9	20	22	50	7	113

Table 4. Frequency of cells with different chromonsome numbers (2*n*) in *Schoenoplectus triangulatus*

1. Culm-apex cell

Individual	Culm No.	Chromosome number (2*n*)								Total of cells
		37	38	39	40	41	42	43	44	
Sa-1	1						8			8
	2						15			15
Td-1	1						5			5
	2						10			10
It-1	1						5			5
	2						10			10
	3						6			6
It-2	1						20			20
It-3	1						13			13
	2						18			18
	3						20			20
Total							130			130

range of variation was 2*n*=32 to 39 in 113 cells of 6 individuals. The most frequent chromosome number was 2*n*=38 and the next frequent was 2*n*=37 The variation in chromosome numbers in *S. triangulatus* is presented in Table 4. Td-3 was the most variable in chromosome number, 2*n*=37 to 44. In 241 cells of 7 individuals of the species, the variation in chromosome number ranged from 2*n*=37 to 44. The most frequent chromosome number was 2*n*=42, the same as found in the culm-apex.

Discussion

Examination of the culm-apexes showed that the chromosome numbers of the 3 species were stable. The chromosome number (2*n*=74) of *S. gemmifer* was reported for the first time. This is the first report of chromosome numbers of 2*n*=38 for *S. mucronatus* var. *mucronatus*. Previous reports were: for *Schoenoplectus mucronatus* (L.) Palla, 2*n*=44 (Jankun 1989) and for *Scirpus mucronatus* L., *n*=21 (Mehra and Sachdeva 1975, Morinaga and Fukushima 1931, Tanaka 1937), *n*=22 (Bir *et al.* 1981, 1988a, 1988b, 1990) and 2*n*=42 (Sanyal and Sharma 1972). The chromosome number (2*n*=42) of *S. triangulatus* identified in this study identical to that found previously (Kitamura *et al.* 1987). *S. gemmifer* was recognized as a new species based on statistical analyses of the morphological characters (Sato *et al.* 2004, Maeda *et al.* 2004) and so was distinguished from *S. mucronatus* and *S. triangulatus*. The chromosome observation in the culm-apexes strongly supports the conclusion that *S. gemmifer* is a new species. *S. gemmifer* may have a polyploidy relationship with *S. mucronatus* and *S. triangulatus*.

Profound variations in chromosome number within inter- and intra-individuals were found in this study. Inter-individual variation of chromosome number *i.e.* intra-specific aneuploidy has been reported frequently in the family Cyperaceae and investigated in *Carex* species (Tanaka 1948, Wahl 1940, Davies 1956, Faulkner 1972, Schmid 1982, Cayouette and Morisset 1986a, b, Hoshino 1981a, b, Witkus 1991, Hoshino *et al.* 1993a, *etc.*). Hoshino and Onimatsu (1994) put forth the idea that aneuploidy was caused by the fusion and fission of chromosomes with diffused centromeres,

Table 4. (Continued.)

2. Root-tip cell

Individual	Root No.	Chromosome number ($2n$)								Total of cells
		37	38	39	40	41	42	43	44	
Sa-1	1						5			5
	2						4			4
	3						6	1		7
	4					1	6			7
	Total					1	21	1		23
Td-1	1					5	1	2	3	11
	2			2	5	3	8	1		19
	3				2		4			6
	4	1				2	4			7
	Total	1		2	7	10	17	3	3	43
Td-3	1		1	1	3	2	1		1	9
	2	2	2	1	4	5	3	1		18
	3			1		1	2			4
	Total	2	3	3	7	8	6	1	1	31
It-1	1			4	4	3	9			20
	2				1	2	11			14
	3					1	3			4
	Total			4	5	6	23			38
It-2	1						4			4
	2					1	3			4
	3					1	9			10
	4						1			1
	Total					2	17			19
It-3	1		2	2	3	4	7	1		19
	2	2		2	3	6	7	2		22
	3		1		1	1	11	2		16
	Total	2	3	4	7	11	25	5		57
It-4	1				1	1	8			10
	2					1	9			10
	3					6	4			10
	Total				1	8	21			30
Grand total		5	6	13	27	46	130	10	4	241

mainly based on the high frequency of heteromorphic trivalents. In the genus *Schoenoplectus*, intraspecific aneuploidy was reported for several species; $2n=38$, 40 and 42 in *S. lacustris* (L.) Palla as *Scirpus lacustris* L. (Tanaka 1938), $2n=40$ and 41 in *S. triqueter* (L.) Palla as *Scirpus triqueter* L. (Hoshino *et al*. 1993b). Different chromosome numbers were reported by Hicks (1928) and

Schuyler (1975) in *S. heterochaetus* (Chase) Soják as *Scirpus heterocharis* Chase, in *S. acutus* (Muhl. ex Bigel.) A. Löve and D. Löve as *Scirpus acutus* Muhl. ex Bigel., in *S. americanus* (Pers.) Volkart as *Scirpus americanus* Pers. The aneuploidy in these species is not clear. In *S. mucronatus*, $n=21$, 22 and $2n=42$ were reported as mentioned above. Aneuploidy, however, is obscure, because *S. mucronatus* contains 3 varieties (Kohno *et al.* 2001) and causes taxonomic problems for the varieties of *S. mucronatus* in Japan (Horiuchi 2001).

Intra-individual variation, mixoploidy or aneusomaty, has been reported in *Carex siderostica* Hance (Cyperaceae) by Tang and Xiang (1989). In this case, the chromosome number varied among both root-tip cells and Pollen Mother Cells (PMCs). Mixoploidy of *Schoenoplectus* occurred in root cells but not in culm-apex cells. Mixoploidy in a paticular tissue was observed in *Xanthisma texanum* D. C. (Compositae) (Berger and Witkus 1954). In this case, mixoploidy was caused by B-chromosomes which were present in shoot cells but not in root cells (Berger *et al.* 1955). It was found that B-chromosomes were eliminated from the cells during the root differentiation but remained in the cells of shoot tissue by lagging at mitosis. The cause of mixoploidy in the *Schoenoplectus* plants has still not been investigated. The cause of mixoploidy in a paticular tissue may be due to differences in cytological events during the developmental process of the primordium as found in *X. texanum*. The chromosomes of the *Schoenoplectus* plants were small and dot-like, similar to other members of the family Cyperaceae. Generally, small, dot-like chromosomes such as the B-chromosome, tend towards elimination, undivision or lagging in mitosis (Haga 1961, Kayano 1961, Jones and Rees 1982, Uchino and Wang 1997). Moreover, the *Schoenoplectus* plants may have chromosomes with diffused centromeres similar to *Luzula* and *Cyperus* of the family Cyperaceae (Braselton 1971). Fusion or fission may occur frequently in their chromosomes.

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