

Karyomorphological Studies on 6 Taxa of *Eleocharis* in Japan

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Abstract

Karyomorphological observations are carried out in 6 taxa of Japanese *Eleocharis*. The following three types are recognized: 1) *Eleocharis mamillata* var. *cyclocarpa* ($2n=16$). Interphase nuclei show round prochromosome type. The 16 metaphase chromosomes show bimodal variation. 2) *E. wichurae* ($2n=20$), *E. tetraquetra* ($2n=20$), *E. acicularis* ($2n=20$) and *E. congesta* var. *thermalis* ($2n=20$). Interphase nuclei show complex chromocenter type. Karyotypes of these four taxa show gradational variation. 3) *E. kuroguwai*, $2n = \text{ca.} 196$. Interphase nuclei show sparsely diffuse type. All metaphase chromosomes are very small, under $0.7 \mu\text{m}$ in length. The karyomorphological characteristics of interphase nuclei and metaphase chromosomes are found to be closely correlated with the taxonomical treatment of section.

Introduction

Chromosome numbers of *Eleocharis* were reported by Tanaka (1948)¹⁾, Håkansson (1954, 1958)²⁾³⁾, Strandhede (1956a,b)⁴⁾⁵⁾, Harms (1968)⁶⁾, Thiébaud (1970)⁷⁾, Nijalingappa (1972)⁸⁾, Schuyler (1977)⁹⁾, etc. The lowest chromosome number of this genus is $2n=10$ and the highest number is $2n=136$ (Lewis et al. 1962).¹⁰⁾ Håkansson (1954)²⁾ reported that the metaphase chromosomes of *E. palustris* possess a non-localized centromere and almost all taxa were supposed to have a diffuse centromere.

Cytological work on Japanese *Eleocharis* was carried out by Tanaka (1948).¹⁾ He observed $2n=16$ in one taxa and $2n=20$ in four taxa. But the karyo-

morphological study of chromosomes of Japanese *Eleocharis* was not fairly carried out, because the root tips of this genus were so hard that they could not be macerated by normal means. In this study, the author could observe the detailed structure of mitotic metaphase chromosomes by using an enzyme treatment to the root tips. The number and length of mitotic metaphase chromosomes and distribution patterns of chromatins at interphase nuclei were observed on six taxa. Pachytene chromosomes in meiotic division were observed in *E. mamillata* var. *cyclocarpa* and *E. wichurae* and the relationship of these taxa was discussed, based on the results.

Materials and Methods

The Localities and Number of clones investigated were shown in Table 1. Voucher specimens of chromosomal observations were deposited in the Herbarium of the Biological Laboratory of Okayama University of Science. The somatic chromosomes were observed in the meristematic cells of root tips. The root tips were pretreated in 0.05% aqueous solutions of colchicine for 3 hours at room temperature. They were then fixed in a 3:1 mixture of absolute ethanol and glacial acetic acid at 5C for over 1 hours and stained by Feulgen's nuclear reaction and then macerated in a mixture of 4% pectinase and 4% cellulase for 1 hour at 30C. After maceration, they were stained in 1% aceto-orcein for 10 or 20 minutes, then squashed. Meiotic chromosomes were observed in the pollen mother cells (PMCs). Staminate spikelets were fixed in a 3:1 mixture of absolute

Table 1. Localities, number of clones and chromosome numbers of 6 taxa of *Eleocharis*.

Taxon	Locality	Chromosome number (n)(2n)	Number of clones studied
<i>Eleocharis mamillata</i> var. <i>cyclocarpa</i>	Hatsuwa, Chuka V., Okayama Pref.	8 16	30
<i>E. wichurae</i>	Ningyotoge, Kamisaihara V., Inubasaritoge, Yatsuka V., Machikanda, Akasaka T.,	" 20 " 20 " 10 20	2 2 24
<i>E. tetraquetra</i>	Masamune, Kagamino T.,	" 10 20	3
<i>E. acicularis</i>	Shiraishi, Okayama C., Ichinomiya, Okayama C.,	" 10 20 " 20	3 2
<i>E. congesta</i> var. <i>thermalis</i>	Kirigamine, Suwa C., Nagano Pref.	20	1
<i>E. kuroguwai</i>	Shimonatsukawa, Okayama C., Okayama Pref.	ca.196	4

ethanol and glacial acetic acid at 5C for over 1 hour. The anthers were stained in 2% aceto-carmin and squashed gently.

Results

The results of the chromosome counts of the 6 taxa are given in Table 1. Among the species observed the following 3 types, based on the chromatic structure of the interphase nuclei and the morphology of somatic metaphase chromosomes, were recognized.

Type 1. Species observed : *Eleocharis mamillata* var. *cyclocarpa*

Thirty clones collected from Okayama Prefecture show $2n=16$ chromosomes (Fig. 2 A).

Interphase chromosomes are observed forming about 10 condensed bodies which stained darkly. The condensed bodies are round in shape with a diameter of about $0.8\ \mu\text{m}$. Small granular, lightly stained spherules of chromatin are observed scattered nuclei (Fig. 1 A). At prophase about ten chromosomes are stained darkly in distal regions.

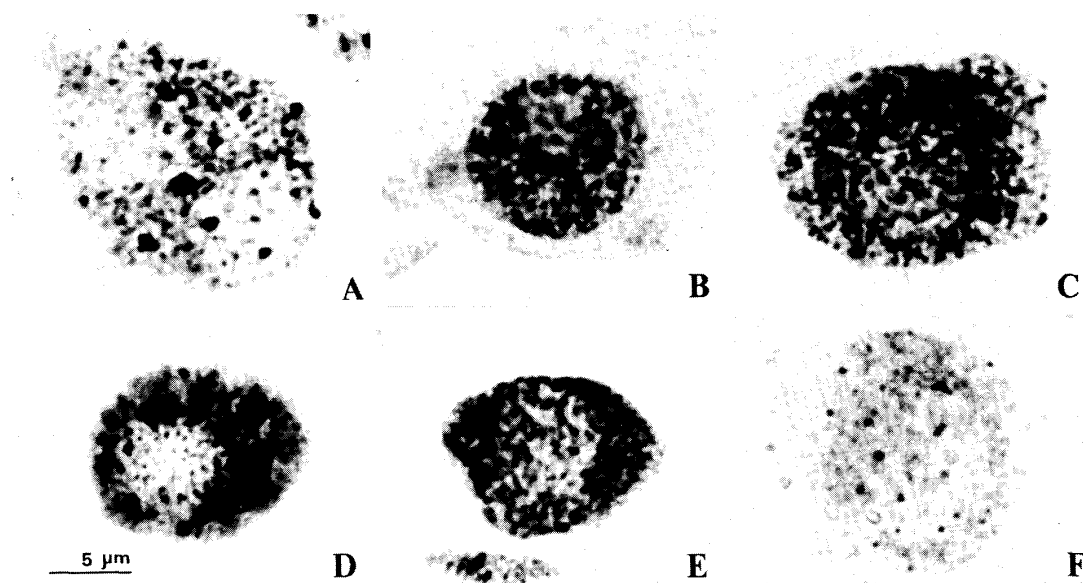


Fig. 1. Photomicrographs of interphase nucleus of *Eleocharis*.

- A. *E. mamillata* var. *cyclocarpa*. B. *E. wichurae*.
 C. *E. tetraquetra*. D. *E. acicularis*. E. *E. congesta* var. *thermalis*.
 F. *E. kuroguwai*.

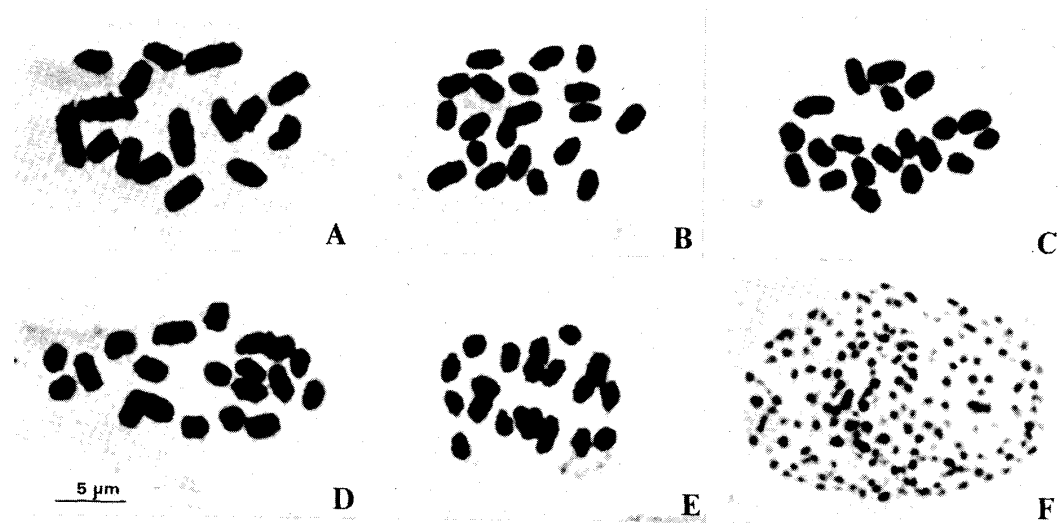


Fig. 2. Photomicrographs of metaphase chromosomes of *Eleocharis*.

- A. *E. mamillata* var. *cyclocarpa* ($2n=16$). B. *E. wichurae* ($2n=20$).
 C. *E. tetraquetra* ($2n=20$). D. *E. acicularis* ($2n=20$).
 E. *E. congesta* var. *thermalis* ($2n=20$). F. *E. kuroguwai* ($2n=ca.196$).

The 1st to 4th chromosomes (1-4 in Fig. 3A) range from $4.3\ \mu\text{m}$ to $4.0\ \mu\text{m}$ in length. These 4 chromosomes are regarded as large ones. The 5th to 16th chromosomes (5-16 in Fig. 3A) range from $2.7\ \mu\text{m}$ to $2.2\ \mu\text{m}$ length. These

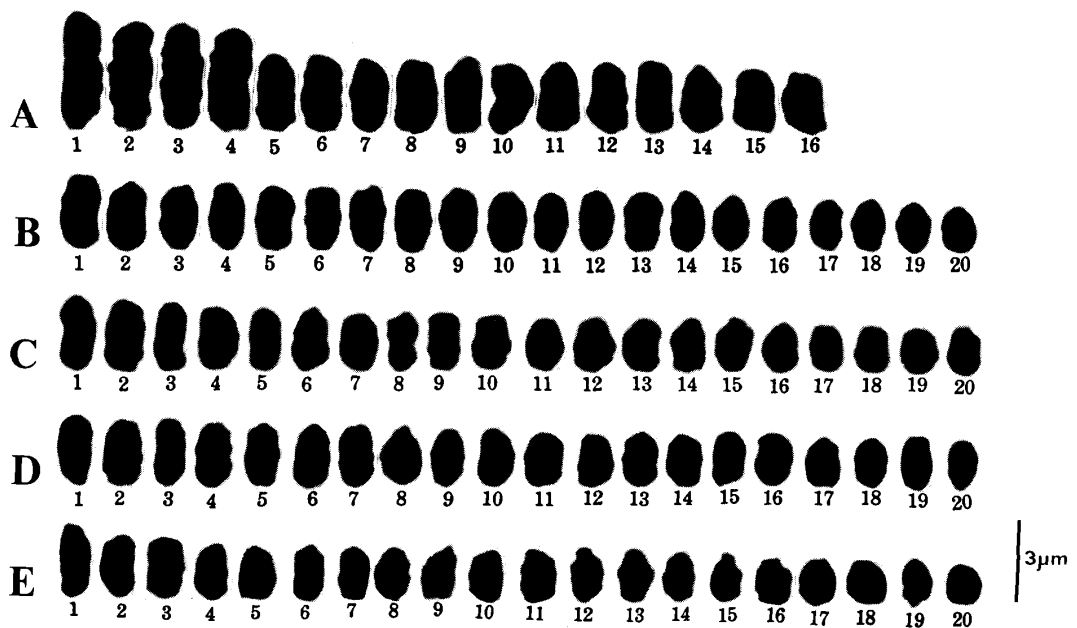


Fig. 3. Karyotypes of mitotic metaphase chromosomes of *Eleocharis*.

- A. *E. mamillata* var. *cyclocarpa* ($2n=16$). B. *E. wichurae* ($2n=20$).
 C. *E. tetraquetra* ($2n=20$). D. *E. acicularis* ($2n=20$).
 E. *E. congesta* var. *thermalis* ($2n=20$).

chromosomes are regarded as small chromosomes. The primary constrictions are not observed in these metaphase chromosomes.

The interphase nuclei of PMCs consist of about 10 condensed bodies which are round in shape. The morphological character of the interphase nuclei of PMCs is the same as that of the root tip cells (Fig. 4A). The pachytene chromosomes have large knobs in distal regions. Two of them are observed attached to a nucleolus by terminal ends (Fig. 4B). During first meiotic division eight bivalents are observed in each pollen mother cell (Fig. 4C). There was no irregular division in anaphase I and II.

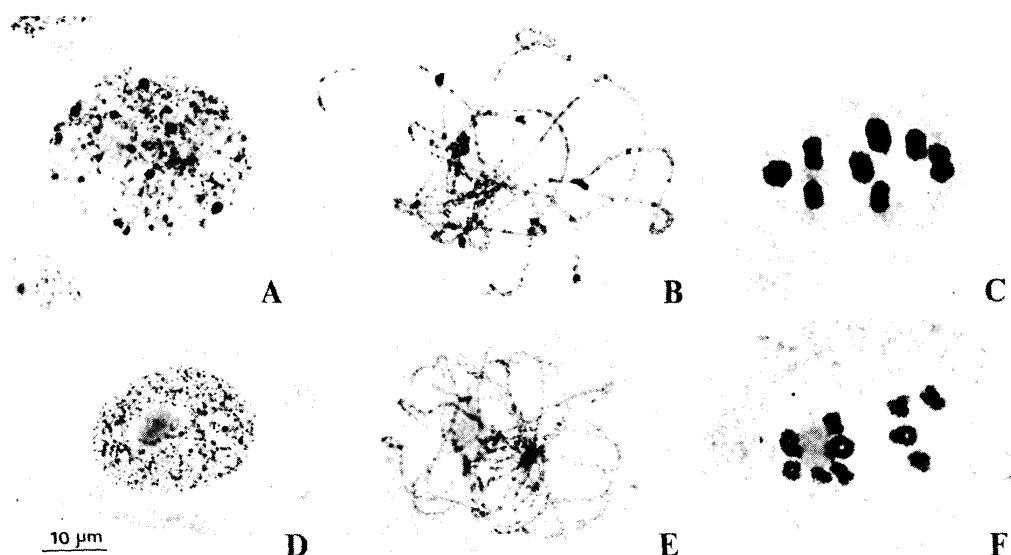


Fig. 4. Photomicrographs of meiotic chromosomes of *E. mamillata* var. *cyclocarpa* (A-C) and *E. wichurae* (D-F).
A, D. Interphase. B, E. Pachytene. C. Metaphase I.
F. Diakinesis.

Type 2. Species observed : *E. wichurae*, *E. tetraquetra*, *E. acicularis*, *E. congesta* var. *thermalis*.

Chromosome number of these four species is $2n=20$ (Figs. 2 B,C,D,E). Interphase chromatins form many small granular spherules of about $0.4\mu\text{m}$. Large condensed bodies are not observed in these species (Figs. 1 B,C,D,E).

The range of mitotic metaphase chromosome length is $2.7-1.7\mu\text{m}$ in *E. wichurae*, $2.7-1.6\mu\text{m}$ in *E. tetraquetra*, $2.5-1.5\mu\text{m}$ in *E. acicularis*, $2.5-1.5\mu\text{m}$ in *E. congesta*. The 20 metaphase chromosomes of each species showed a gradual reduc-

tion in length (Figs. 3 B,C,D,E).

In *E. wichurae*, interphase nuclei of PMCs have many small granular spherules as mitotic interphase nuclei (Fig. 4 D). At pachytene, a large knob is not found in these species (Fig. 4 E). Ten bivalents are found during first meiotic division in each pollen mother cell (Fig. 4 F).

Type 3. Species observed : *E. kuroguwai*.

Four clones collected from Okayama Pref. have $2n = \text{ca.}196$ chromosomes.

The lightly stained interphase chromatins form many small granular spherules about $0.2\mu\text{m}$ in diameter. The whole nuclear region stained very lightly and could not be distinguished from the cytoplasm (Fig. 1 F).

The length of all mitotic metaphase chromosomes are under $0.7\mu\text{m}$ (Fig. 2 F).

Discussion

Chromosome counts for *Eleocharis mamillata* var. *cyclocarpa*, *E. wichurae*, *E. tetraquetra*, and *E. acicularis* reported here are in conform to previous reports (Tanaka 1948)¹⁾. The chromosome number of $2n=20$ for *E. congesta* var. *thermalis* and $2n = \text{ca.}196$ for *E. kuroguwai* are determined for the first time in this study. The chromatin pattern of the interphase nuclei of 6 taxa is classified into three types. The morphological characteristics of the interphase nuclei of Type 1 are comparable to the round prochromosome type, those of Type 2 to the complex chromocenter type and those of Type 3 to the sparsely diffuse type defined by Tanaka (1971)¹¹⁾. The 16 chromosomes of *E. mamillata* var. *cyclocarpa* grouped into two types, 4 large and 12 small, showing bimodal variation. The chromosome number of *E. wichurae*, *E. tetraquetra*, *E. acicularis* and *E. congesta* var. *thermalis* are $2n=20$ and the karyotypes of these four taxa show gradational variation. *E. kuroguwai* has a very characteristic karyotype and is distinguished from 5 other taxa. In this genus the chromatin pattern of the interphase nuclei is supposed to be closely correlated with the karyotype of metaphase chromosomes.

Genus *Eleocharis* was divided by C. B. Clarke (1909)¹²⁾ into two Subgenera, *Limnochloa* and *Eu-eleocharis*, chiefly by the thickness of culms and the shape of spikelets. However, Koyama (1961)¹³⁾ moved *E. margaritacea* and *E. parvula* from genus *Scirpus* to *Eleocharis* and made a new Section *Pauciflorae* in *Eleocharis*.

The genus *Eleocharis* is therefore divided into three groups as Sect. *Pauciflorae*, Sect. *Eleocharis* and Sect. *Limnochloa*. *E. kuroguwai* belongs to the Sect. *Limnochloa* and the 5 other taxa to Sect. *Eleocharis*. The karyomorphology of these species and taxonomical treatment of sections suggest that *E. kuroguwai* and the 5 other taxa were derived through different courses of evolution.

The 5 taxa of the Sect. *Eleocharis* are found to fall into two types. One of them is *E. mamillata* var. *cyclocarpa* which shows bimodal karyotype. The other type is $2n=20$ and shows a gradational karyotype. Many workers have shown that the chromosomes of *Eleocharis* possess diffuse centromeres (Nordenskiöld 1961, 1962, Strandhede 1956 a,c, Harms 1968).^{14) 15) 4) 5) 6)} Agmatoploidal change of chromosomes may be present in this genus as *Luzula* and *Carex*. Fragmentations of four large chromosomes of *E. mamillata* var. *cyclocarpa* is seen to produce gradational karyotype of the four other taxa. However, these two groups are quite different in karyomorphology of pachytene chromosomes and the chromatin pattern of interphase nuclei. This fact suggests that gradational karyotype of $2n=20$ is not produced by the simple structural change of four large chromosomes of *E. mamillata* var. *cyclocarpa*.

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