Chromosomal evolution in genus *Carex* as viewed from nuclear DNA content, with special reference to its aneuploidy¹⁾

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ABSTRACT

In order to consider characteristic chromosomal evolution, agamaploidy in genus *Carex*, nuclear DNA content of metaphase plates in root tip of 50 cytologically stable strains belonging to 26 species of *Carex* collected in Japan was measured Feulgen cytophotometrically.

Considerably large variation was observed in six species with rather high chromosome number. There was 7.7 times variation in DNA content among species analyzed in the present study. As assumed by earlier reports, it is presumed that *C. siderosticta* 4x and *C. humilis* were derived from *C. siderosticta* 2x and *C. humilis* ssp. *nana*, respectively.

Among diploid species, 4.66 times but continuous variation was observed. There were no significant differences in nuclear DNA among four aneuploid of *C. oxyandra* and five of *C. conica*, respectively. Neither of five couples of strains, each belonging to a single species and having the same chromosome number but collected at different sites revealed any differences, indicating intraspecific aneuploid resulted from simple change of chromosome number. Three pairs of close relatives, *C. foliosissima* vs. *C. morrowii*, *C. paxii* vs. *C. nubigera* and *C. pumila* vs. *C. kobomugi*, were similar to each other in DNA content. It is clear that the increase in chromosome number is accompanied by the decrease in nuclear DNA content; negative correlation and regression between DNA content and chromosome number were confirmed to be highly significant in 45 diploid species.

Based on the evidence of various kinds of earlier investigations and the present study, it is suggested that species with many small chromosomes would be derived from species having small number of large chromosomes by means of fragmentation followed by slight reduction of DNA.

1. INTRODUCTION

Family *Cyperaceae* comprises about 45 genera including more than 4000 species. One of them, genus *Carex* contains about 1800 species which are distributed throughout the world, predominantly in Asia and northern America. The plants in this genus are characterized by chromosomes with non-localized centromere or holocentric chromosomes and wide range of variation in chromo-

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some number ($n=6$ to 68), resulting in so-called *Carex* type aneuploidy or agamaploidy (Tanaka 1939, 1948, Devies 1956). Tanaka (1948) pointed out that total chromosome volume in species with the small number of chromosomes was remarkably larger than that in species of high chromosome number. Recently, Hoshino (1981b) reported that the size of chromosome in intraspecific aneuploids decreased according to the increase in chromosome number and that polyploidy might have played only a minor role in the speciation of this genus. But he supported the theory of chromosome differentiation by agamaploidy proposed by Devies (1956) and Faulkner (1972).

In order to consider this characteristic chromosomal evolution in genus *Carex*, nuclear DNA content of 50 strains belonging to 26 species of *Carex* collected in Japan was measured by Feulgen-cytophotometry.

2. MATERIALS AND METHODS

Cytologically stable 50 strains of 26 species shown in Table 1 were kindly provided by Dr. T. Hoshino, Okayama University of Science and used in the present study. Root tips were fixed in fresh acetoethanol fluid in May to June or September to October, rinsed in ethanol series and stocked in 75% ethanol in refrigerator. Root tips of *C. ciliato-marginata* used as the standard and five strains to be compared with the standard were hydrolyzed together in 5N HCl at 25°C for 30 minutes, stained in Schiff's reagent (25°C) for 3 hours in dark, washed in potassium pyrosulfite solution three times for 15 minutes each and squashed. Immediately after staining, spherical metaphase cells were measured at 556nm by Nikon-Vickers M85 Scanning Microdensitometer. When it was possible, ten metaphase plates of each root tip and three root tips of each strain per replication were selected for measurement and three replications were carried out.

3. RESULTS AND DISCUSSION

Fig. 1 is the histogram of nuclear DNA content at various mitotic stages in root tip of *C. ciliato-marginata*. The DNA values measured in interphase and prophase nuclei widely varied, while those of nuclei at metaphase and anaphase, particularly at metaphase, converged on the limited range of variation (Fig. 1). Then, it is apparent that metaphase plate is suitable for measurement of DNA content of each nucleus. DNA content of nucleus in 50 strains belonging to 26 species in *Carex* is shown in Table 1. Considerably large variation was observed in six species, *C. tristachya* (coefficient of variation, 18%), *C. capillacea* (13%), *C. brownii* (15%), *C. thunbergii* (14%), *C. paxii* (17%) and *C. nubigera* (14%). These species are rather high in chromosome number and include intraspecific aneuploids. But, it is not explainable why DNA content of nucleus is so variable even within a given individual plant.

Table 1. Nuclear DNA content at metaphase in strains belonging to 26 species of genus *Carex* (arbitrary unit)

Strain number	Section and species	2n	No. of nuclei	Nuclear DNA content (4C)		
				Mean±SE	CV*	Significant at 5%
Laxiflorae						
1	<i>C. ciliato-marginata</i>	12	1000	225±1	7	d**
2	<i>C. pachygyna</i>	12	90	296±2	6	b
3	<i>C. pachygyna</i>	12	75	285±2	5	bc
4	<i>C. siderosticta</i> 4x	24	90	471±3	6	a
Digitatae						
5	<i>C. oxyandra</i>	18	80	162±1	7	jklm
6	<i>C. oxyandra</i>	20	90	166±1	3	jkl
7	<i>C. oxyandra</i>	20	60	155±1	5	lmnop
8	<i>C. oxyandra</i>	24	90	162±1	6	jklm
9	<i>C. oxyandra</i>	26	90	161±1	6	jklm
	<i>C. humilis</i>					
10	ssp. <i>lanceolata</i>	72	90	292±2	6	bc
11	ssp. <i>lanceolata</i>	72	60	269±3	8	c
Decorae						
12	<i>C. reinii</i>	26	90	197±2	10	defghi
13	<i>C. chrysolepis</i>	28	90	160±1	6	jklmn
14	<i>C. makinoensis</i>	30	90	163±1	7	jklm
15	<i>C. blepharicarpa</i>	30	90	182±1	7	ghijk
Praecoces						
16	<i>C. conica</i>	32	90	175±1	7	ijkl
17	<i>C. conica</i>	32	90	176±1	7	hijkl
18	<i>C. conica</i>	36	80	181±1	7	ghijk
19	<i>C. conica</i>	36	90	179±1	8	ghijkl
20	<i>C. conica</i>	38	90	183±1	7	fghij
21	<i>C. conica</i>	?	60	178±1	6	ghijkl
22	<i>C. conica</i>	?	60	173±2	7	ijkl
23	<i>C. foliosissima</i>	30	90	208±1	5	def
24	<i>C. morrowii</i>	38	90	200±1	5	defgh
25	var. <i>temnolepis</i>	38	90	208±2	8	defg
26	var. <i>albo-marginata</i>	30	80	198±2	8	defghi
	<i>C. dolichostachya</i>					
27	var. <i>glaberrima</i>	62-70	90	157±1	8	klmno
28	<i>C. laticeps</i>	58	90	132±1	9	opqr
	<i>C. oahuensis</i>					
29	ssp. <i>robusta</i>	62	80	106±1	8	stu
	<i>C. tristachya</i>					
30	ssp. <i>pocilliformis</i>	?	60	124±3	18	qrst

(to be continued)

Table 1. (Continued)

Strain number	Section and species	2n	No. of nuclei	Nuclear DNA content (4C)		
				Mean±SE	CV*	Significant at 5%
31	<i>C. pisiformis</i>	68	90	138±1	7	mnopq
32	ssp. <i>alterniflora</i>	60-84	60	131±1	7	pqrs
33	ssp. <i>duvaliana</i>	76	60	125±1	9	qrst
34	ssp. <i>stenostachya</i>	58	90	131±1	8	pqrs
35	var. <i>ikegamiana</i>	58	90	130±1	9	pqrs
	Extensae					
36	<i>C. bostrychostigma</i>	46	90	222±1	6	de
37	<i>C. capillacea</i>	60-66	80	101±1	13	tu
38	<i>C. ischnostachya</i>	62	60	110±2	10	rstu
39	<i>C. brownii</i>	72	90	87±1	15	uv
	Atratae					
40	<i>C. curviollis</i>	56	90	135±1	9	nopqr
	Graciles					
	<i>C. sendaica</i>					
41	ssp. <i>nakiri</i>	62	90	133±1	7	opqr
	Carex					
42	<i>C. shimidzensis</i>	68	60	126±2	9	qrst
43	<i>C. maximowiczii</i>	68-74	60	118±1	7	qrst
44	<i>C. kiotensis</i>	74	70	121±1	9	qrst
45	<i>C. thunbergii</i>	78-90	70	119±2	14	qrst
	Paludosae					
46	<i>C. pumila</i>	82	60	110±1	9	rstu
	Vigneae					
47	<i>C. omiana</i>	48-58	90	118±1	9	qrst
48	<i>C. kobomugi</i>	88	80	89±1	10	u
49	<i>C. pazii</i>	76	70	62±1	17	vw
	<i>C. nubigera</i>					
50	ssp. <i>albata</i>	112	60	61±1	14	w

* coefficient of variation (%)

** no difference between strains with the same alphabet.

Variance analysis revealed highly significant difference among strains. The result of test of significance among means of strains is shown in the rightmost column in Table 1. Nuclear DNA content of the highest species, *C. siderosticta* 4x (DNA content, 471) was 7.72 times that of the least DNA species, *C. nubigera* (61). Mello-Sampayo (1961) and Halkka (1964) reported 6 times variation in DNA content among species of *Luzula* with holocentric chromosomes. Tanaka (1940) frequently observed tetravalents at meiosis in species with the highest DNA value, *C. siderosticta* 4x and assumed that this plant

Fig. 1. Distribution of nuclear DNA content at four stages of mitosis in root tip of *C. ciliato-marginata* ($2n=12$).

was autotetraploid derived from *C. siderosticta* $2x$ which is distributed in Kanto district of Japan, but unfortunately was not available in the present study. Moreover, Hoshino (1981b) supported Tanaka's assumption by identifying duplication in the number of darkly stained segments in distal region of mitotic prophase chromosomes. DNA value in this tetraploid (471) was nearly twice of that of *C. ciliatomarginata* ($2n=12$, 225) which is a close relative to diploid strain of *C. siderosticta*. Another tetraploid, *C. humilis* (Tanaka 1984) had also indeed more DNA (269 or 292). Tanaka (1948) mentioned that this tetraploid species might be derived from *C. humilis* ssp. *nana*, which was not available in the present study.

Among diploid species, nuclear DNA content varied from 285 in *C. pachygyna* ($2n=12$) to 61 in *C. nubigera* ($2n=112$) or 62 in *C. paxii* ($2n=76$), indicating 4.66 times variation in diploid species of this genus. In genus *Carex*, a number of intraspecific aneuploids and the geographical cline in their distribution were reported (Hoshino 1980, 1981 a and b). Hoshino (1981b) pointed out the difference in total volume of metaphase chromosome among intraspecific aneuploids. There were, however, no significant differences in nuclear DNA content among four different kinds of aneuploid of *C. oxyandra* ($2n=18$ to 26) or five of *C. conica* ($2n=32$ to 38) (Table 1). Additionally, neither of five couples of strains, each belonging to a single species and having

Fig. 2. Relationship between chromosome number and nuclear DNA content in 47 strains of the genus *Carex*. Circle and square show diploid and tetraploid, respectively. Number in circle or square indicates strain number listed in Table 1.

the same chromosome number but collected at different sites (*C. pachygyna* ($2n=12$), *C. oxyandra* ($2n=20$), *C. humilis* ($2n=72$) and 2 pairs of *C. conica* ($2n=32, 36$)) revealed any differences in DNA content (Table 1). At least, it seems that these intraspecific aneuploids resulted from simple change of chromosome number caused by fragmentation or fusion, but without deficiency and/or duplication of chromosome segment. DNA content of three pairs of close relatives, *C. foliosissima* ($2n=30$, DNA value; 208) vs. *C. morrowii* (38, 200), *C. paxii* (76, 62) vs. *C. nubigera* (112, 61) and *C. pumila* (82, 110) vs. *C. kobomugi* (88, 89) was similar to each other, respectively.

Nuclear DNA contents of 47 strains were plotted against their chromosome number (Fig. 2). *C. pachygyna* with minimum number of chromosome ($2n=12$) had the highest DNA content, contrastingly the least amount of DNA was found in *C. paxii* ($2n=76$) and *C. nubigera* with the highest chromosome number ($2n=112$). Hoshino (1981b) concluded that total chromosome volume decreased slightly with the increase of chromosome number. At a glance of Fig. 2, it is clear that the increase in chromosome number is accompanied by the decrease in nuclear DNA content. Namely, highly significant negative correlation and regression between DNA content and chromosome number were confirmed in 45 diploid strains of the genus *Carex* used in the present study. Although Tanaka (1952) reported two peaks at $2n=38$ and 68 in relation to distribution of chromosome number of 203 species and few species between $2n=44$ to 50, belonging to subgenus *Eucarex*, nuclear DNA content varied continuously among species used in this study (Table 1, Fig. 2).

According to karyological study (Tanaka 1948; Hoshino 1981b), morpho-taxonomical study (Koyama 1957) and morphological study of development of inflorescence (Smith 1966), it is likely that *C. pachygyyna* ($2n=12$), *C. ciliat-marginata* (12) and *C. siderosticta* (12) belonging to section Laxiflorae are primitive, while that *C. paxii* (76) and *C. nubigera* (112) of section Vigneae are the advanced type. In the major course of speciation of genus *Carex*, it seems to be a logical conclusion that species with many small chromosomes would be derived from species having the small number of large chromosomes by means of fragmentation followed by slight reduction of DNA content (La Cour 1952, Castro *et al.* 1954). Hoshino (1981b), classified 49 taxa of *Carex* into two types of interphase nuclei, complex and simple chromocenter types, by double staining of leucobasic fuchsin and aceto-orcein. Complex chromocenter type was found in taxa below $2n=76$ and simple chromocenter type in those over $2n=58$. Number and/or size of chromocenter may be at least partially related to nuclear DNA content. The duplication of genetic materials was pointed out to have played an important role in evolution (Ohno 1970), but it should be noticed that the reduction of DNA content took place in the course of speciation of some kinds of plant, for example, genera *Lathyrus* (Rees and Hazarika 1969), *Vicia* (Chooi 1971), *Crepis* (Jones and Brown 1976), *Atriplex* (Belford and Thompson 1981) and subtrib Microseridinae (Price and Bachmann 1975).

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