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Genome Analysis and Variation of 4c DNA Content in the Subtribe Carinae

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In situ estimation of 4c DNA content have been made extensively at intergeneric, interspecific levels by different workers (Nagato *et al.* 1981, Dhillon and Miksche 1982, Sharma and Mukhopadhyay 1984, Mukherjee and Sharma 1986, Watson 1987, Das and Mallick 1989a, b, Chattopadhyay and Sharma 1990). The study of DNA content in different taxa is of much importance in different aspects of chromosomal research (Price 1976, Sharma 1983). Interspecific variations of nuclear DNA amount to a great extent depend on repetitive and non-repetitive sequences of the genome (Bannett *et al.* 1977, Rees and Narayan 1977). In order to ascertain precisely the importance of DNA in diversity of species in the subtribe Carinae under the family Umbelliferae, a full understanding of the interspecific variation, if any, is necessary. Cytological studies, so far, carried out earlier in the different species of Carinae showed 2n=14 chromosomes in *Cuminum cyminum* (Sharma and Ghosh 1954); 2n=18 in *Carum copticum* (Subramanian 1986); 2n=20 in *Carum carvi* (Crawford and Hartman 1972) and 2n=22 in *Petroselinum crispum* (Hiroe 1955). Interspecific chromosomal data are also very scanty in the above stated species. Nuclear DNA content have not yet been studied in these species too. The present study principally deals with the 4C DNA estimation in relation with their genomic behaviour in six species of Carinae.

Materials and methods

The seeds of different species of the subtribe Carinae namely, *Cuminum cyminum* var. RSI, *C. cyminum* var. RSII, *C. cyminum* var. UC19, *Carum copticum*, *Carum carvi* var. KBI 1434 and *Petroselinum crispum* var. Sutton 1810 were obtained through the courtesy of the Department of Genetics and Plant Breeding, Jobnar, India; Komarov Botanical Institute, USSR; Sutton Seed Nursery, Calcutta. Seeds were grown in experimental plot of the Department of Botany, University of Calcutta.

Fresh young root-tips were pretreated in saturated solution of paradichlorobenzene and aesculine mixture for three and half hours at 14°C followed by 1 : 3 propionic ethanol fixation. Chromosome staining was made in 2% propionic orcein after the cold hydrolysis in 5N HCl at 12°C for 7 min. Chromosome squash preparation was done in 45% propionic acid. Total chromosomal length and volumes were carried out by adding the length of all chromosomes in the karyotype and by applying the formula $\pi r^2 h$ respectively, where, r=radius of the chromosome and h=length of the chromosome.

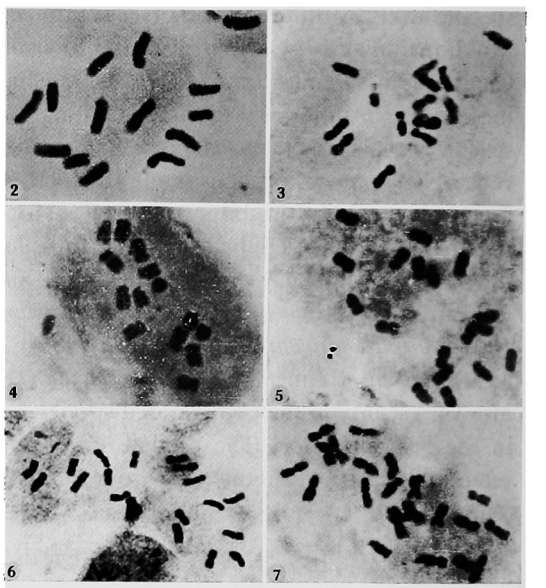
For Feulgen cytophotometric estimation of 4C DNA, ten fixed root-tips from each species were hydrolysed in 1N HCl for 12 min at 60°C; washed in distilled water; stained in Schiff's reagent for 2 hr at 14°C. Each root-tip squash was made in 45% acetic acid separately and ten scorings were taken from each slide. 4C DNA was estimated from metaphase chromosomes using Leitz Wetzler Aristophot with microspectrophotometer following the method of Sharma and Sharma (1980) applying monochromatic light of 550 nm. *In situ* DNA

values were obtained on the basis of optical density, then converted to picograms (pg) by using Van't Hof's (1965) 4C nuclear DNA value 67.1 pg for *Allium cepa* as standard. To investigate the significant differences of 4C DNA content, if any, in the subtribe Carinae ANOVA test (Sokal and Rohlf 1973) was performed followed by Duncan's multiple range tests (Harter 1960).

Observations

The somatic chromosome number ranged from 2n=14 in *Cuminum cyminum* to 2n=22 in *Petroselinum crispum* were observed in the different members of the subtribe Carinae. On the basis of the size of the chromosome and position of the constriction a number of chromosome

Fig. 1. Standard types of chromosomes present in different members of Carinae.



Figs. 2–7. Somatic metaphase plates of different members of Carinae. ×2139
2 *Cuminum cyminum* var. RSII, 2n=14; 3 *C. cyminum* var. UC19, 2n=14; 4 *C. cyminum* var. RSI, 2n=14; 5 *Carum copticum*, 2n=18; 6 *Carum carvi* var. KBI 1434, 2n=20; 7 *Petroselinum crispum* var. Sutton 1810, 2n=22.

types were common in all of them though they differed from one another in their minute details of the karyotype. Different types of chromosomes which were noted in the studied members of Carinae were expressed in Fig. 1. On the basis of the chromosomal types karyotype formula revealed clear differences in minute structural details of chromosomes in the subtribe (Table 1, Figs. 2–7). A remarkable feature was noted in *C. cyminum* var. RSI where A type of chromosomes were completely omitted. Moreover, *C. cyminum* var. UC19 showed absence of D type of chromosomes and presence of again B types of chromosomes. The chromosome length varied from 43.26 μm in *C. cyminum* to 71.18 μm in *P. crispum*. The highest chromosome volume noted so far in *C. carvi* (Table 1) whereas minimum was found in *C. cyminum*. A singificant interspecific variation was observed regarding chromosome length and volume. The TF% also varied subsequently. The karyotype showed nearly medium type chromosome to nearly submedian chromosomes in *P. crispum* and *C. cyminum* respectively. Gradual

Table 1. 4C DNA amount in different species of the subtribe Carinae along with the values of other cytological parameters

Species	Chromosome number (2n)	No. of secondary constricted chromosomes	Karyotype formula	Total chromosome length ±S.E. (μm)	Total chromosome volume ±S.E. (μm^3)	4C DNA amount ±S.E. (picograms)	Total F%	Average chromosome length (μm)	Average chromosome volume (μm^3)	DNA amount per chromosome (pg)
Cuminum cyminum var. RSII	14	2	$A_2+C_{10}+D_2$	45.84±0.29	23.00±0.25	18.252±0.012	23.60	3.28	1.64	1.30
C. cyminum var. UC19	14	4	$A_2+B_2+C_{10}$	43.26±0.03	20.28±0.21	17.250±0.006	25.59	3.09	1.45	1.23
C. cyminum var. RSI	14	2	$B_2+C_{10}+D_2$	43.26±0.02	33.58±0.01	17.995±0.002	26.19	3.09	2.40	1.29
Carum copticum	18	2	$A_2+C_{10}+D_6$	47.90±0.01	30.84±0.09	18.783±0.004	35.76	2.66	1.71	1.04
C. carvi var. KBI 1434	20	4	$A_4+C_{10}+D_6$	70.08±0.03	52.10±0.26	19.051±0.005	37.47	3.50	2.61	0.95
Petroselinum crispum var. Sutton 1810	22	4	$A_4+C_8+D_{10}$	71.18±0.05	29.35±0.52	17.225±0.002	38.02	3.24	1.33	0.78

shifting of the position of primary constrictions in accordance with their numerical changes in the somatic chromosomes were noted within the members (Table 1). The nuclear DNA amount differed significantly (Tables 2, 3) in the subtribe ranging from 17.225 pg in *P. crispum* to 19.051 pg in *C. carvi*. Applying ANOVA test and multiple comparisons test of means of 4C DNA values it was noted that the critical differences of the DNA values were significant at 5% level or 1% level except in between *P. crispum* and *C. cyminum* var. UC19. DNA values not show any definite correlation with total chromosome length or total chromosome volume of the species.

Table 2. Analysis of variance (ANOVA) of the 4C DNA amounts among the species of Carinae

Source of variation	D.F.	S.S.	M.S.	F
Between species	5	28.928	5.785	81.478[a]
Within species	54	3.837	0.071	
Total	59			

[a] Significant: $P<0.01$

Table 3. Multiple comparisons of the means of 4C DNA amounts for the species of Carinae. Values are difference between pairs of means (pg.)

C. cyminum var. UC19	*C. cyminum* var. RSI	*C. cyminum* var RSII	*C. copticum*	*C. carvi* var. KBI 1434	Species
0.025 NS	0.770**	1.027**	1.558**	1.826**	*P. crispum* var. Sutton 1810
	0.745**	1.002**	1.533**	1.801**	*C. cyminum* var. UC19
		0.257*	0.788**	1.056**	*C. cyminum* var. RSI
			0.531**	0.799**	*C. cyminum* var. RSII
				0.268*	*C. copticum*

* Indicates significance at 5% level
** Indicates significance at 1% level
NS indicates not significant: $P<0.05$
C.D. values at 5%=0.237
C.D. values at 1%=0.316

Discussion

The karyotype analysis in six members of the subtribe Carinae revealed some remarkable findings. The variation of chromosome numbers 2n=14, 18, 20 and 22 were noted in different taxa (Table 1, Figs. 2–7). But an overall homogeneity in the chromosome behaviour grouped them under the single subtribe. In all the studied species A, C and D types of chromosomes were common except two varieties of *C. cyminum* viz. UC19 and var. RSI. In addition, complete omission of D types and A types of chromosomes were noted in these species respectively. Furthermore, B type was also introduced in these species. Evidently, the B type of chromosome originated from A and D types during microevolution. The total form percentage (TF%) values ranged from nearly submedian i.e. 23.60 in *C. cyminum* var. RSI to nearly median i.e. 38.02 in *P. crispum* var. Sutton 1810 which maight be due to gradual alteration of chromosome types within the subtribe. However, detailed structural alteration of the chromosome morphology as well as variation of secondary constricted chromosomes in the species might be due to duplication of chromosomes or translocation between the chromosomes with or without secondary constrictions at a very early stage of evoltion. The total chromosome length varied markedly in between species though that was not reflected in the average chromosome length

(Table 1). Out of six species except *C. cyminum* var. RSI and *P. crispum* var. Sutton 1810 showed proportional increase of chromosome length with the increase of chromosome volume. Perhaps average chromosome volume showed remarkable variation viz., *C. cyminum* var. UC19 and var. RSI having the same chromsome number 2n=14 chromosomes showed average chromosome volume 1.45 μm^3 and 2.40 μm^3 respectively, though both species possessed chromosome length 3.09 μm per chromosome. These indicate the predetermined genetic control of chromosome coiling. Evidently differences in chromosome length or chromosome volume might be due to differential condensation and spiralization of chromosome arms. In addition, the specific genomic compaction of DNA threads along with nucleosomes or the additional gene sequences (Das and Mallick 1989b) with altered nonhistone proteins (Chattopadhyay and Sharma 1990) in the chromosomes played an important role for chromosomal architecture of the species.

Critical investigation on 4C DNA content showed significant variation within the subtribe Carinae (Tables 1, 2). The reports regarding DNA values though for the first time in these species but such type of interspecific variations were noticed earlier in several other species (Price *et al.* 1980, Mukherjee and Sharma 1986, Das and Mallick 1989a, b, 1991 and Chattopadhyay and Sharma 1990). The maximum DNA amount was noted 19.051 pg in *C. carvi* where the somatic chromosome number 2n=20 chromsomes. Whereas *P. crispum* showed 17.225 pg DNA having 2n=22 chromosomes as like as *C. cyminum* var. UC19 with 2n=14 chromosomes only. Average DNA content also varied markedly (Table 1). Duncan's multiple range test did not show any significant difference in between *C. cyminum* var. UC19 and *P. crispum*. Furthermore, *C. copticum* and *C. carvi* differed only in 5% CD level as like as *C. cyminum* var. RSII and *C. cyminum* var. RSI (Table 3). However, such type of fluctuation in DNA amount might be dependent on the amount of repetitive DNA sequences (Price *et al.* 1980, Mukhopadhyay and Sharma 1987, Das and Mallick 1991) in the genome. The variability of DNA amount often been attributed to loss or addition of high repeates which was adapted in micro- and macro-environment during speciation in evolution. Their stable DNA values confirm species identity within the subtribe.

Summary

A detailed karyotypic analysis and cytophotometric estimation of 4C DNA amount was carried out in six species of the subtribe Carinae of Umbelliferae. Intergeneric and interspecific chromosome number viz., 2n=14, 18, 20, 22 were varied in the subtribe level. Critical analysis of chromosome morphology revealed the structural alteration of chromosomes along with their changed DNA amount. Significant variation of DNA amount having numerical, gross or minor chromosomal alteration leads to the genetic drift in between the species of the subtribe suggesting the compromise between the structural and biochemical changes of the genome during macro- and micro-evolution.

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Literature

Bennett, M. D., Gustafson, J. P. and Smith, J. B. 1977. Variation in nuclear DNA in the genus *Secale*. Chromosoma **61**: 149–176.

Chattopadhyay, D. and Sharma, A. K. 1990. Chromosome studies and microspectrophotometric estimation of nuclear DNA in different strains of *Coriandrum sativum* L. Cytobios **64**: 43–51.

Crawford, D. J. and Hartman, R. L. 1972. Chromosome numbers and taxonomic notes for Rocky Mountain Umbelliferae. Am. J. Bot. **59**: 386–392.

Das, A. B. and Mallick, R. 1989a. Variation in karyotype and nuclear DNA content in different varieties of *Foeniculum vulgare* Mill. Cytologia **54**: 129–134.

— and — 1989b. Variation in 4C DNA content and chromosome characteristics in different varieties of *Coriandrum sativum* L. Cytologia **54**: 609–616.

— and — 1991. Correlation between genomic diversity and asiaticoside content in *Centella asiatica* (L.) Urban. Bot. Bull. Academia Sinica **32**: 1–8.

Dhillon, S. S. and Miksche, J. P. 1982. DNA content and heterochromatin variation in various tissues of peanut (*Arachis hypogea*). Am. J. Bot. **69**(2): 219–226.

Harter, H. L. 1960. Critical values for Duncan's multiple range test. Biometrics **16**: 671–685.

Hiroe, M. 1955. A cytotaxonomic comparison of Parsley and Celery. Bot. Mag. (Tokyo) **68**: 805–806.

Mukherjee, S. and Sharma, A. K. 1986. Intervarietal relationships between chromosome size, nuclear DNA amount and protein content in *Cajanus cajan* (L.) Mill sp. Perspective in Cytology and Genetics (eds. G. K. Manna and U. Sinha) **5**: 735–740.

Mukhopadhyay, S. and Sharma, A. K. 1987. Karyomorphological analysis of different species and varieties of *Calathea*, *Maranta* and *Stormanthe* of Marantaceae. Cytologia **52**: 821–831.

Nagato, Y., Yamamoto, K. and Yamashita, H. 1981. Variation of DNA content in Asian Rice. Jap. J. Genet. **56**: 483–493.

Price, H. J. 1976. Evolution of DNA content in higher plants. Bot. Rev. **42**: 27–52.

—, Bachman, K., Chambers, K. L. and Riggs, J. 1980. Detection of intraspecific variation in nuclear DNA content in *Microseris douglasii*. Bot. Gaz. **141**: 195–198.

Rees, H. and Narayan, R. K. J. 1977. Evolutionary DNA variation in *Lathyrus*. Chromosome Today **6**: 131–139.

Sharma, A. K. 1983. Additional genetic materials in chromosomes: Kew Chromosome Conf. II (eds) P. E. Brandham and M. D. Bennett (UK: George Allen and Unwin), pp. 35–42.

— and Ghosh, C. 1954. Cytogenetics of some of the Indian Umbellifers. Genetica **27**: 17–44.

— and Mukhopadhyay, S. 1984. Feulgen microspectrophotometric estimation of nuclear DNA of species and varieties of three different genera of Marantaceae. Proc. Indian Acad. Sci. (Plant Sci) **93**(3): 337–347.

— and Sharma, A. 1980. Chromosome Technique: Theory and Practice. 3rd edn. (London): Butterworths.

Sokal, P. R. and Rohlf, F. J. 1973. Introduction to Biostatistics. Freeman, San Francisco.

Subramanian, D. 1986. Cytotaxonomical studies in South Indian Apiaceae. Cytologia **51**: 479–488.

Van't Hof, J. 1965. J. 1965. Relationships between mitotic cycle duration, S period duration and the average rate of DNA synthesis in the root meristem cells of several plants. Expt. Cell Res. **39**: 48.

Watson, E. M. 1987. Nuclear DNA content in the Australian *Bulbine* (Liliaceae). Genome **29**(2): 225–234.