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Chromosomal DNA variation in *Cucumis*

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Summary. Variation in nuclear DNA amounts found in different species of *Cucumis* was surveyed. The DNA amounts varied from 1.373 to 2.483 pg in diploids and from 2.846 to 3.886 pg in tetraploids. DNA amount was not correlated with chromosome number and periodicity. Tetraploids were found to have double the quantity of nuclear DNA of diploids. A positive linear relationship was established between the nuclear DNA amounts and volume of chromosomes. The botanical varieties within a particular species do not differ significantly for 2C DNA amounts. A comparison of the distribution of DNA amounts among different chromosomes of haploid complement in different species revealed that the quantitative DNA changes associated with speciation affected all chromosomes. DNA changes were not however, of the same magnitude in all chromosomes of the complement. Speciation in *Cucumis* thus seems to have occurred through amplification or diminution of DNA proportionate to the size of chromosomes. The relationship between the basic numbers, x = 7 and x = 12, will have to be considered relative to the high DNA amount noticed in some species with x = 12.

Key words: *Cucumis* – Chromosomal DNA-Nuclear DNA – Speciation – DNA amplification – DNA diminution

Introduction

The genus *Cucumis* of the Cucurbitaceae family includes about 30 species with two basic chromosome numbers: x = 7 and x = 12. While the species with haploid chromosome number 7 are indigenous to India (De Candolle 1882), the species with the haploid chromosome number 12 are distributed naturally in Africa (Leppik 1966; Dane et al. 1980). Chemotaxonomical research has shown that the Asian and African species differ in their flavonol patterns and in the distribution of isozymes (Brown et al. 1969; Dane 1976). Cytologically, the genus *Cucumis*, like all other cucurbits, is a less studied genus. Even though reports on interspecific hybridisation are available (Deakin et al. 1970; Dane 1976; Kroon et al. 1979), the taxonomic relationship between the two basic chromosome numbers (x = 7 and x = 12) has yet to be established. Also, the pattern of divergence and evolution within the genus *Cucumis* is not clear. Such information is essential for successful gene transfer between cultivated and feral species. This investigation deals with the variation in DNA content among the chromosome complements of different *Cucumis* species and also with the variation in the distribution of DNA among different chromosomes within the complements.

Materials and methods

Twenty *Cucumis* species, including 16 diploids and four tetraploids, were studied for nuclear DNA variation (Table 1). The chromosome number of one species, *C. meeusi*, has not been reported earlier. It is now established to be a tetraploid with 2n = 48 chromosomes.

Chromosome volume

Actively growing root tips in distilled water were cold treated in an ice box for 24 h. They were subsequently fixed in acetic alcohol (1:3) for 48 h. Roots were then hydrolysed in 5 N HCl for 1 h at room temperature and stained in feulgen solution for 1 h. Roots were then softened with enzymes (2.5% pectinase + 2.5% cellulase) for 20 min and kept in distilled water for 20 min before squashing. The

Table 1. Nuclear DNA amount in *Cucumis* species. A = annual; P = perennial

Species	2n	Periodicity	Total nuclear DNA in picograms
Asiatic			
C. trigonus (syn. *C. callosus*)	14	A	1.590
C. sativus	14	A	1.777
C. sativus var. hardwickii	14	A	1.798
African			
C. melo var. 'agrestis'	24	A	2.483
C. melo var. 'utilissimus'	24	A	2.358
C. melo var. 'momordica'	24	A	2.291
C. metuliferus	24	A	2.391
C. anguria var. 'longipes'	24	A	1.587
C. africanus	24	A	1.782
C. ficifolius	24	P	1.373
C. meeusi	48	P	3.203
C. dinteri	24	A	2.167
C. dipsaceus	24	A	2.448
C. figarei	48	P	3.886
C. zeyheri	24	P	1.682
C. zeyheri	48	P	2.846
C. sagitattus	24	P	1.571
C. prophetarum	24	A	1.656
C. humifructus	24	A	2.455
C. heptadactylus	48	A	2.225

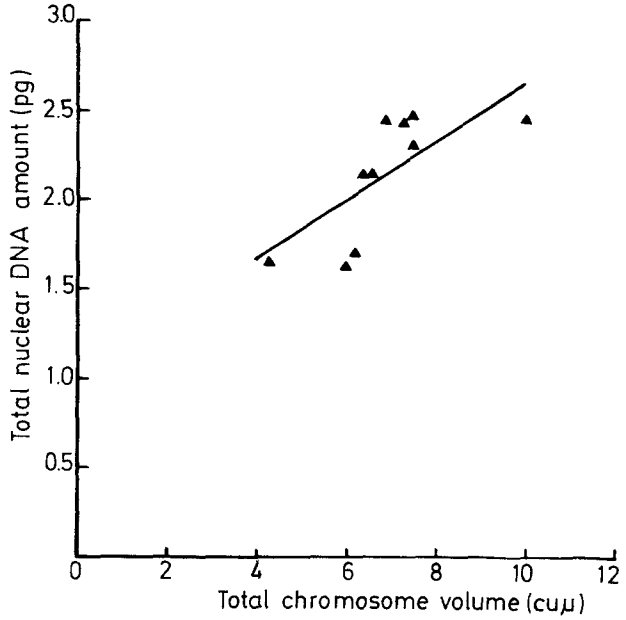

Fig. 1. Total DNA amounts (pg) of 10 *Cucumis* species ($n = 12$) plotted against the total chromosome volumes (cuμ) at mitotic metaphase

chromosomes in each cell were measured individually for length and five chromatids in each cell were taken at random to obtain the mean chromatid width. Mean chromosome volumes were computed from five cells in each species. The volume of each chromosome was calculated from total chromatid length (2 × chromosome length) and average chromatid width, assuming the chromatids to be cylindrical.

Total nuclear DNA

Total amount of DNA was determined using Feulgen microphotometry. Root from germinating seeds were fixed and feulgen stained using the method first suggested by McLeish and Sunderland (1961) and later modified by Teoh and Rees (1976). The DNA measurements were made on a Vickers M85 microdensitometer. Fifteen 2C nuclei were measured in each of the three replicates in each species. The estimated DNA values were corrected to picograms using *Allium cepa* (2 C DNA = 33.5 pg) as a standard.

DNA amount in individual chromosomes

A clear linear relationship was observed in the genus *Cucumis* between chromosome volume and DNA amounts (Fig. 1). Knowing the total nuclear DNA amount and the volume of individual chromosomes, the DNA amount in each chromosome was estimated as a proportion of total DNA (Raina and Rees 1983).

Results and discussion

In Table 1 are the chromosome numbers, periodicity and 2C DNA amounts of different *Cucumis* species. From these data, the following inferences can be drawn: 1. the species fall into two groups with respect to basic chromosome numbers; x = 7 and x = 12; 2. nuclear DNA amount among the diploid species varies from 1.373 to 2.483 pg; 3. tetraploids show almost double the quantity of nuclear DNA amount as diploids with a mean value of 3.04 pg; 4. nuclear DNA amounts are not correlated with chromosome number. Hence the change in DNA amounts was achieved independently of change in number; 5. there is no obvious difference in nuclear DNA amount between annual and perennial species.

The volume of individual chromosomes and the total chromosome volume of the haploid complement in each species are given in Table 2. Total chromosome volume was found to have a positive linear relationship with total nuclear DNA amounts (Fig. 1). The volume of individual chromosomes was also positively correlated with DNA content of individual chromosomes. In other words, one can estimate directly the DNA content in individual chromosomes (Table 3) provided the volume and the total DNA amount of a particular species are known (Raina and Rees (1983).

The botanical varieties, 'agrestis' (wild melon), 'utilissimus' (long melon), 'momordica' (Phut or Snap melon) of *Cucumis melo* do not differ significantly in nuclear DNA amount. Similarly, *C. sativus* var. 'hardwickii' recently classified as a botanical variety of *C. sativus,* has a similar DNA concentration as *C. sativus.* Even though these botanical varieties differ morphologically, they are grouped under a single species,

Table 2. Volume of chromosomes in different *Cucumis* species

Species	Chromosome volume (Cuμ)												Total volume (Cuμ)
	1	2	3	4	5	6	7	8	9	10	11	12	
Asiatic													
C. sativus	1.93	1.68	1.39	1.17	1.08	1.03	0.83						9.11
C. trigonus	1.08	0.95	0.85	0.85	0.77	0.61	0.59						5.70
African													
C. melo var. 'agrestis'	1.00	0.69	0.67	0.63	0.62	0.59	0.58	0.57	0.53	0.52	0.49	0.41	7.30
C. melo var. 'utilissimus'	0.74	0.64	0.63	0.57	0.56	0.56	0.53	0.51	0.48	0.46	0.38	0.37	6.43
C. melo var. 'momordica'	0.94	0.70	0.69	0.69	0.67	0.67	0.67	0.58	0.54	0.52	0.49	0.35	7.51
C. metuliferus	1.14	1.09	1.05	0.99	0.92	0.89	0.85	0.79	0.72	0.64	0.56	0.52	10.16
C. dipsaceus	1.00	0.81	0.69	0.65	0.63	0.60	0.57	0.53	0.47	0.45	0.42	0.39	7.21
C. dinteri	0.84	0.73	0.62	0.58	0.54	0.54	0.49	0.47	0.45	0.43	0.39	0.36	6.44
C. humifructus	0.86	0.73	0.66	0.61	0.60	0.60	0.57	0.54	0.51	0.45	0.43	0.33	6.89
C. prophetarum	0.51	0.47	0.45	0.42	0.38	0.36	0.34	0.31	0.28	0.26	0.25	0.24	4.27
C. anguria	0.68	0.62	0.58	0.55	0.53	0.52	0.50	0.47	0.46	0.43	0.36	0.35	6.05
C. zeyheri	0.73	0.73	0.68	0.57	0.55	0.53	0.50	0.47	0.43	0.39	0.35	0.27	6.20

Table 3. Mean DNA amounts (in picograms) of individual metaphase chromosomes in 12 *Cucumis* species

Species	2n	DNA in chromosomes (pg)											
		1	2	3	4	5	6	7	8	9	10	11	12
Asiatic													
C. sativus	14	0.377	0.328	0.272	0.229	0.211	0.201	0.162					
C. trigonus	14	0.301	0.265	0.237	0.237	0.215	0.170	0.165					
Mean		0.339	0.297	0.255	0.233	0.213	0.185	0.164					
African													
C. melo var. 'agrestis'	24	0.340	0.235	0.228	0.214	0.211	0.201	0.197	0.194	0.180	0.177	0.167	0.140
C. melo var. 'utilissimus'	24	0.248	0.215	0.211	0.191	0.188	0.188	0.178	0.171	0.161	0.154	0.127	0.124
C. melo var. 'momordica'	24	0.287	0.214	0.211	0.211	0.204	0.204	0.203	0.177	0.165	0.159	0.150	0.106
C. metuliferus	24	0.268	0.257	0.247	0.233	0.217	0.209	0.200	0.186	0.169	0.151	0.132	0.122
C. dipsaceus	24	0.340	0.275	0.234	0.221	0.214	0.204	0.194	0.180	0.159	0.153	0.142	0.132
C. dinteri	24	0.283	0.246	0.208	0.195	0.182	0.182	0.165	0.158	0.151	0.145	0.131	0.121
C. humifructus	24	0.307	0.260	0.235	0.218	0.214	0.214	0.203	0.193	0.182	0.161	0.153	0.117
C. prophetarum	24	0.198	0.182	0.175	0.163	0.147	0.140	0.132	0.120	0.109	0.101	0.096	0.093
C. anguria	24	0.178	0.163	0.152	0.144	0.139	0.136	0.131	0.123	0.121	0.113	0.094	0.093
C. zeyheri	24	0.198	0.198	0.185	0.155	0.149	0.144	0.135	0.128	0.117	0.106	0.094	0.073
Mean		0.267	0.224	0.209	0.194	0.186	0.182	0.174	0.163	0.151	0.142	0.129	0.111

mainly because they hybridise fairly easily between them. The nuclear DNA measurements in these botanical varieties support their varietal nomenclature.

The genus *Cucumis* seems to be quite distinct from the genera *Lathyrus Nicotiana, Clarkia, Allium* and *Vicia* (Narayan 1982; Raina and Rees 1983). The variation in nuclear DNA amounts among different species of *Cucumis* is not as large as in other genera, even though there are effective barriers to crossing between species (Deakin et al. 1971; Dane 1976). The DNA distribution among diploid species is limited to approximately 1 picogram. Therefore, the interspecific divergence in *Cucumis* would have resulted not only from changes in chromosome number and nuclear DNA amount, but also from reproductive isolation, gene mutation, karyotype rearrangements or rapid change subsequent to random genetic drift. The latter factors also had major role in the speciation in *Cucumis*.

The DNA amounts in individual chromosomes of haploid complement of the different species are given in the descending order of amounts in Table 3. In Fig. 2 and Fig. 3 the DNA amounts of individual chromosomes are plotted against the mean of chromosomes for $x=7$ and $x=12$ species, respectively. The linear regression analysis shows that the regression slopes for different species are strikingly dissimilar and the slopes diverge significantly. The joint regression analysis has also revealed a significant difference between the

regression lines for different species. Since the heterogeneity of means and regressions are significant, it can be inferred that the quantitative DNA changes associated with speciation have affected all chromosomes within the complements (Table 4a, b). If we assume that the rank in order of size of chromosomes corresponds to rank in order of homology or rather homeology of chromosomes (Seal and Rees 1982) then the joint regression analysis (heterogeneity of regression) shows that the DNA changes were not of the same magnitude in all the chromosomes of the complement.

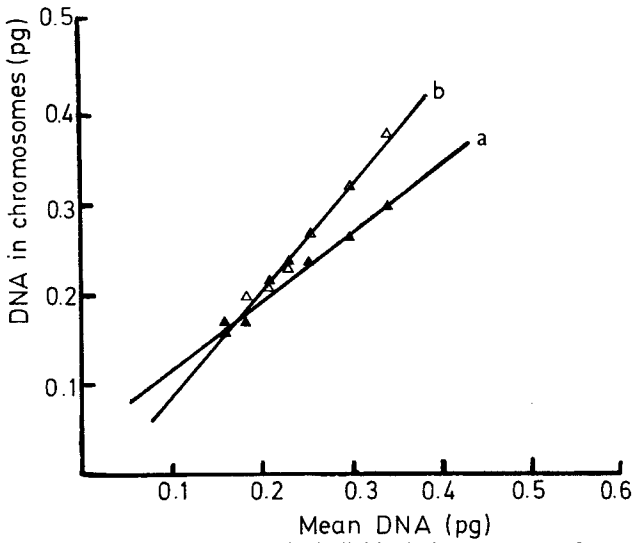

Fig. 2. DNA amounts (pg) in individual chromosomes of two *Cucumis* species with $n=7$ plotted against the mean amounts of both species: *a* (▲——▲) = *C. trigonus*; *b* (△——△) = *C. sativus*

For analysing the situation more clearly, two species differing in DNA amounts were compared (Fig. 4). In this, a low DNA species (*C. prophetarum* – a) is taken as a basic species from which the high DNA species (*C. metuliferus* – d) is assumed to have evolved. If the excess DNA in the high DNA species is equally distributed to all chromosomes of the low DNA species, the expected regression slope (b) will be parallel to a. But if the increased DNA is distributed according to the size of chromosomes, then the regression slope will diverge (c). It was found that the DNA distribution within the chromosomes of high DNA species (*C. metuliferus*) follows a pattern similar to c ($P > 0.20$). Hence, speciation in the genus *Cucumis* among both the x = 7 chromosome species and x = 12 chromosome species was not achieved by equal DNA increments to all chromosomes of the complement, as reported in *Lathyrus* (Narayan 1982) and *Vicia* (Raina and Rees 1983). In *Cucumis,* the larger chromosomes in the high

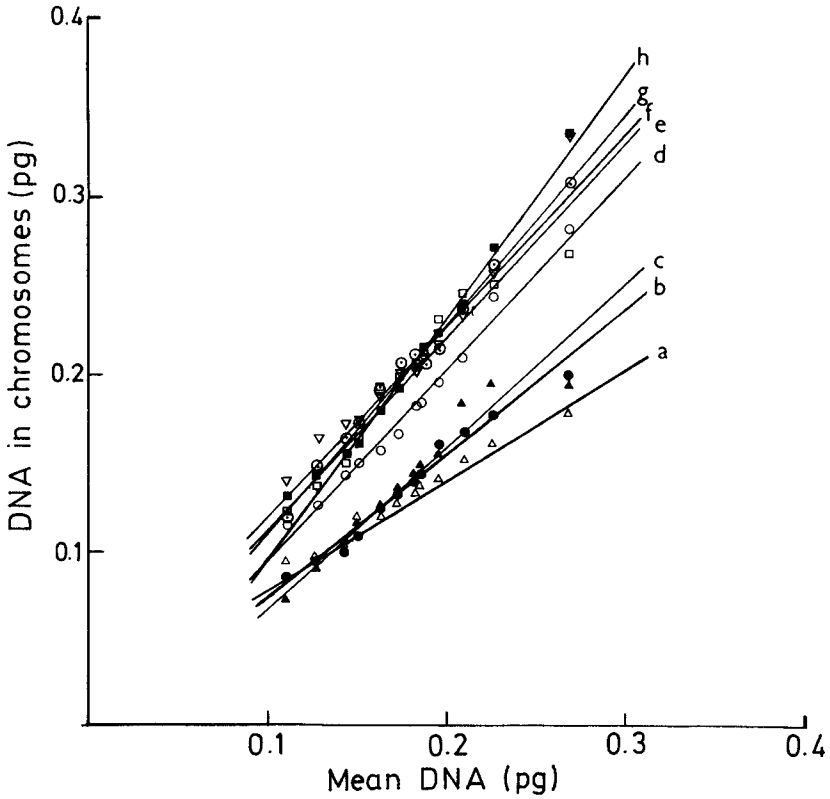

Fig. 3. DNA amounts (pg) in individual chromosomes of eight *Cucumis* species with $n=12$ plotted against the mean amounts for the eight species: *a* (△——△) = *C. anguria*; *b* (●——●) = *C. prophetarum*; *c* (▲——▲) = *C. zeyheri*; *d* (○——○) = *C. dinteri*; *e* (□——□) = *C. metuliferus*; *f* (▽——▽) = *C. melo* var. 'agrestis'; g (○——⊙) = *C. humifructus*; *h* (■——■) = *C. dipsaceus*

Table 4. **a** Joint regression analysis of species with $n=7$ chromosomes; **b** Joint regression analysis for species with $n=12$ chromosomes

a	Degrees of freedom	Mean squares	Variance ratio
Heterogeneity – regression	1	2.261×10^{-3}	19.325*
Heterogeneity – means	1	2.579×10^{-3}	22.043*
Error	10	0.117×10^{-3}	
b	Degrees of freedom	Mean squares	Variance ratio
Heterogeneity – regression	9	0.967×10^{-3}	9.036*
Heterogeneity – means	9	10.576×10^{-3}	98.841*
Error	100	0.107×10^{-3}	

* Significant at $P=0.01$

Table 5. Analysis of variance of DNA amounts for diploid species of *Cucumis*

	Degrees of freedom	Mean squares	Variance ratio
Total	47		
Species	15	0.4875	161.441*
Replication	2	0.0003	0.1149
Error	30	0.0030	

* Significant at $P=0.01$; SE (m) = 0.0320; CD (1%) = 0.1970

DNA species have acquired more DNA than the smaller chromosomes. Brandham (1983) has observed a disproportionate amplification of DNA sequences in the larger chromosomes of complements in the genus *Aloe*.

Attempts are being made in the genus *Cucumis* to transfer desirable genes from feral or semiferal species to cultivated species (*Cucumis sativus* – cucumber and *C. melo* – muskmelon) and also between cultivated species. However, there is a shortage of information on the evolution, cytology, systematics and taxonomy of the genus. The relationship between the two basic chromosome numbers x = 7 and x = 12 is also not clear. Two different hypotheses have been put forward to explain the relationship between the two basic chromosome numbers. The fragmentation hypothesis suggests that *C. melo* (x = 12) has derived from *C. sativus* (x = 7) or a closely related species by fragmentation of particular chromosomes followed by de novo regeneration of centromeres (Bhaduri and Bose 1947; Ayyangar 1967). The fusion hypothesis on the other hand says that the basic number x = 7 might have arisen from x = 12 possibly by unequal translocation or fusion of non-homologous chromosomes (Trivedi and Roy 1970).

The survey of nuclear DNA amounts and of distribution pattern of DNA in chromosomes shows that amplification or diminution of DNA is also involved in the evolution of these species. If we have to assume the relationship between x = 7 and x = 12, we have to take into account the high nuclear DNA amounts observed in certain species with x = 12 (Table 5). On the contrary, if x = 7 is derived from x = 12, one has to assume the diminution of DNA. The interesting fact remains that while the 14 chromosome species are Asian in origin,

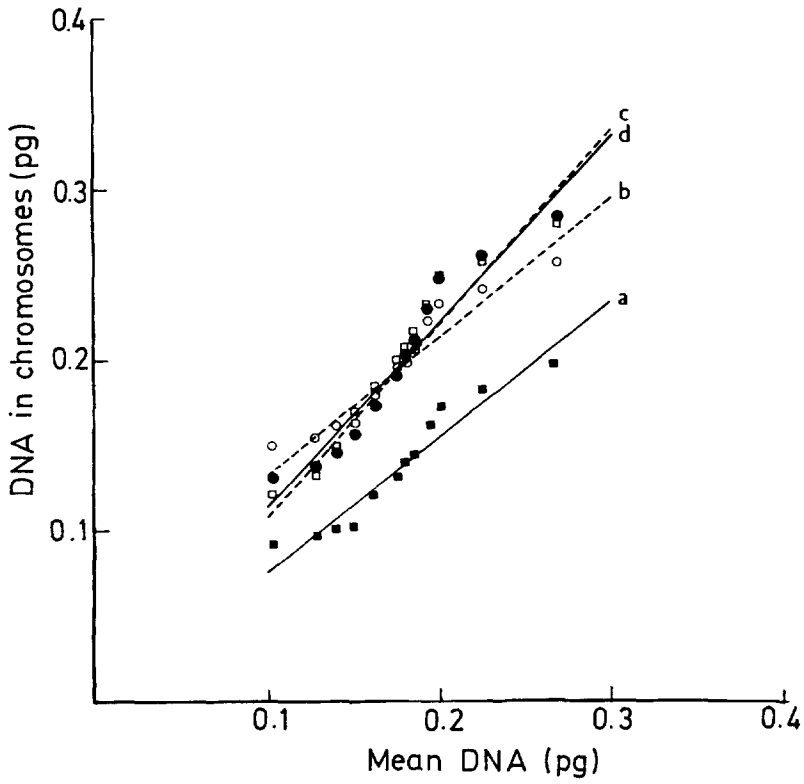

Fig. 4. Distribution of DNA amounts in two species compared with two expected distributions. *a* (■——■) = distribution of DNA amounts in chromosomes of *C. prophetarum* (a low DNA species); *b* (○---○) = expected distribution of DNA amounts in chromosomes assuming equal increment for each chromosome relative to *a*; *c* (●---●) = expected distribution of DNA amounts in chromosomes assuming the increase is proportional to DNA amounts in *a*; *d* (□——□) = distribution of DNA amount in chromosomes of *C. metuliferus* (a high DNA species)

the 24 chromosome species are believed to be native of Africa. How these two groups of species with different chromosome numbers are related, is a problem to be solved from the ecological and phylogenetical point of view. A detailed quantitative estimation of different nuclear components (satellite sequence, middle repetitive DNA and non repetitive DNA) would be useful for assessing the taxonomical relationship between the Asiatic and African species. Also, the distribution of these sequences as revealed by in situ hybridisation experiments would give valuable information regarding the chromosome evolution in these species.

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