RELATIVE AMOUNTS OF NUCLEAR DNA IN POPULATIONS OF *COSTUS SPECIOSUS* (KOEN.) SM.

A. K. SHARMA AND SUCHITRA CHATTOPADHYAY
Centre of Advanced Study in Cell and Chromosome Research, Department of Botany, University of Calcutta, Calcutta 700 019, India.

ABSTRACT

Cytological and cytochemical investigations, involving determination of somatic chromosome number, karyotype formula, total chromosome length, volume and 4C nuclear DNA amount, were carried out on 20 different populations of *Costus speciosus* (Koen.) Sm. The 4C nuclear DNA amount was estimated through Feulgen microspectrophotometry and expressed in arbitrary units of relative absorbances. Variations between the 4C DNA amounts were quite distinct within the tetraploid populations but were significantly less between the diploid and triploid cytotypes.

INTRODUCTION

THE quantitation of DNA in different species has become a fascinating subject of research in recent years. The role of evolution of DNA content in higher plants has been extensively reviewed[1]. Studies have also been carried out on the correlation between nuclear volume, cell volume and the content of DNA[2-4] Variation in DNA content during organogenesis, attributed to the amplification of DNA during differentiation, has also been recorded[5-7] However, such variations have been associated with differences in protein content as well[8,9].

Extensive studies on the intra- and interspecific DNA content have been carried out by different workers[10-16]. It has also been recorded that variations between genotypically related taxa may involve supplementary DNA with specific non-repetitive to repetitive fractions[14,16-20]. The association of such variations with heterochromatin content has been studied[21]. In majority of the cases, however, the data have been scored principally through *in situ* estimation of DNA followed by spectrophotometric estimation of sequence pattern. The importance of such studies, at least as specific parameters for identification of genotypes, is clearly established.

Costus speciosus (Koen.) Sm., one of the diosgenin yielding species, occurs widely in different parts of India and populations are distributed at diploid, triploid and tetraploid levels. The genotypic variations amongst the populations have been established through karyotype analysis aided by refined methods. No data is yet available on the amount of DNA in different genotypes of *C. speciosus*. In view of the wide spectrum of genotypes recorded in the species, and also in view of the fact that such data may be utilised as characteristic parameters, the present investigation was undertaken. It deals with *in situ* estimation of the relative amount of DNA in 20 different populations of *C. speciosus*, including diploid, triploid and tetraploid cytotypes.

For *in situ* quantitation of the relative DNA amounts, 20 different populations of *Costus speciosus* (Koen.) Sm., collected from different geographical regions of India and Nepal, were analysed following the usual Feulgen staining procedure. The quantities were measured in Reichert Zetopan Microspectrophotometer through single wave length (550 nm) method[22] from 4C nuclei at metaphase and the amounts were expressed in arbitrary units of relative absorbances. Fixation of root tips in neutral formalin (40%) under the same experimental conditions was also carried out for confirmation.

Cytological studies, dealing mainly with the karyotype analysis and determination of

TABLE 1

Relative amounts of nuclear DNA in different populations of Costus speciosus (Koen.) Sm. along with the values of geographical and other cytological parameters.

Population number	Altitude (in metre)	Somatic chromosome number (2n)	Karyotype formula	Total chromosome length (μm)	Total chromosome some volume (Cu μm)	Nuclear DNA amount (in arbitrary units)
I	366	$2n = 36$	$A_2B_4C_2D_{28}$	95.44 ± 0.08	82.43 ± 0.10	0.1410 ± 0.0022
II	128	$2n = 36$	$A_2B_4C_2D_{28}$	89.46 ± 0.07	105.58 ± 0.17	0.1683 ± 0.0024
III	111	$2n = 36$	$A_2B_4C_2D_{28}$	79.04 ± 0.06	102.38 ± 0.14	0.1540 ± 0.0037
IV	111	$2n = 36$	$A_2B_4C_2D_{28}$	99.80 ± 0.08	110.59 ± 0.12	0.1610 ± 0.0038
V	5.5	$2n = 36$	$A_2B_4C_4D_{26}$	109.22 ± 0.07	122.30 ± 0.15	0.1123 ± 0.0025
VI	98.0	$2n = 36$	$A_2B_4C_2D_{28}$	96.62 ± 0.08	108.48 ± 0.13	0.1673 ± 0.0031
VII	152.4	$2n = 36$	$B_6C_2D_{28}$	101.08 ± 0.09	107.54 ± 0.12	0.1598 ± 0.0025
VIII	134.0	$2n = 36$	$A_2B_4C_2D_{28}$	104.40 ± 0.09	97.93 ± 0.10	0.1473 ± 0.0037
IX	152	$2n = 36$	$A_2B_2C_2D_{30}$	107.80 ± 0.10	108.70 ± 0.37	0.1020 ± 0.0012
X	980	$2n = 18$	$B_2C_2D_{14}$	45.18 ± 0.06	78.84 ± 0.30	0.0880 ± 0.0032
XI	1011	$2n = 18$	$A_2B_2D_{14}$	57.92 ± 0.17	78.31 ± 0.30	0.0763 ± 0.0021
XII	1011	$2n = 27$	$A_1B_3C_3D_{4(3)+4(2)}$	69.54 ± 0.09	73.70 ± 0.23	0.1140 ± 0.0022
XIII	1011	$2n = 36$	$A_2B_2C_4D_{28}$	112.90 ± 0.10	138.63 ± 0.17	0.1228 ± 0.0026
XIV	32	$2n = 27$	$A_1B_6D_{4(3)+4(2)}$	81.83 ± 0.11	97.92 ± 0.22	0.1135 ± 0.0036
XV	1749	$2n = 18$	$A_2B_2D_{14}$	44.80 ± 0.08	78.34 ± 0.33	0.0773 ± 0.0019
XVI	1225	$2n = 18$	$A_2B_2D_{14}$	58.76 ± 0.14	65.96 ± 0.23	0.0830 ± 0.0024
XVII	913	$2n = 36$	$A_2B_4C_2D_{28}$	103.64 ± 0.10	113.88 ± 0.17	0.1565 ± 0.0026
XVIII	1500	$2n = 36$	$A_2B_2C_4D_{28}$	101.64 ± 0.11	74.16 ± 0.14	0.1228 ± 0.0023
XIX	409	$2n = 36$	$A_2B_6C_2D_{26}$	112.94 ± 0.10	123.05 ± 0.17	0.1235 ± 0.0027
XX	1402.08	$2n = 27$	$A_1B_3C_2D_{3(3)+6(2)}$	81.24 ± 0.11	72.80 ± 0.13	0.1067 ± 0.0016

chromosome length and volume, were carried out following the normal acetic-orcein schedule[22].

RESULTS

The somatic chromosomes of 20 different populations of *Costus speciosus* (Koen.) Sm. were analysed in detail. The observed intra-specific chromosomal races were diploids ($2n = 2x = 18$), triploids ($2n = 3x = 27$) and tetraploids ($2n = 4x = 36$) of which the tetraploids predominated. The karyotype formulae of the different populations revealed several chromosome types (figure 1) and also structural and numerical alterations of the nucleolar chromosome both at the intra- and inter-cytotypic levels (table 1).

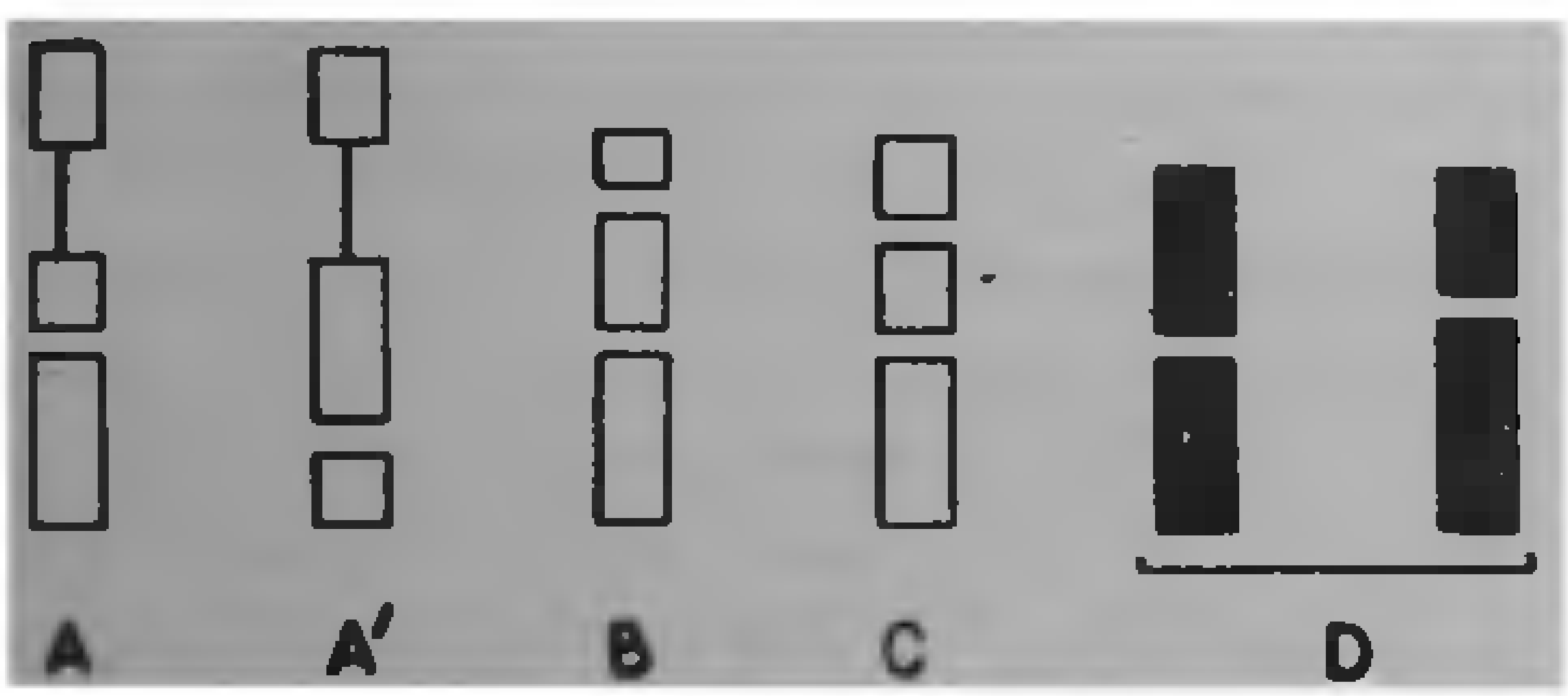

Figure 1. Different chromosome types common to twenty different populations of *Costus speciosus* (Koen) Sm.

The total chromosome lengths of the triploid and tetraploid populations were not the exact multiples of the diploid set. However, they did not differ significantly between cytotypes (table 1).

So far as the total chromosome volume was concerned, a significant variation was noted within and between the different cytotypes (table 1 and figure 3). Amongst the diploids, the volume was found to range from 65.96 to 78.84 cu μm. In the triploids and tetraploids on the other hand, the ranges observed were from 72.8 to 97.92 cu μm and from 74.16 to 138.63 cu μm respectively (table 1 and figure 3).

In the present investigation the amount of 4C nuclear DNA was estimated and expressed in arbitrary units of relative absorbances in the 20 different populations of *Costus speciosus* (Koen.) Sm.

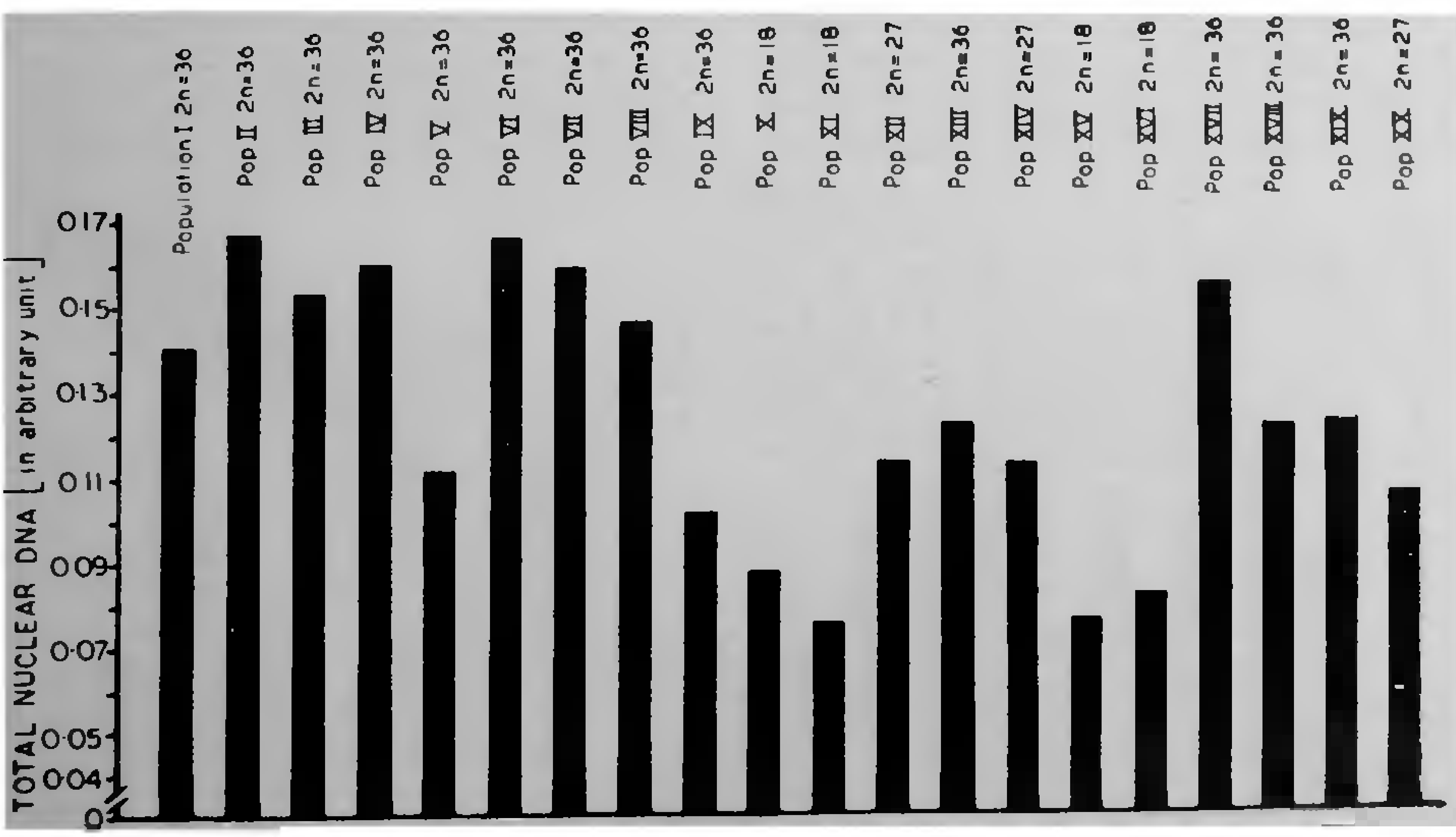

Figure 2. Histogram plate of nuclear DNA amount in twenty different populations of *Costus speciosus* (Koen) Sm.

Variations between the 4C DNA amounts were significantly less between the diploid and triploid cytotypes but was quite distinct amongst the tetraploids (table 1 and figure 2). In the four diploid populations, despite the minute variations in their total chromosome length and volume, the amount of DNA remained more or less the same, ranging from 0.0763 to 0.088 (table 1 and figure 2, 3).

Amongst the three triploids, two populations from Mungpoo and Burdwan contained almost the same amount of DNA, though their total chromosome length and volume differed considerably (table 1 and figures 2, 3). In the remaining triploid population from Nepal, the 4C nuclear DNA amount, being 0.1067, revealed slight variation from the other two.

In the tetraploids, on the other hand, irrespective of the similarities and dissimilarities in the total chromosome length and volume, the amounts of DNA varied significantly, ranging from 0.102 to 0.1683 (table 1 and figures 2, 3).

DISCUSSION

Out of the total of 20 populations studied, 13 are tetraploids, 3 triploids and 4 diploids. In the case of tetraploids, several populations could be collected because of their wide occurrence as compared to others. While analysing the difference in amounts of DNA, calculated in arbitrary units, both chromosome length and volume have been included in table 1. So far as this genus is concerned, differential spiralisation and consequent condensation, as well as the association of protein, may be regarded to contribute to a significant extent to the length and volume.

Nuclear DNA in diploids, triploids and tetraploids

A comparative analysis of the amount of nuclear DNA in diploids, triploids and tetraploids clearly indicates the absence of clear multiplicity. In the diploid population X, the value is 0.088 whereas in triploid and tetraploid populations XX and V, the values are 0.1067 and 0.1123 respectively. In addition to these cases, there are striking differences in other populations showing no clear relationship with direct multiplication. In certain cases, the triploids and tetraploids, *viz.* populations XX and IX, show more or less similar values, *i.e.*, 0.1067 and 0.102 respectively. This has a slightly lower value in the tetraploid. It is true that there has been differences even in populations with the same chromosome number, which may indicate that triploidy and tetraploidy might have originated from different populations altogether. But even then, the absence of any marked duplication of chromo-

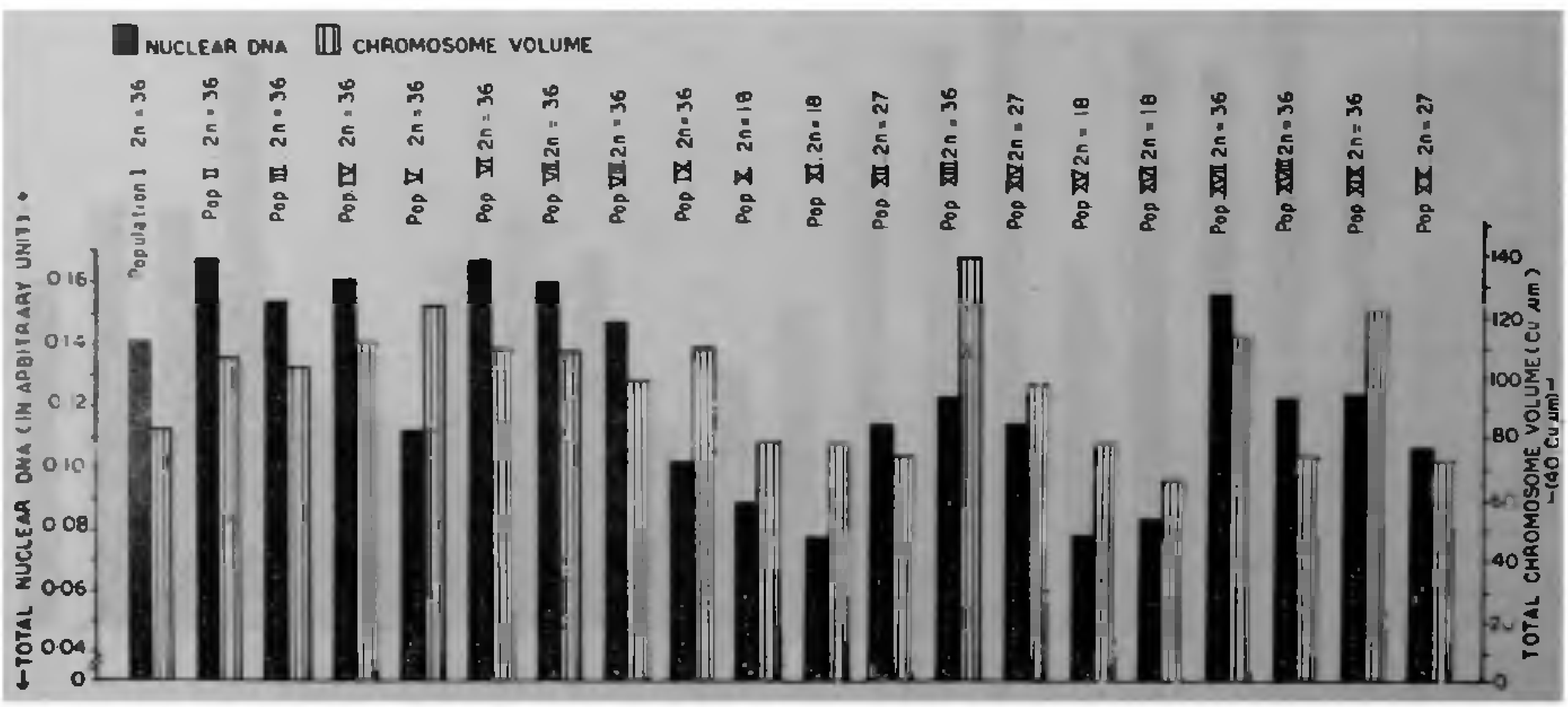

Figure 3. Comparative histogram of nuclear DNA amount and total chromosome volume in twenty different populations of *Costus speciosus* (Koen) Sm.

somes, though belonging to different populations, needs to be accounted for.

All these facts clearly suggest that there have been large scale structural changes in the chromosomes, associated with polyploidy, which possibly has also involved diminution in the amount of DNA. There are ample cases on record where there has been diminution of DNA in evolution[1].

The extent to which such changes in DNA values involve repeated sequences needs to be explored. Such repeated sequences may include heterochromatin segments as well. In *Allium stracheyii*, the elimination of heterochromatic B chromosomes has been recorded associated with induction of polyploidy[23]. It would be worthwhile to record a detailed analysis of the repetitive DNA sequences in diploid, triploid and tetraploid genotypes of *Costus speciosus* (Koen.) Sm. as well. The presence of supplementary DNA with distinct non-repetitive and repetitive sequences, responsible for the differences in the amount of DNA between related species, has been suggested[19].

Variation in the nuclear DNA with constant chromosome number

Over and above the curious differences in the amounts of DNA between diploids, triploids and tetraploids, population-wise analysis has also been made at the intra-diploid, intra-triploid and intra-tetraploid levels.

At the diploid level, of which only 4 populations have been studied, the range is between 0.0763 in population XI to 0.088 in population X. It is not very marked. On the other hand, the total chromosome lengths in these two populations are 45 and 58 μm respectively. On the basis of such heavy difference in the total chromosome length but with little divergence in the amount of DNA, it may be reiterated once more that in this genus the amount of DNA is not necessarily reflected in the length of the chromosome. The differential condensation may be one of the factors associated with this process, which no doubt, is also under genetic control.

As far as the triploids are concerned, the range of difference is between 0.1067 in population XX to 0.114 in population XII— not a very marked one. The explanation suggested for diploids holds good for triploids as well.

On the other hand, in the tetraploids, of which 13 populations have been studied, the range is between 0.102 in population IX to 0.1683 in population II. The difference is rather wide. Intermediate values have also been recorded in other populations.

Chromosome length, karyotype and the nuclear DNA amount

The total chromosome length reflecting the chromosome size, ranges between 89.46 μm in population II to 107.80 μm in population IX, the latter containing the least amount of DNA. The population IX represents an example where the amount of DNA is the least, whereas the total chromosome length is highest. In this genus, this is possibly the best example where chromosome size can not be taken as an index of the amount of DNA. As a large number of individuals were studied for each population, population-wise constancy can be safely claimed. It is remarkable that this population represents a striking difference in the karyotype, specially in the 'A' chromosome where the middle arm is longer as compared to the other two arms, a feature quite different from the rest of the 'A' chromosomes. The correlation of this karyotype with a very low DNA amount is worth attention.

At the tetraploid level, the amount of DNA may be utilised as a parameter for identification, at least for certain populations. Leaving aside the chromosome size, there have been minute changes in the karyotypes of the different populations as well. The importance of structural alteration is clear.

Nuclear DNA amount in relation to the ecological variation

The two extremes in the amount of DNA represented in populations II and IX are also associated with their wide ecological differences. The former was collected from the dry belt of the United Provinces whereas the latter from the Terai region of the Himalayas (Naxalbari). This is a very noteworthy correlation. The mechanism through which this heavy difference in the amount of DNA is concerned with adaptive features in their ecology, is yet to be precisely investigated. A study of the repeated sequences, on which investigation is in progress, may throw significant light in this direction.

The absence of any marked difference in the amount of DNA in the diploid and triploid populations is a feature quite in contrast to the tetraploids. It is true that in the case of tetraploids, a large number of populations could be obtained because of its wide occurrence whereas in the diploids and triploids the number of populations were few in view of their limited distribution. A search into the unexplored regions of the Himalayas, eastern and western Ghat forests as well as other primary forests may reveal significant data in relation to the amount of DNA at the diploid level.

ACKNOWLEDGEMENT

The financial help received from the Department of Science and Technology, New Delhi, in the form of Fellowship to the second author is gratefully acknowledged.

1. Price, H. J., *The Botanical Review*, 1976, **42**, 27.
2. Price, H. J. and Neuman, A. E., *Experientia*, 1973, **29**, 1028.
3. Edwards, G. A. and Endrizzi, J. E., *Can. J. Genet. Cytol.*, 1975, **17**, 181.
4. Gupta, P. K., *Chromosoma*, 1976, **54**, 155.
5. Sen, S., *Nucleus*, 1974, **17**, 40.
6. Nagl, W., *Nature (London)*, 1976, **261**, 614.
7. Banerjee, M. and Sharma, A. K., *Experientia*, 1979, **35**, 42.
8. Martini, G. and Brunori, A., *Caryologia*, 1973, **26**, 101.
9. Sau, H., Sharma, A. K. and Chaudhuri, R. K., *Indian J. Exp. Biol.*, 1980, **18**, 1519.
10. Rees, H. and Walters, M. R., *Heredity*, 1965, **20**, 73.
11. Rees, H., Cameron, F. M., Hazarika, M. H. and Jones, G. H., *Nature (London)*, 1966, **211**, 828.
12. Rees, H. and Hazarika, M. H., *Chromosomes today* 1967, **2**, 158.
13. Rees, H. and Jones, G. H., *Heredity*, 1967, **22**, 1.
14. Rees, H. and Jones, G. H., *Int. Rev. Cytol.*, 1972, **32**, 53.
15. Jones, R. N. and Rees, H., *Heredity*, 1968, **23**, 591.
16. Bullen, M. R. and Rees, H., *Chromosoma*, 1972, **39**, 93.
17. Ayonoadu, U. W. U., *Chromosoma*, 1974, **48**, 41.
18. Bennett, M. D., Gustafson, J. P. and Smith, J. B., *Chromosoma*, 1977, **61**, 149.
19. Rees, H., Narayan, R. K. J. and Hutchinson, J., *Nucleus*, 1979, **22**, 1.
20 Resslar, P. M., Stucky, J. M. and Miksche, J. P., *Am. J. Bot.*, 1981, **68**, 149.
21. Nagl, W., *Nature (London)*, 1974, **249**, 53.
22. Sharma, A. K. and Sharma, A., *Chromosome techniques: Theory and Practice*, 3rd ed. Butterworths and Co. Pvt. Ltd., London, 1980.
23. Sharma, A. K. and Aiyangar, H. R., *Chromosoma*, 1961, **12**, 310.