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Intraspecific Variations in Motherwort (*Leonurus sibiricus* L) — Chromosome Structure and Amount of Nucleic Acid

ANADI KUMAR KUNDU* and A K SHARMA
Centre of Advanced Study in Cell and Chromosome Research, Department of Botany, University of Calcutta 700 019

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Cytological investigation including structure and behaviour of somatic chromosomes, total chromosome length, total chromosome volume and *in situ* estimation of 4C nuclear DNA amount were carried out on nine different populations of motherwort (*Leonurus sibiricus*). All the populations reveal a common number of $2n=20$ chromosomes. However, they differ from one another in minute karyotypic details. In general, the meiosis is normal in all the populations studied. Inter-populational variation in the amount of DNA, viz., 2.36 fold, exists in diploid *L. sibiricus*. The amount of DNA does not show direct correlation with the total chromosome length and chromosome volume. The differences in the amount of DNA among different populations could be correlated with certain ecological preferences.

Key Words: Chromosome, Nuclear DNA, *Leonurus*

Introduction

The nuclear DNA content at any level of taxonomic hierarchy in animal or plant is determined and governed by the process of natural selection. As such the amount of DNA is to some extent marked by intraspecific constancy. The amount does to some extent vary among different individuals of a given taxon but the limits of such variations are rather narrow.

Closely related species of the same genus may often have different content. This interspecific variation in the amount of DNA may hold true, irrespective of whether the related species has the same number of chromosomes or not. A 6-fold differences in nuclear DNA content among different species of *Vicia* has been recorded by Chooi (1971a), a 3-fold variation among species of *Lathyrys* (Narayan & Rees 1976) and a 5-fold variation has been recorded among different species of *Anemone* (Rothfels et al. 1966). Edwards et al. (1974) measured more than a 2-fold differences between the lowest and highest values among diploid *Gossypium* species with the same chromosome number. The associations of such variations with highly redundant base sequences (Britten & Davidson 1969, Chooi 1971b, Miksche & Hotta 1973, Price 1976, Sharma 1984) and heterochromatin content (Nagl 1974) has also been advocated. Reports are also available in literature on the correlation between nuclear volume, cell volume, chromosome volume, mitotic cycle time and DNA content (Price et al. 1973, Edwards et al. 1975, Gupta 1976).

That the genome size varies to a considerable extent among closely related plant species has been recorded in several taxa whereas there are only a few confirmed reports as to the intraspecific variation in DNA content, independent of polyploidy,

* *Present address:* Assistant Professor of Botany, Darjeeling Govt. College, Darjeeling

aneuploidy or 'B' chromosomes (cf. Miksche 1968, 1971 Bennett & Smith 1976, Price et al. 1980, 1981a, 1981b, Sharma et al. 1983). The exact mechanism or evolutionary significance of intraspecific variability in DNA content is not precisely understood, although, ecological correlations at intraspecific level in *Picea glauca* and *Picea sitchensis* have been observed (Miksche 1968, 1971). Reports also exist on the induced changes of DNA content (Durrant 1962, 1971).

The species of *Leonurus sibiricus* was selected as material for cytological and cytophotometric analyses. The genus *Leonurus* belongs to the family Lamiaceae and includes a total of 14 species (Willis 1973). *L. sibiricus* along with other species of *Leonurus* are used in the indigenous system of medicine in India. It has a wide distribution in India and found in various parts of West Bengal representing different ecological zones. The present investigation gives a detailed cytological and Feulgen microspectrophotometric analysis of nine differemt populations of *L. sibiricus* collected from different parts of West Bengal.

Material and Methods

Nine different populations of *Leonurus sibiricus*, belonging to the family Lamiaceae were chosen. The plants were collected from different places of West Bengal and grown in earthen pots containing sand and soil mixture in the experimental garden of the Department of Botany, University of Calcutta (table 1).

For the study of somatic chromosomes, root tips were treated in half-saturated aqueous solution of paradichlorobenzene with a trace of aesculine and saponin at 16°C for 45 min, fixed in acetic-ethanol (1:3) for 1 hr, kept in 45% acetic acid for 5 min: hydrolysed in 2.5 N HCl at 4°C for 5 min; kept in 2% acetic-orcein stain for 2 hr and squashed in 45% acetic acid.

Meiotic studies were carried out from fixed flower buds (1:2 acetic-ethanol) following the acetic-carmine technique.

Total chromosome length of each species was calculated by adding the whole length of all the chromosomes present in a complement at metaphase.

For determination of chromosome volume, the breadth of individual chromosomes at metaphase was measured from karyogram plates. Assuming the chromosome as cylindrical, the volume was calculated from the following formula: Chromosome volume $(V) = \pi r^2 h$, where r is the radius of the chromosome = breadth/2 and h, the whole length of the chromosome. The total chromosome volume was then calculated by summing up the volumes of all the chromosomes of a complement.

For estimation of 4C nuclear DNA amount, root tips were fixed in acetic-ethanol (1:2) for 24 hr; kept in 45% acetic acid for 5 min; hydrolyzed in 1N HCl at 60°C for 11 min; rinsed in distilled water; stained for 2 hr in Schiff's reagent, and tips were finally squashed in 45% acetic acid. The nuclear DNA content was measured by the single wavelength method at 550 μm and thirty nuclei at metaphase were scored by using three root tip smears from each of three plants per population. The zone for count of the nuclei was 2 to 3 mm. from the apex including both epidermal and cortical cells. The relative absorbances on the basis of optical density, in terms of arbitrary units of absorbance have been presented, to indicate the relative amounts of DNA.

Table 1 *Habitat characteristics of the 9* Leonurus sibiricus *populations studied*

Population	Area of collection	Altitude (in (meters)	Nature of soil	Soil *p*H	Average annual rainfall (in mm)
I	Calcutta	6	Alluvial	6.6	1440
II	Serampore	8	Alluvial	6.9	1550
III	Hasimara	137	Tarai soil	5.8	4440
IV	Sonarpur	8	Silty clay loam	7.5	1582
V	Bankura	62	Lateritic	6.3	960
VI	Bongaon	7	Alluvial	7.1	1625
VII	Burdwan	32	Red, sandy	6.5	1404
VIII	Janai	5.5	Alluvial	6.9	1550
IX	Bandel	10.8	Alluvial	6.8	1400

Observations

Mitotic Studies

All the nine populations studied showed a common number of $2n = 20$ chromosomes. The morphology of the chromosomes showed little variation in having median to nearly submedian primary constrictions in all the populations. The karyotype analysis revealed the presence of four different chromosome types viz, Types A, B and C (with two constructions) and Type D (with median to nearly submedian primary constrictions) (table 2 and figure 1). The number of nucleolar chromosomes varies from four to six. The chromosome size ranged from 1.25 to 2.85 μm. The total chromosome length ranged from 32.04 to 36.60 μm and

Table 2 *Relative amount of nuclear DNA in different populations of* Leonurus sibiricus *L. along with the values of other cytological parameters*

Population	Somatic chromosome number (2n)	**Total chromosome length** (μm)	Total **chromosome volume** (cu. μm)	4C DNA amount (Mean ± SE) In arbitrary unit of absorbance
I	20	34.78±0.48	10.16±0.43	0.1494±0.0029
II	20	36.25±0.53	11.14±0.38	0.1377±0.0029
III	20	32.04±0.43	8.55±0.51	0.2440±0.0054
IV	20	36.60±1.36	11.52±0.43	0.1630±0.0036
V	20	35.49±1.55	8.80±0.37	0.2730±0.0036
VI	20	32.79±1.43	9.55±0.74	0.2136±0.0043
VII	20	32.55±1.25	8.19±0.32	0.2203±0.0040
VIII	20	32.71±1.07	10.36±0.97	0.1156±0.0030
IX	20	34.35±0.46	9.28±0.74	0.1504±0.0031

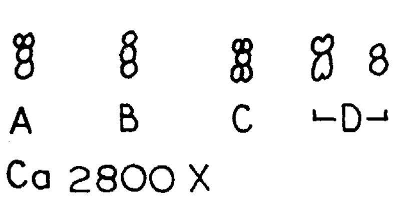

Figure 1

the total chromosome volume from 8.19 to 11.52 cu. μm (table 2).

Meiotic Studies

In all the populations clear 10 bivalents were observed at first meiotic metaphase. Anaphase I segregation was found to be normal.

In situ Estimation of DNA

The 4C DNA content per nucleus varies approximately 2.36 fold from 0.1156 in population VIII to 0.2730 in population V (table 2). The DNA values along with other cytological parameters, such as the total length and volume of the chromosome are given in table 2.

Discussion

Chromosome Study and Its Implications

The nine different populations of *L. sibiricus* studied showed a common number of $2n = 20$ chromosomes. The present observation of $2n = 20$ chromosomes in this species tallies with that of previous records (Chuang et al. 1963, Bhattacharya 1968). In view of this numerical constancy, no correlation exists between the chromosome number and the detectable phenotypic variations. But the structure and behaviour of chromosomes have revealed certain interesting facts.

Populations have been shown to differ from one another with respect to minute structural differences in the chromosome complements, indicating their status as distinct cytotypes. The number and structure of the nucleolar chromosomes vary from population to population, suggesting the role of both numerical and structural changes of nucleolar chromosomes in evolution.

There is a basic resemblance in the gross appearance of the karyotypes of all the populations studied. The continued accumulation of minute structural alterations has possibly been principally responsible for the origin of different cytotypes. Meiotic behaviour is more or less normal with distinct bivalent formation. These observations may indicate that the structural changes in the satellited chromosomes have attained homozygosity in evolution.

The total chromosome length does not reveal any marked variation in the cytotypes (table 2). The minute differences in chromosomal morphology occurring in the course of chromosomal evolution in *L. sibiricus* as such, might not have involved a marked increase in the chromatin content.

Amount of DNA and Its Implications

A 2.36-fold variation in 4C nuclear DNA content per cell exists among the nine different populations of *L. sibiricus*. As all the nine populations contain the same chromosome number $2n = 20$, the variability in DNA amount can hardly be attributed to aneuploidy or B-chromosomes.

It is evident from the table 2, that the amount of 4C nuclear DNA per cell is in no way correlated with the total chromosome volume per cell. For instance, the DNA amounts in population V and population IX are 0.273 and 0.1504, though the chromosome volumes remain more or less intact being 8.8 cu. μm and 9.28 cu. μm respectively. Price et al. (1974) and others observed a positive correlation between nuclear DNA amount and chromosome volume. But nuclear DNA amount is not the sole factor that determines the chromosome volume. The chromosomal proteins, both basic and non-basic, do also play a significant role in determining the chromosome volume. Differential polynemy too has been suggested to be responsible for changes in chromosome volume. The total chromosome volume of a species is, moreover, genetically controlled. As such, any mutation in such a gene may naturally alter the total chromosome volume without involving changes in the total amount of nuclear DNA. The fact that no correlation between DNA content and chromosome volume has been recorded indicates that the amount of DNA may not necessarily be the sole important factor determining chromosome volume.

The amount of DNA too, does not reveal any correlation with the total chromosome length (table 2). The total chromosome length in populations V and II is 35.49 μm and 36.25 μm though the DNA content is different being 0.273 and 0.137 respectively. In absence of any such correlation, the difference in length may be attributed to the differential condensation and spiralization of the chromosome arm.

The exact role of the intraspecific variation in DNA amount in evolution and the mechanism involved for such a variation are yet to be worked out. In eukaryotic system, the amount of nuclear DNA is not directly related to the total number of structural genes present *vis-a-vis* the complexity of the organism. Some plants (e.g. lilies) contain 20 times as mush DNA as mammals (Lewin 1980) and yet latter are normally accepted as comprising a more complex group of organisms. This variability in the amount of DNA among the closely related individuals has often been attributed to repetitive base sequences in DNA (Raina & Rees 1983, Sharma 1984). Chooi (1971b) observed that 15 to 38% of the DNA of six *Vicia* species was composed of repetitive base sequences. Flavell et al. (1974) suggested that most of the changes in the amount ot DNA during evolution have resulted from addition or deletion of repeated sequences. The functional significance of such repeats is, however, not fully known. It has also been recently reported that keeping the total amount of DNA constant at the intraspecific level, the moderate and minor repeats may undergo changes. The fast repeating fraction remains constant in all populations (Sharma et al. 1986). The addition or deletion of such repeated base sequences may be attributed to the variability in the amount of DNA in different populations of *L. sibiricus*.

Miksche (1968, 1971) reported a correlation between the amount of DNA and altitudinal distribution of species. He observed that the nuclear DNA content of plants from higher altitudes was about 1.5 times that of plants from lower altitudes. Durrant (1962, 1971) was able to induce changes of upto 10% in DNA content in the flax variety "Stormont cirris" by way of changing certain environmental conditions like temperature and fertilizer. Price et al. (1981a) suggested that the smaller genomes in certain geographically disjunct populations of *Microseries bigelovii* might have resulted from selection for low DNA content in response to environmental stress.

Considerable variation in the amount of DNA in the nine different populations of *L. sibiricus* correlates with differences in ecological parameters (table 1). The two extremes in DNA content recorded in populations V and VIII may suggest adaptiveness to distinct ecological niches with specific soil compositions, *p*H and annual rainfall. These ecological parameters are quite different in areas where populations V (Bankura) and VIII (Janai) were recorded (table 1).

There are indications that ecological adaptations seem to be associated with the acquisition of specific amount of DNA; however, possibility of differential distribution of block of repeated sequence at intraspecific level can not be ruled out. But the precise extent and basis of such a correlation and the exact mechanism of acquisition of a large amount of DNA in some of the populations of the same species are still not precisely understood and need further studies.

References

Bennett M D and Smith J B 1976 Nuclear DNA amounts in angiosperms; *Phil. Trans. Roy. Soc. Lond.* **B274** 227–274

Bhattacharya R 1968 Cytotaxonomic studies on different taxa of Labiatae and Zingibaranceae and an investigation on the response of chromosomes to hormone treatment; Ph.D. Thesis, University of Calcutta

Britten R J and Davidson E H 1969 Gene regulation for higher cells: a theory; *Science* **165** 349–357

Chooi W Y 1971a Variation in nuclear DNA content in the genus *Vicia; Genetics* **68** 195–211

—— 1971b Comparison of the DNA of six *Vicia* species by the method of DNA-DNA hybridization; *Genetics* **68** 213–230

Chuang T I, Chao C Y, Hu W W I and Kwan S C 1963 Chromosome numbers of the vascular plants of Taiwan I; *Taiwania* **1** 51

Durrant A 1962 The environmental induction of heritable change in *Linum; Heredity* **17** 27–61

—— 1971 Induction and growth of flax genotrophs; *Heredity* **27** 277–298

Edwards G A, Endrizzi J E and Stein R 1974 Genome DNA content and chromosome organization in *Gossypium; Chromosoma* **47** 309–326

—— and Endrizzi J E 1975 Cell size, nuclear size and DNA content relationships in *Gossypium; Can. J. Gen. Cyt.* **17** 181–186

Fedoroff N V 1979 On spacers; *Cell* **16** 697–710

Flavell R B, Benett M D, Smith J B and Smith D B 1974 Genome size and the proportion of repeated nucleotide sequence DNA in plants; *Biochem. Genet.* **12** 257–269

Gupta P K 1976 Nuclear DNA, nuclear area and nuclear dry mass in thirteen species of *Crotalaria* (Angiospermae, Leguminosae); *Chromosoma* **54** 155–165

John B and Miklos C L G 1979 Functional aspects of satallite DNA and heterochromatin; *Int. Rev. Cytol.* **58** 1–114

Lewin B 1980 *Genes* (New York: John Wiley and Sons Inc)

Miksche J P 1968 Quantitative study of intraspecific variation of DNA per cell in *Picea glauca* and *Pinus banksiana; Can. J. Genet. Cytol.* **10** 590–600

—— 1971 Intraspecific variation of DNA per cell between *Picea sitchensis* (Bong.) Carr. Provenances; *Chromosoma* **32** 343–352

—— and Hotta Y 1973 DNA base composition and repetitious DNA in several conifers; *Chromosoma* **41** 29–36

Narayan R K J and Rees H 1976 Nuclear DNA variation in *Lathyrus; Chromosoma* **54** 141–154

Nagl W 1974 Role of heterochromatin in the control of cell cycle duration; *Nature* **249** 53–54

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

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

—— , Chambers K L and Bachmann K 1981a Genome size variation in diploid *Microseries bigelovii* (Asteraceae); *Bot. Gaz.* **142** 156–159

—— , —— and —— 1981b Geographic and ecological distribution of genomic DNA content variation in *Microseries douglasii* (Asteraceae); *Bot. Gaz.* **142** 415–426

—— , Sparrow A H and Naumann A F 1973 Correlations between nuclear volume, cell volume and DNA content in meristematic cells of herbaceous angiosperms; *Experientia* **29** 1028–1029

—— , —— and —— 1974 Evolutionary and developmental considerations of the variability of nuclear parameters in higher plants. I. Genome volume, interphase chromosome volume and estimated DNA content of 236 gymnosperms; *Brookhaven Symp. Biol.* **25** 390–421.

Raina S N and Rees H 1983 DNA variation between and within chromosome complements of *Vicia* species; *Heredity* **51** 335–346

Rothfels K, Sexsmith E, Heimburger M and Krause M O 1966 Chromosome size and DNA content of species of *Anemone* L. and related genera (Ranunculaceae); *Chromosoma* **20** 54–74

Sharma A K 1984 Dynamic DNA and chromosome structure; *Genetics: New Frontiers.* (Proceedings of the XV International Congress of Genetics). eds V L Chopra, B C Joshi, R P Sharma and H C Bansal Vol. **1** 205–210 (New Delhi: Oxford & IBH Publishing Co.)

—— and Chattopadhyay 1983 Relative amounts of nuclear DNA in populations of *Costus speciosus* (Koen.) Sm; *Curr. Sci.* **52** 653–658

—— , Chaudhuri R K and Mukherjee S 1986 DNA content and repetitive DNA in *Lathyrus sativus* at intraspecific level; Proceedings of *Lathyrus* Colliquie at University of Pau, France (In press)

Willis J C 1973 *A Dictionary of Flowering Plants and Ferns*: 8th edn (London: Cambridge University Press)