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DNA estimation in mitotic and amitotically dividing nuclei in species of *Chara* and *Nitella* (Characeae) by feulgen cytophotometric method

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Abstract. The relative amount of DNA was measured by microspectrophotometric method from amitotically and mitotically dividing nuclei of twelve species of *Chara* and *Nitella* (Characeae). The relative amount of DNA in amitotically dividing nuclei was significantly higher than that in mitotically dividing nuclei. Mitotic metaphase of reproductive structure showed 2C amount of DNA as the plant body was haploid, whereas the amitotic nuclei of vegetative body showed variation at 4 to 6C level. It implies that the vegetative nuclei of *Chara* and *Nitella* acquire a larger amount of DNA when dividing amitotically.

Key words: Amitosis; *Chara*; Cytophotometry; DNA; Mitosis; *Nitella*.

Introduction

Feulgen microspectrophotometric DNA determination helps in studying the DNA heterogeneity among different parts of the same plant body in lower groups of plants (Shen, 1967; Therrien, 1967; Maszewski and Kwiatkowska, 1982). It also helps to get a clear picture of life history based on DNA amount, where chromosome count is not possible (Bryant and Howard, 1969; Hurdelbrink and Schwantes, 1972; Lee and Kemp, 1975; Hopkins and McBride, 1976; Breeman, 1979). Such studies are important, as it throws some light on the vegetative body at different ploidy level and it also helps to find out the site of meiosis in the life cycle of those plants, where the chromosome counts are very difficult (Hurdelbrink and Schwantes, 1972; Lee and Kemp, 1975; Hopkins and McBride, 1976).

Both *Chara* and *Nitella* from Characeae are interesting having both amitosis and mitosis within the plant body during its cell cycle. The plant body is haploid (1C) and develops reproductive units (sperm and ovum) by mitotic process. But the vegetative body shows amitosis very frequently. A thorough investigation of amitosis in *Chara* and *Nitella* were carried out by many authors (Sundaralingam, 1946; Gillet, 1959; Shen, 1967a; Roberts and Chen, 1975). In the present study variation in DNA content was measured cytophotometrically of nuclei of different divisional cycles in twelve species of Characeae.

Materials and Methods

Seven species of *Chara* and five species of *Nitella* were taken as experimental materials, viz.

C. corallina Klein ex Willd. var. & f. *corallina*, *C. zeylanica* Klein ex Willd. var. & f. *zeylanica*, *C. setosa* Klein ex Willd. f. *setosa*, *C. globularis* Thuill. var. & f. *globularis*, *C. vulgaris* L. var. *inconnexa* (T.F.A.) R.D.W. f. *hippelliana* (Vihl) R.D.W., *C. fibrosa* Ag. ex Bruz. var. & f. *fibrosa*, *C. braunii* Gm. f. *braunii*, *N. hyalina* (DC) Ag. var. & f. *hyalina*, *N. furcata* (Roxb. ex Bruz.) Ag. subsp. *furcata* var. & f. *furcata*, *N. stuartii* A. Br., *N. furcata* (Roxb. ex Bruz.) Ag. subsp. *flagelliformis* (A. Br.) R.D.W. and *N. acuminata* A. Br. ex Wallm. var. & f. *acuminata*.

Plants were collected from West Bengal, India, and were grown and maintained in soil water biphasic medium in natural condition (14-24°C, light intensity 4,000 lux) in glass jars. No artificial light was used. All the specimens cultured in the laboratory showed vigorous growth and were similar in all morphological aspects to that of plants collected from natural habitats.

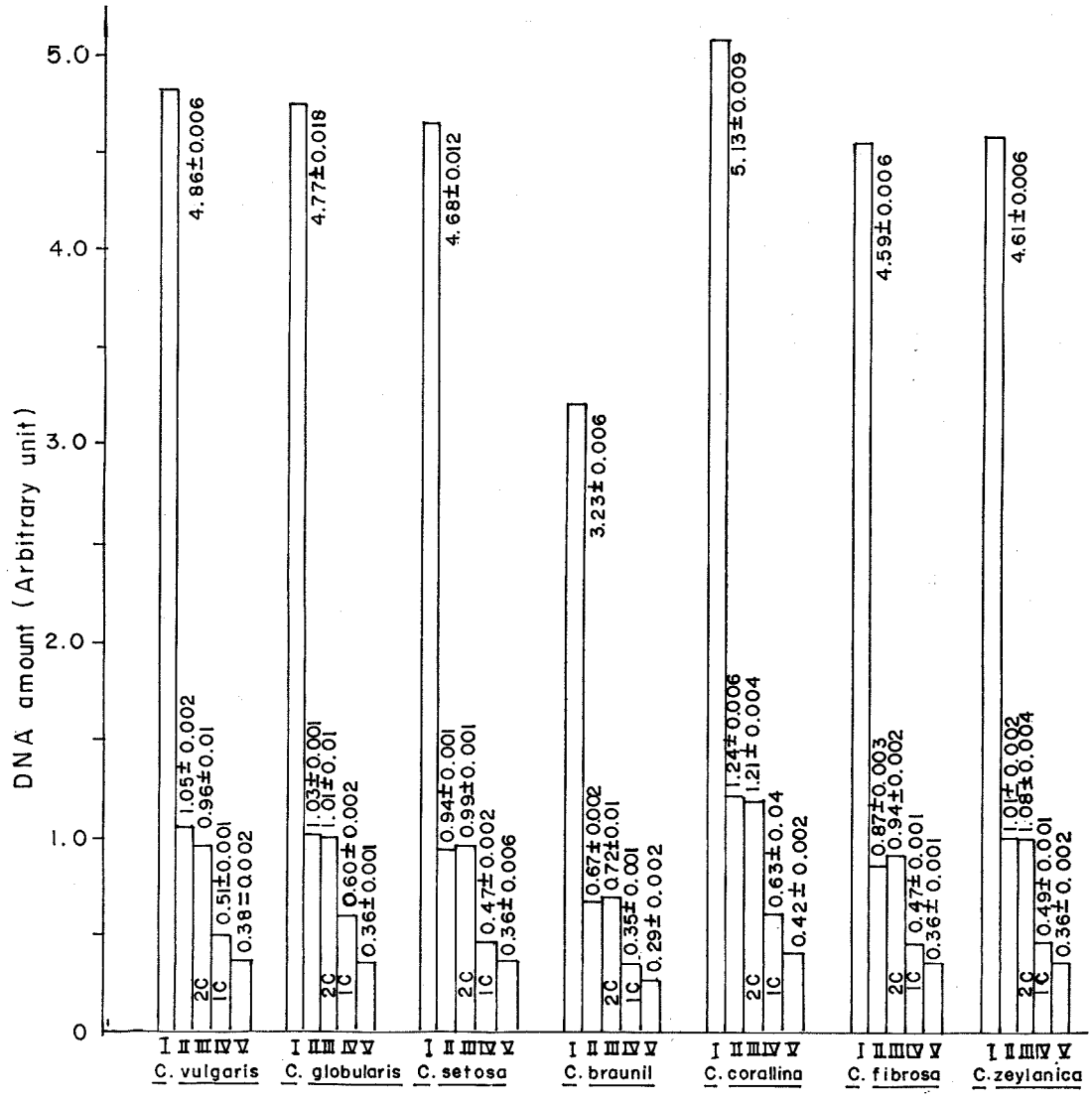

Fig. 1. Histogram showing DNA contents in different divisional stages in seven species of *Chara*. I-Vegetative nucleus, II-Interphase, III-Metaphase, IV-Telophase, V-Mature sperm.

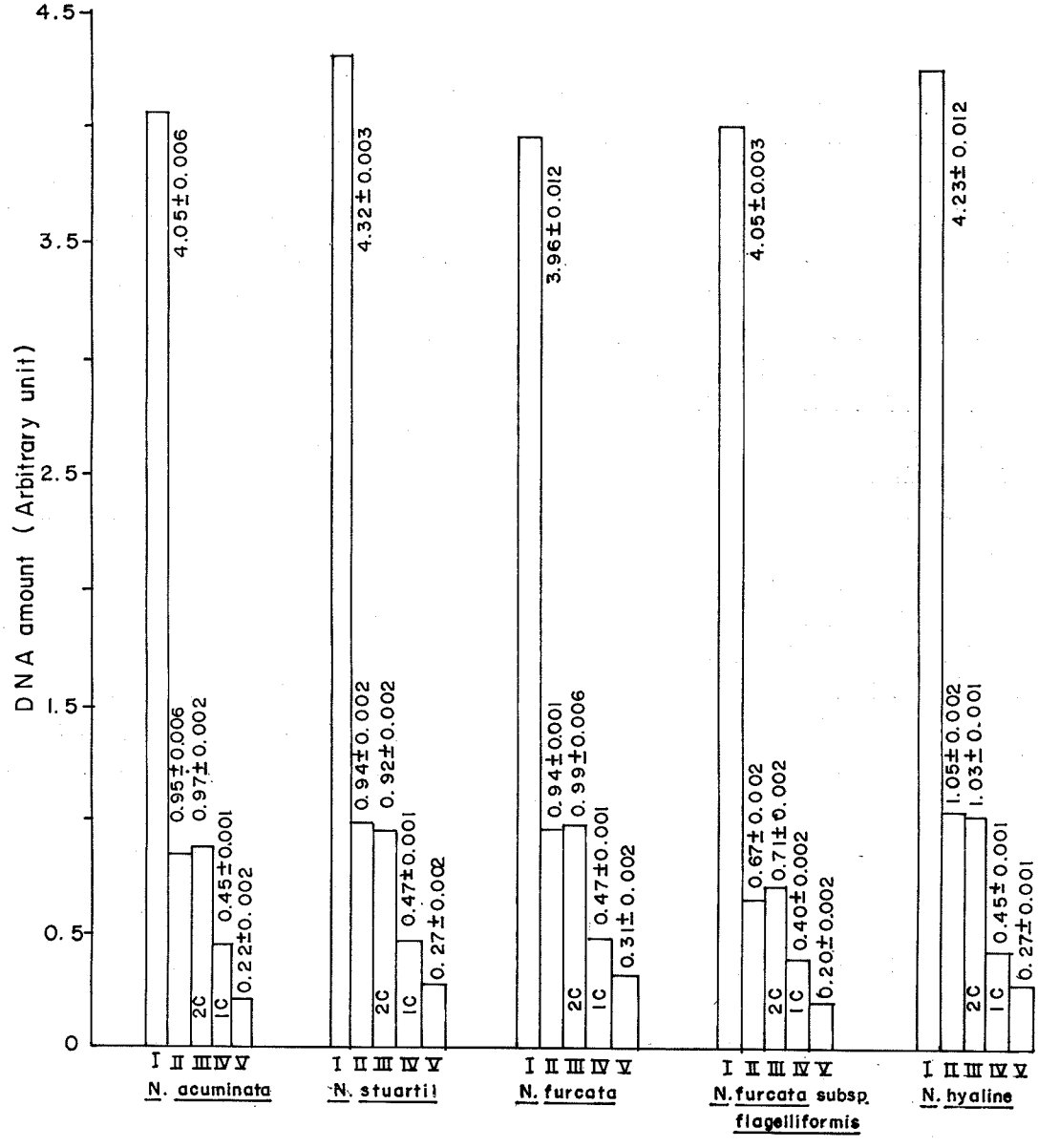

Fig. 2. Histogram showing DNA contents in different divisional stages in five species of *Nitella*. I-Vegetative nucleus, II-Interphase, III-Metaphase, IV-Telophase, V-Mature sperm.

Young, fresh shoot tips were stained by basic fuchsin solution following Pal and Chatterjee's method (1986a, b). Cytophotometric analysis was done in a Reichert Zetopan microspectrophotometer following single wavelength method (550 nm). Readings were taken from vegetative nuclei, both at the mitotic and amitotic divisional stages, and from nuclei of antheridial filaments at different stages of divisional cycle and mature sperms. 100 readings were taken for each cases.

The nuclear DNA amount was measured on the basis of optical density in terms of arbitrary units (A.U.) of relative absorbances. Statistical analysis was done among the metaphase DNA value (2C) of *Chara* and *Nitella* separately and it was found to be significant within the species level.

Results

The DNA content of vegetative nuclei was found to be maximum in *C. corallina* (5.13±0.009). The other species of *Chara* with 28 chromosomes showed little variation in DNA amount in amitotically dividing nuclei, lying between 4.59±0.006 to 4.86±0.006 arbitrary units, *C. braunii* showed comparatively less amount of DNA than the other species 3.23±0.006 (Fig. 1). Among the five species of *Nitella* the variation in DNA was from 3.96±0.012 to 4.23±0.012 A.U. (Fig. 2).

Variation in DNA content in interphasic nuclei in four species of *Chara* and one species of *Nitella* (Fig. 3) were between 1C and 2C level.

The DNA value of post-synthetic phases like prophase and metaphase were almost similar and showed little variation, in anaphase or telophase the amount of DNA was almost half of DNA content of metaphase. In mature sperms the DNA content was a little less than that of telophasic (1C) nuclei (Figs. 1 and 2).

Discussion

The amitotically dividing nuclei of all the twelve species were larger in size than the mitotically dividing nuclei of antheridial filaments and contained larger amount of DNA also. So there is a direct relation between the nuclear volume and DNA content (Pal and Chatterjee, 1986a, b). DNA content varied from 4C to 9C level. Shen (1967) also obtained higher ploidy level of DNA in amitotically dividing nuclei in *C. zeylanica*. The maximum value was 32C from second and third internodal cells. In this investigation, the readings were taken from actively growing shoot tips. It implies that the vegetative nuclei of *Chara* acquire a large amount of DNA in the older portions of the main axis.

The variation in DNA content (from 1C to 2C level) in interphase nuclei of antheridial filaments of five species viz., *C. vulgaris*, *C. corallina*, *C. zeylanica*, *C. globularis* and *N. hyalina* implied distinct presynthetic and synthetic phases. In the

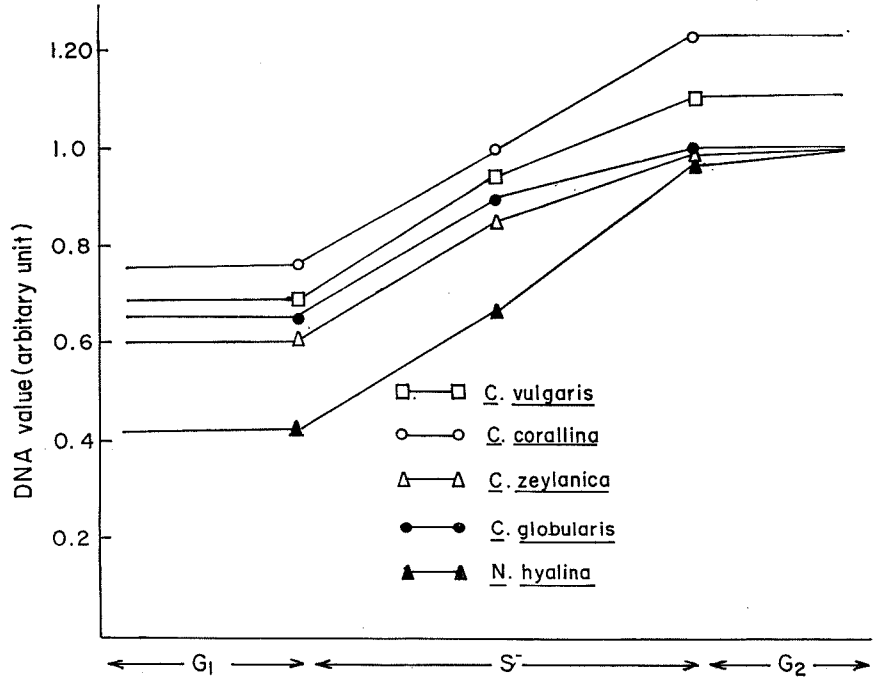

Fig. 3. Graph showing DNA variation in different substages of interphase.

rest of the species studied, the interphase DNA value was exactly similar to that of prophase, and no significant DNA variation was obtained. This means the post-synthetic phase (G_2) was persisting for longer period of time than the G_1 or S-phase or the duration of interphase is less and prophase occurs soon after. In other words, DNA synthesis occurred quickly after completion of one mitotic division, hence the G_1 phase or S-phase were not detectable. It can be pointed out that the five species with distinct G_1 and S-phase contained much more amount of DNA than the rest of the species. It is known that in diploid plants the amount of nuclear DNA influences the length of the mitotic cycle. A positive relationship is present between DNA content and the minimum mitotic cycle time (Van't Hof and Sparrow, 1963; Van't Hof, 1965; Evans and Rees, 1971). Therefore, from the present observation it can be said that duration of interphase was longer since G_1, S, G_2 stages were detectable.

Chara and *Nitella* being haploid plants, showed 2C DNA at prophase and metaphase stage showing little variation. Consequently, anaphase and telophase showed 1C level of DNA.

Marked differences in amount of DNA between telophasic nuclei and the mature sperm suggested a certain amount of loss in the DNA during final spermatization process, which tallies with the ultrastructural study of the spermatogenesis made by Cocucci and Caceres (1976). Thus from our data also we fully confirm the Shen's (1967) conclusions that the life cycle of *Chara* is of haplo-haplobiontic type.

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Chara 和 *Nitella* 在有絲與無絲分裂之 DNA 定量

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取 *Chara* 和 *Nitella* (Characeae) 共12品系之有絲和無絲分裂的分裂細胞核，用微分光光度計 (microspectrophotometer) 測其 DNA 相對量。無絲分裂的分裂細胞核中的 DNA 量明顯高於有絲分裂的分裂細胞核。單倍體 (haploid) 的生殖體中複製組織在有絲分裂 metaphase 含有 2C 的 DNA 量，而營養體之無絲分裂的細胞核約含有 4C～6C 的 DNA 量。這表示 *Chara* 和 *Nitella* 之細胞核在進行無絲分裂時需要大量的 DNA。