# THE AMOUNT OF NUCLEAR DNA IN *CHARA ZEYLANICA* MEASURED BY MICROSPECTROPHOTOMETRY[1]

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## INTRODUCTION

*Chara* is an old genus and has attracted the interest of many former investigators, yet still a number of aspects of its biology remains unsolved. Among these, the site of meiosis has never been reported definitely in any member of the Charophyta. The measurements of the nuclear DNA is an efficient means of clearing the nuclear history of an organism, especially with those in which the definite chromosome figures are difficult to observe. The validity of DNA measurements by the use of Feulgen-stained preparations has been well established and extensively discussed (Ris and Mirsky, 1949; Lessler, 1953; Swift, H., 1953 Kasten, 1958; Garcia, 1965). In the present investigation the two-wavelength microspectrophotometric method (Patau, 1952; Ornstein, 1952) has been employed for the determination of the amount of nuclear DNA in the materials of *Chara zeylanica* Willd.

## MATERIALS AND METHODS

The materials of *C. zeylanica* were obtained from an aquarium at the University green house in Austin, Texas and was identified by Mrs. Fey K. Daily of Butler University, Indianapolis, Indiana, and Professor R. D. Wood of the University of Rhode Island. The writer wishes to acknowledge with gratitude their help.

Antheridia and sperms were smeared on a previously albuminized slide on which a smear of the erythrocytes of the frog *Rana pipiens* has been made. After being dried, the slide with the smears was hydrolyzed with 1 N HCl at 60°C for 12 minutes, stained for 1 hour in Schiff's reagent (Lillie, 1951), washed twice for 5 minutes each in bisulfite solution[3] and then washed with distilled water and dried.

The wavelength used for the present investigation was selected from a Feulgen spectral-absorption curve plotted from the extinction value against the wavelength from the measurements of a single homogenous erythrocyte nucleus. From this curve, wavelengths at the maximum absorption and the half-maximum absorption

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(3) Prepared by adding 5 ml of 1 N HCl and 5 ml of 10% aqueous solution of potassium metabisulfite to 100 ml of distilled water.

were selected as the two wavelengths to be used for measuring the DNA content in the nuclei. To make the DNA measurement of nucleus, two readings, $I_1$ and $I_{o1}$ were taken from the galvanometer at the wavelength at half-maximum absorption; another two readings, $I_2$ and $I_{o2}$, were taken at maximum absorption. Calculating by the formula, these readings were converted to DNA value (Patau, 1952):

$$DNA = KBL_1C$$

where $K$ = the extinction coefficient

$B$ = the area of the exposed field

$L_1 = 1 - T_1 \qquad L_2 = 1 - T_2$

$T_1 = I_1/I_{o1} \qquad T_2 = I_2/I_{o2}$

$Q = L_2/L_1$

$$C = \frac{1}{2-Q} \ln \frac{1}{Q-1}$$

The extinction coefficient (K) was calculated by using as a standard material, the erythrocytes of the frog, *Rana pipiens*. Sze (1953) determined biochemically the amount of DNA per nucleus in *R. pipiens* and reported a mean value of $1.04 \times 10^{-11}$ g.

A Feulgen spectral-absorption curve was plotted from data obtained from the nucleated erythrocytes of the frog, *R. pipiens*. The maximum absorption of this curve was at wavelength 562 m$\mu$ and the half-maximum value at a wavelength of 497 m$\mu$. These two wavelengths were used with the erythrocytes to determine the value of K, and this K was used to calculate the absolute amounts of DNA in the sperm of *Chara zeylanica*.

## RESULTS AND CONCLUSION

Thirty five measurements of *Chara zeylanica* sperms and twenty five measurements of *Rana pipiens* erythrocytes have been made with wavelengths of 562 m$\mu$ and 497 m$\mu$. The mean value of the *C. zeylanica* sperms is 11.4 K and the mean value of the frog erythrocytes is 12.5 K. Since the mean value of DNA in the erythrocyte nucleus of *Rana pipiens* has been determined by Sze as $1.04 \times 10^{-11}$ g, from these data the extinction coefficient (K) in this Feulgen absorption spectrum can be calculated as follows:

$$12.5\,K = 1.04 \times 10^{-11}\,g$$

$$K = \frac{1.04}{12.5} \times 10^{-11}\,g$$

$$K = 0.83 \times 10^{-12}\,g$$

The average mean value of the sperm nuclei of *C. zeylanica* stained on the same slide with the frog erythrocytes can be calculated as follows:

$$11.4\,K = 11.4 \times 0.83 \times 10^{-12}\,g$$

$$= 9.46 \times 10^{-12}\,g$$

The absolute mean value of sperm nuclear DNA of *C. zeylanica* is $9.46 \pm 0.25$[4] micrograms.

DNA duplication and the life cycle of the cell has been extensively discussed (Robertis, Nowinsky & Saez 1965). The amount of DNA in an interphase nucleus of vegetative cells in the diploid phase would be 2C and 4C, and it would be 1C and 2C in the haploid phase. The diploid telophase nucleus in somatic mitosis contains half the DNA content (2C) of the subsequent prophase (4C) and twice the amount of DNA present in the haploid (C) sperm nucleus of the same species (Wilson & Morrison, 1966). The replication of the DNA actually occurs in the cell long before any division processes take place (Trumbore, 1966). The doubling of DNA-takes place only between divisions. If a given cell is not destined to divide again, DNA synthesis does not begin (Mazia, 1961).

In the present investigation an absolute amount of nuclear DNA in the sperm of *C. zeylanica* has been determined. In another paper (Shen, 1967) the writer has comparatively analyzed the DNA content of nuclei in various parts of *C. zeylanica*, and found that the interphase nuclei of vegetative cells contain the same amount of nuclear DNA as in the metaphase stage and has twice as much DNA as in the sperm. The measurements of the interphase nuclei of the antheridial filaments show that no meiosis takes place during the process of spermatogenesis. It indicates that the sperm nucleus contains the haploid amount of DNA (C), since it is not destined to divide again, DNA synthesis does not begin, and thus it kept constant in 1C amount and does not change; while the interphase nuclei of vegetative cells measured are in the $G_2$ period of the DNA cycle of dividing cells. DNA synthesis takes place in the interphase nucleus of the vegetative cells shortly after division; thus the interphase nucleus usually contains the 2C, or twice haploid, amount of DNA. The plant appears to have a haplo-haplobiontic life cycle. The plant body is haploid, and the haploid amount of DNA of *C. zeylanica* is $9.46 \pm 0.25 \times 10^{-12}$ g.

(4) The standard error from the 35 measurements made on *Chara* sperms is 0.25.

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