MICROSPECTROPHOTOMETRIC ANALYSIS OF NUCLEAR DNA IN *CHARA ZEYLANICA*

EUGENE Y. F. SHEN

From the Department of Botany, The University of Texas, Austin, Texas. Dr. Shen's present address is the Department of Biology, Wisconsin State University, Platteville, Wisconsin 53818

ABSTRACT

Microspectrophotometric analysis of the DNA content of nuclei in various parts of *Chara zeylanica* Willd. revealed that the amount of DNA in the nucleus of an internodal cell equals twice the amount of DNA in the nucleus of a sperm, while the half-anaphase stage of the same nodal cells contains the same amount of DNA as the nuclei of the male gametes. The DNA content of the nuclei of internodal cells may rise as much as 50 times higher than that of the gametes. However, in the oldest (most basal) internodal cells, the DNA content of the minute nuclei falls again to the basic (1 C) amount. Measurements of sister nuclei derived by amitosis indicated that both nuclei have equal amounts of DNA; this was interpreted as further evidence that amitosis is not a disorganized process or manifestation of degeneration. The bearing of these analyses on the question of the site of meiosis in these plants is discussed.

INTRODUCTION

Although *Chara* is a linnaean genus and has been the subject of many types of investigations, the definite pattern of its chromosome cycle has not been clear. The nuclear division in internodal cells is often cited in cytology texts as an example of amitosis, but no one has ever described this process in any detail. In another paper (Shen, 1967), the writer has described the history of the nucleus and its mode of division during ontogeny. The development of the multinucleate condition in the internodal cells of *Chara zeylanica* and *Chara contraria* does show a regular pattern of amitotic division, and the two daughter nuclei are always similar in shape and size (Fig. 21).

To obtain further support for this conclusion, to make clear the chromosome cycle of this plant, and to elucidate the pattern of DNA synthesis in the internodal cells, we subjected the material to microspectrophotometric analysis.

MATERIALS AND METHODS

In the present investigation, nuclei of *Chara zeylanica* were stained by using the Feulgen technique, and many nuclei were measured microspectrophotometrically by using the two-wavelength method (Patau, 1952; Ornstein, 1952) so that the amount of DNA could be determined. The validity of DNA determination 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).

Shoot apices and older internodes of *C. zeylanica* were fixed overnight in 10% neutral-buffered formalin, washed, and hydrolyzed with 1 N hydrochloric acid at 60°C for 12 min. They then were stained for 1 hr in Shiff's reagent (Lillie, 1951), were washed twice for 5 min each in a bisulfite solution (prepared by adding 5 ml of 1 N HCl and 5 ml of a 10% aqueous solution of potassium meta-bisulfite to 100 ml of distilled water), and then were washed with distilled water. The older internodal cells were ecorticated under the stereoscopic binocular microscope, dehydrated, and mounted on Permount (Fisher Scientific Company, Pittsburgh, Pa.). Sperms were smeared on previously albuminized slides. Shoot apices were dehydrated, embedded in tissue mat, and cut into 25-μ sections with the rotary microtome. The paraffin sections were then affixed to albuminized

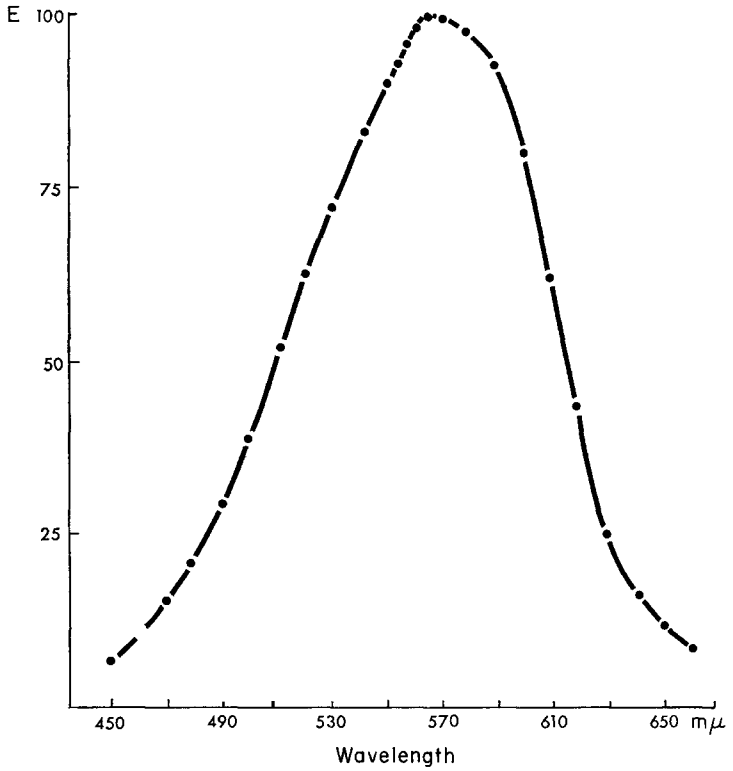


FIGURE 1 Feulgen spectral absorption curve of the nucleus of a nodal cell of *Chara zeylanica*. *E*, extinction.

slides, dried, deparaffinized in xylene, and mounted on Permount. It should be emphasized that all the *Chara* materials used for the microspectrophotometric study have been fixed, hydrolyzed, and treated *in toto* by using the Feulgen method. As a result, the intensity of the Feulgen coloration was relatively uniform and suitable as a basis for the DNA analysis in all parts of the plant.

A Feulgen spectral-absorption curve was obtained (Fig. 1) from a *Chara* nodal cell nucleus which had been fixed and stained *in toto* with all the *Chara* materials used for this experiment. The maximum and half-maximum absorption values are 564 and 509 mμ, respectively. These two wavelengths were used for all the measurements in this experiment.

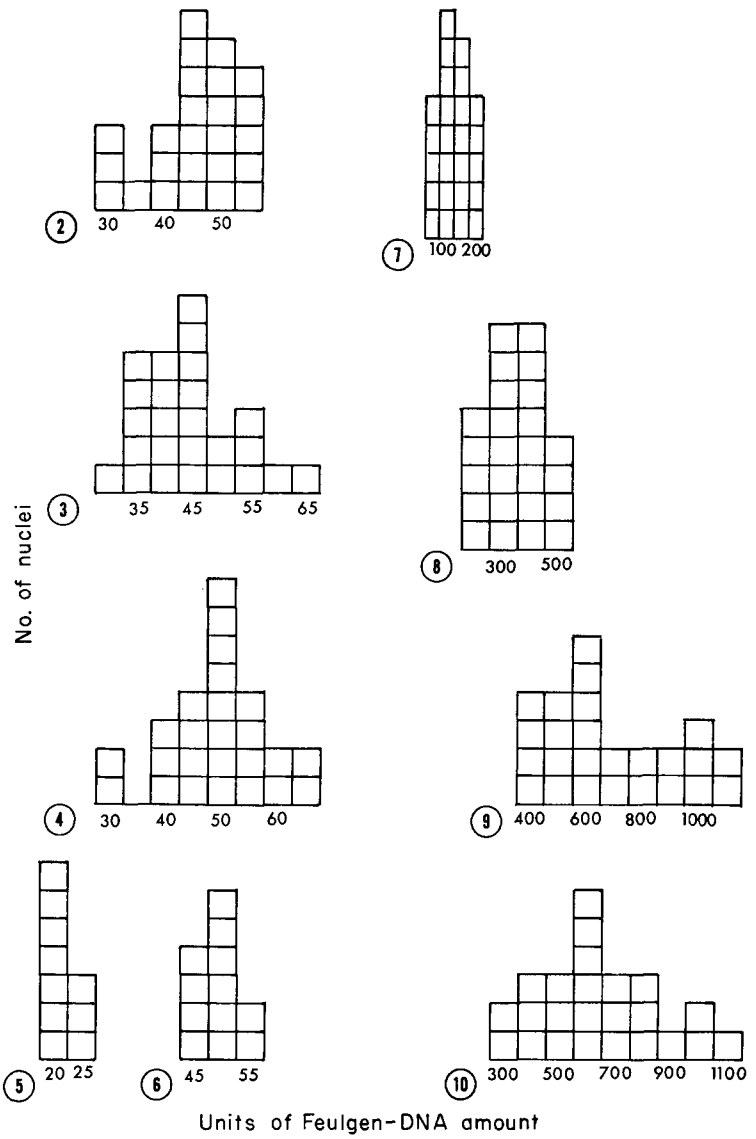
The amounts of Feulgen-DNA in the materials measured were calculated according to the following formula: $DNA = KBL_1C$ (Patau, 1952). The data presented in tables (Tables I and II) and histograms (Fig. 2–15) are given in arbitrary units (BL_1C), without a calculated value for K.

RESULTS

The measurements of nuclei of *C. zeylanica* in various parts of the organism (Figs. 16–21) are presented with mean values in Table I; the frequency–distribution of the DNA content in relation to the number of nuclei is shown in the histograms of Figs. 2–15.

The frequency-distribution charts (Figs. 2–6 and 11–13) show that a somatic nucleus typically has the same amount of DNA at interphase and metaphase, and twice as much at "half-anaphase" and that it also contains twice as much DNA as a gametic nucleus. Hence DNA synthesis is carried on early during interphase. DNA increases in amount in the nuclei of the internodal cells. It can exceed 20 times the DNA content of the nucleus of the internodal initial. The frequency–distribution chart (Fig. 15) of the spherical nuclei in the internodal cells shows two peaks; these peaks coincide with the peaks of the typical somatic nodal nuclei and with those of the gametic nuclei. The difference in the DNA content between the typical interphase nucleus and the spherical nucleus in the old internodal cell may

[1] Half-anaphase refers to each daughter group of chromosomes in late anaphase.



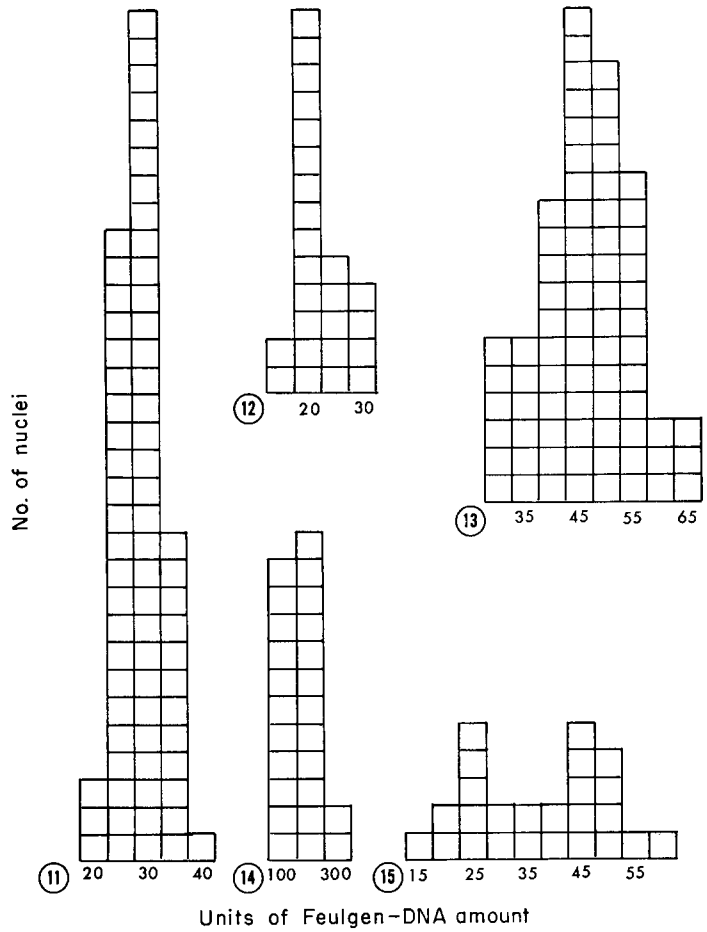
FIGURES 2–10, Frequency-distribution of amounts of Feulgen-stained DNA in nuclei of various parts of *Chara zeylanica*. Each square equals one nucleus.

Fig. 2, apical cells; Fig. 3, subapical cells; Fig. 4, nodal cells; Fig. 5, half-anaphase of nodal cells; Fig. 6, metaphase of nodal cells; Fig. 7, first internodal cells; Fig. 8, second internodal cells; Fig. 9, third internodal cells; Fig. 10, fourth internodal cells.

be due to the fact that the typical somatic interphase nucleus, which is actively growing, shows the 2 C amount of DNA,[2] while the arrest of division and of DNA synthesis in the older cells results in the accumulation of a population of nuclei with 1 C values.

Sister nuclei, formed after amitotic nuclear division, which were still closely associated were selected and measured with the microspectrophotometer; the measurements are recorded in

[2] C = haploid amount of DNA according to Swift (1950). This designation is used on the assumption that the plant is haploid. The haploid amount of DNA in *C. zeylanica* was found to be 9.46×10^{-12} g.



FIGURES 11–15, Frequency distribution of amounts of Feulgen-stained DNA in nuclei of various parts of *Chara zeylanica*. Each square equals one nucleus.

Fig. 11, antheridial filament; Fig. 12, sperm; Fig. 13, vegetative cells (combined apical, subapical, and nodal cells); Fig. 14, crescentic nuclei in old internodal cell; Fig. 15, spherical nuclei in the old internodal cell.

Table II. The results indicate that two sister nuclei always contain almost equal amounts of DNA. This identity reflects equipartition of DNA and also a high degree of synchrony in the subsequent DNA synthesis by the daughter nuclei.

DISCUSSION

The data summarized above have shed some light on the following phenomena: (*a*) the sequence of "polyploidy" or "polyteny" and somatic reduction of the nuclei in the internodal cells of *Chara*; (*b*) the symmetrical division pattern of the nucleus and equipartition of DNA in amitosis in the internodal cells; (*c*) the site of meiosis.

In the present investigation, DNA measurements show that the nuclei in the internodal cells of *Chara zeylanica* increase their DNA content during their proliferation; this increase results in an increase in nuclear DNA and in the number of individual nuclei in a cell. Although no chromosomes could be seen in the internodal cells, the data on DNA in the internodes (Table I; Figs. 7–10) clearly suggest the occurrence of a phenomenon similar to the endomitosis that Berger observed as leading to endopolyploidy in insects (Berger, 1938, 1941; White, 1954).

DNA synthesis and nuclear division are two independent functions of cellular metabolism.

TABLE I

DNA Measurements of Nuclei of C. zeylanica in Various Parts of the Organism

Parts	Number	Mean value ± S.E.	Probable polyploid series, c
Sperm*	25	22.5 ± 0.78	1
Interphase nuclei of antheridial filament*	70	29.16 ± 3.53	1
Apical cell	25	45.7 ± 1.6	2
Subapical cell	25	44.4 ± 1.74	2
Nodal cell	25	48.9 ± 1.59	2
Metaphase of nodal cell	12	49 ± 1.23	2
Half-anaphase of nodal cell	8	21.9 ± 2.1	1
First internode	25	124.2 ± 10.3	4
Second internode	25	342.9 ± 22.6	16
Third internode	25	694.8 ± 45.8	32
Fourth internode	25	655.2 ± 45.3	32
Crescentic nuclei in old internode	25	174.2 ± 12.2	8
Spherical nuclei in old internode‡	12	25.4 ± 1.3	1
	13	46.6 ± 1.5	2

* The difference of the DNA amount between sperm and interphase nuclei of antheridial filaments may be due to some systematic error of unknown source.

‡ The spherical nuclei in the old internode have two peaks; thus the two means.

Either function may be blocked in the course of normal development in a tissue in which the other process may still take place (Patau, 1952; Patau and Swift, 1953; Patau and Das, 1961). In the early development of *Chara* internodes, the rate of DNA synthesis exceeds that of nuclear division. Therefore, the nuclei in the internodal cell enlarge. As these nuclei reach an upper limit in size, the rate of nuclear division becomes higher; thus, in the older internodal cells the nuclei become smaller. It is very interesting that the small, spherical nuclei in the old internodal cells have the same amount of DNA as do the nuclei of the apical cells and the nuclei of the nodes in the vegetative axes and reproductive cells. This suggests a process of somatic reduction (Berger, 1938; Grell, 1946; Patau and Das, 1961); it also gives strong evidence that nuclear division in the internodal cells of *Chara* is as highly regulated as the more conventional mitotic division, and is not a degenerative process. The newly divided daughter nuclei, which could easily be recognized by their similarity in size and shape (Fig. 21), also proved to have equal amounts of DNA (Table II), as has been observed in protozoa (Kimball and Prescott, 1962) and mammals (Cleland 1961; Lapham, 1962), both of which also exhibit amitotic division (although the phenomenon in protozoa is not strictly comparable to that in the present case).

The supposed site of meiosis in *Chara* is controversial. No one has ever reported definite meiotic figures in any member of the Charophyta. Oehlkers (1916), who studied *Chara foetida*, reported that meiosis occurs just before the germination of the oospores. He stated that he found four nuclei in the oospore before its germination. Thus, the plant would be haplo-haplobiontic, and the only diploid stage in its life cycle would be the fertilized oospore. However, Tuttle (1924, 1926) studied an undetermined species of *Nitella* and reported that meiosis occurs during the process of gametogenesis; thus, the gametes would be the only stage in the life cycle to contain a haploid number of chromosomes. Accordingly, the *Nitella* plant would be diplo-haplobiontic.

Gonçalves da Cunha (1936, 1942) studied *Chara vulgaris* var. *longibracteata* and reported that meiosis occurs in the protonema. To substantiate this theory would prove that the plant is haplobiontic with diploid oospores and protonema and that it is a haploid adult plant with haploid sexual organs.

Mendes (1946) studied *Chara vulgaris* var. *longibracteata* and made the following statements. "The fact of having encountered the same num-

TABLE II

Measurements of DNA in the Sister Nuclei of Internodal Cells

Location	Size*	DNA‡
	μ	
Second internode	24	169
		180
	20	314
		326
	20	357
		373
	20	431
		438
	24	524
		526
Third internode	20	412
		423
	30	420
		431
	28	536
		547
	28	559
		561
	23	609
		616
Fourth internode	28	391
		407
	32	541
		557
	25	595
		595
	30	652
		664
	32	745
		752

* Size in diameter of the crescentic nucleus.
‡ Amount of DNA in arbitrary units.

bers of chromosomes in the cauloid apex, as well as in the spermatogenous cells, proves that, contrary to the opinion of Tuttle, the pregametic chromatic reduction is not verified in the case of *Chara vulgaris* var. *longibracteata*." "The technical difficulties concerning the observation of the nuclei of the oospore have not yet enabled me to find out whether the haploid life cycle described by Oehlkers for *Chara foetida* is valid, or not, for the strain on which I have worked." "Gonçalves da Cunhas opinion . . . cannot be taken into consideration as this author probably has not seen real chromosomes." These reports from the literature reveal the unsatisfactory condition of our knowledge regarding meiosis in Charophyta.

From the present microspectrophotometric measurements of DNA in various parts of *Chara zeylanica*, it is clear that the interphase nuclei of nodal cells contain twice as much DNA as do the sperm and that the anaphase stages of the nodal cells have the same amount of DNA as the sperm. The measurements of the interphase nuclei of the antheridial filament show that no meiosis takes place during the process of spermatogenesis. They indicate that the gamete nucleus contains a 1 C amount of DNA and that the plant body is haploid. DNA synthesis takes place in the interphase nucleus of the vegetative cells shortly after division; thus the interphase nucleus usually contain the 2 C, or twice haploid, amount of DNA. The plant appears to have a haplo-haplobiontic life cycle. The site of meiosis should be either just before the germination of the oospores (zygotes), as reported by Oehlkers, or immediately after fertilization, as in the case of *Spirogyra crassa* (Godward, 1961). Further studies on the zygote development will be necessary to determine which of these alternatives is correct.

This investigation was supported, in part, by National Science foundation grant GB 1323 to Dr. Harold C. Bold, and by grant GM 09654 from the United States Public Health Service to Dr. David Bloch.

The aid of Doctors Bold and Bloch during the investigation and preparation of the manuscript is gratefully acknowledged.

Received for publication 10 March 1967; revision accepted 19 July 1967.

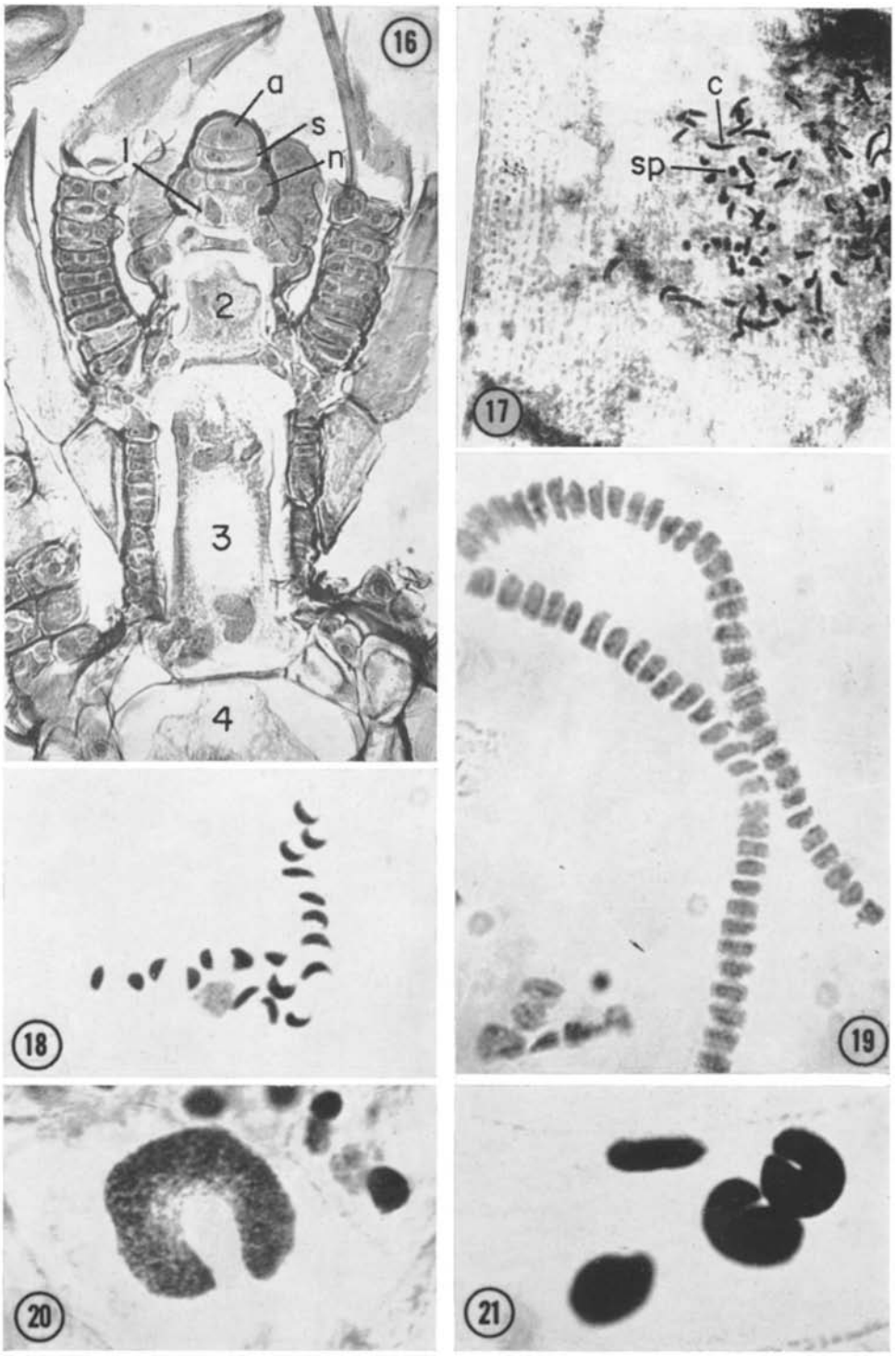
REFERENCES

BERGER, C. A. 1938. Multiplication and reduction of somatic chromosome groups as a regular developmental process in the mosquito, *Culex pipiens*. *Carnegie Inst. Wash. Publ.* **496**:209.

BERGER, C. A. 1941. Multiple chromosome complexes in animals and polysomaty in plants. *Cold Spring Harbor Symp. Quant. Biol.* **9**:19.

CLELAND, K. W. 1961. Deoxyribonucleic acid content of nuclei dividing amitotically. *Nature*. **191**:504.

GARCIA, A. M. 1965. A one-wavelength, two-area



Figs. 16–21, various parts of *Chara zeylanica* stained with the Feulgen reaction.

FIGURE 16 Median longitudinal section of apex of main shoot. *a*, apical cell; *s*, subapical cell; *n*, nodal cell; *1*, first internodal cell; *2*, second internodal cell; *3*, third internodal cell; *4*, fourth internodal cell. × 120.

FIGURE 17 A portion of an old internodal cell with a cluster of small nuclei. *c*, crescentic nucleus; *sp*, spherical nucleus. × 120.

FIGURE 18 Sperms. × 500.

FIGURE 19 Antheridial filament. × 500.

FIGURE 20 Large crescentic nucleus of internodal cell. × 600.

FIGURE 21 Two pairs of sister nuclei in internodal cell. × 400.

method in microspectrophotometry for pure amplitude objects. *J. Histochem. Cytochem.* **13**:161.

GODWARD, M. B. E. 1961. Meiosis in *Spirogyra crassa*. *Heredity*. 16(1):53.

GONÇALVES DA CUNHA, A. 1936. Sur la position de la meiose dans le cycle evolutif des Characées. *Bull. Soc. Port. Sci. Natl.* **12**:175.

GONÇALVES DA CUNHA, A. 1942. Contribuiçao para o estudo dos Carofilos em Portugal. *Trabal. Inst. Botany Lisboa.* 6 (trabalho n. °4).

GRELL, M. 1946. Cytological studies in *Culex*. I. Somatic reduction division. *Genetics.* **31**:60.

KASTEN, F. H. 1958. The Feulgen-DNA absorption curve in situ. *Histochemie.* **1**:123.

KIMBALL, R. F., AND D. M. PRESCOTT. 1962. Deoxyribonucleic acid synthesis and distribution during growth and amitosis of the macronucleus of *Euplotes*. *J. Protozool.* **9**:88.

LAPHAM, L. W. 1962. Cytologic and cytochemical studies of neuroglia. I. A study of the problem of amitosis in reactive protoplasmic astrocytes. *Am. J. Pathol.* **41**:1.

LESSLER, M. A. 1953. The nature and specificity of the Feulgen nucleal reaction. *Intern. Rev. Cytol.* **2**:231.

LILLIE, R. D. 1951. Simplification of the manufacture of Schiff reagent for use in histochemical procedures. *Stain Technol.* **26**:163.

MENDES, E. J. 1946. Mitosis in the spermatogenous threads of *Chara vulgaris* K. var. longibracteata Kutz. *Port. Acta Biol.* **1**:251.

OEHLKERS, J. 1916. Beitrag zur Kenntnis der Kernteilungen bei den Characeen Ber Deutsch. *Botan. Ges.* **34**:233.

ORNSTEIN, L. 1952. Distributional error in microspectrophotometry. *Lab. Invest.* **1**:250.

PATAU, K. 1952. Absorption microphotometry of irregular-shaped objects. *Chromosoma.* **5**:341.

PATAU, K., AND H. SWIFT. 1953. The DNA-content (Feulgen) of nuclei during mitosis in a root tip of onion. *Chromosoma.* **6**:149.

PATAU, K., AND N. K. DAS. 1961. The relation of DNA synthesis and mitosis in tobacco pith tissue cultured in vitro. *Chromosoma.* **11**:553.

RIS, H., AND A. E. MIRSKY. 1949. Quantitative cytochemical determination of desoxyribonucleic acid with the Feulgen nuclear reaction. *J. Gen. Physiol.* **33**:125.

SHEN, E. Y. F. 1967. Amitosis in *Chara*. *Cytologia (Tokyo)*.

SWIFT, H. 1950. The constancy of desoxyribose nucleic acid in plant nuclei. *Proc. Natl. Acad. Sci. U. S.* **36**:643.

SWIFT, H. 1953. Quantitative aspects of nuclear nucleoproteins. *Intern. Rev. Cytol.* **2**:1.

TUTTLE, A. H. 1924. The reproductive cycle of the Characeae. *Science.* **60**:412.

TUTTLE, A. H. 1926. The location of the reduction division in a Charophyta. *Univ. Calif. (Berkeley) Publ. Botany.* **13**:227.

WHITE, M. J. D. 1954. Animal Cytology and Evolution. Cambridge University Press, London. 2nd edition.