haploid number of chromosomes for both wild and cultivated forms of *P. campestris* as nine gemini.

Details of the work will be published elsewhere.

S. R. Bose*

Botanical Laboratory,
R. G. Kar Medical College,
Calcutta, India. May 23.

* Present address: Laboratoire de Cryptogamie, Jardin des Plantes, 12 rue de Buffon, Paris 5.

[1] Colson, Barbara, *Ann. Bot.*, **39**, 1 (1935).

An Acetocarmine Squash Technique for the Fucales

Acetocarmine techniques have only been used to a limited extent in cytological studies of the Phaeophyta, and only in examination of filamentous forms, or filamentous phases in the life-history of the larger forms. Investigations of the Fucales have relied on techniques of embedding and microtoming, followed by staining with Heidenhain's hæmatoxylin[1] or crystal violet[2].

The chief obstacles to the use of acetocarmine squash techniques in the Fucales are the pigmentation, which may obscure or block the staining, and the nature of the cell wall, which is insufficiently macerated by the acetic acid to allow the necessary degree of squashing. Both these difficulties can be overcome by pretreatment with hydrogen peroxide and sodium carbonate according to the following schedule.

Fixation. Small portions of the material, either apices or receptacles, are fixed for 24 hr. in Karpechenko fixative[3] and then washed thoroughly in water.

Bleaching. Bleaching is carried out immediately after fixation: in all the species examined, 4 hr. in 20 per cent hydrogen peroxide produces a suitable degree of bleaching and also causes a preliminary softening of the cell walls, which helps in the subsequent spreading of the squash. After bleaching, the material is thoroughly washed and then treated with acetocarmine.

Acetocarmine treatment. Hand-sections of the region to be examined are mounted on a filmed slide in a drop of 6 per cent sodium carbonate. This converts any alginate into sodium alginate, and as the preparation is gently warmed, the weight of the coverslip squashes and spreads the section: manual pressure can be applied if further spreading is required.

The preparation is irrigated with distilled water to remove excess sodium carbonate, and then with acetocarmine, after which it is gently boiled until the stain is sufficiently differentiated. It may then be examined as a temporary mount in the acetocarmine, or made permanent by allowing the coverslip to fall off in acetic alcohol, dehydrating and mounting in 'Euparal'.

Storage. If the material is not to be examined immediately after bleaching, it may be stored in acetic alcohol made up of one part of glacial acetic acid to three parts of absolute alcohol, with ferric acetate dissolved in 45 per cent acetic acid added until it is a light amber colour. After rinsing in alcohol to remove excess acetate, acetocarmine squashes can be made as above.

Using this technique, growing apices and developing receptacles of several species of *Fucus* and *Cystoseira*, *Himanthalia elongata* and *Halidrys siliquosa* have been examined, and many stages of division found. Fig. 1*a* shows a metaphase plate in a vegetative cell in the apical groove of *Himanthalia elongata* obtained

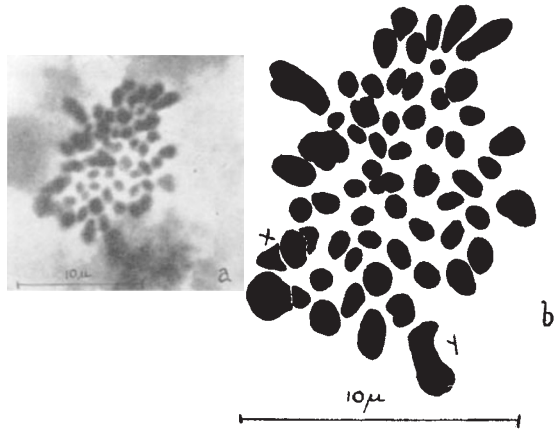

Fig. 1. (*a*) Photomicrograph of a metaphase plate from a cell in the apical groove of *Himanthalia elongata*. (*b*) Analysis of plate shown in (*a*). At *x* one chromosome is at a higher focal level; at *y* the chromosome is scarcely in the focal plane of (*a*)

by this method, and Fig. 1*b* an analysis of this plate made by enlarging the negative to a greater degree, inking in the chromosomes and bleaching the print. This is then checked visually against the preparation. Fig. 1 gives a count of 60 chromosomes. A number of metaphases have been examined in this way and give counts ranging between 56 and 62—a variation possibly due to the fact that many of the chromosomes show separation into chromatids and it is not always possible to distinguish them.

Thus, by using this technique on actively growing apices and developing conceptacles, nuclear divisions in the Fucales may be obtained with little more trouble involved than in making root-tip squashes or pollen grain smears.

Margaret Naylor

Department of Botany,
University of Hull.
March 21.

[1] Hiroe, M., and Inoh, S., *La Kromosomo*, **27-28**, 942 (1956). (This lists all relevant literature.)
[2] Moss, B. L., and Elliot, E., *Ann. Bot.*, N.S., **21**, 143 (1957).
[3] Langlet, O., *Svensk. Bot. Tidsk.*, **26**, 381 (1932).

Chromosome Numbers in Some Members of the Ulotrichales

Chromosome numbers in three species of *Uronema* were reported earlier by me[1]. Subsequent study of the mitotic divisions in some other members of the Ulotrichales resulted in establishing the chromosome numbers as listed below.

		Previous counts
**Uronema terrestre* Mitra	$n = 16$	—
Microspora amoena (Kütz.) Rabenh.	$n = 32$	$n = 8$–12 (Neuenstein, ref. 2)
**Microspora* sp.	$n = 18$	—
Ulothrix zonata Kütz.	$n = 10$	$n = 4$ (Schussnig, ref. 3, Gross, ref. 4)
**Ulothrix subtilissima* Rabenh.	$n = 14$	—
**Sphaeroplea annulina* (Roth) Ag.	$n = 16$	—

The chromosome number for *Sphaeroplea annulina* given above is the first record for the genus. Among other species of *Microspora* and *Ulothrix*, Cholnoky[5] recorded $n = 8$–10 for *M. stagnorum* and $n = 7$–8 for *U. variabilis*, and Lind[6] gave $n = 5$ for *U. rorida*. It is noteworthy that the numbers given here for *M. amoena* and *U. zonata* do not confirm the earlier