the 5 percent level of confidence and are therefore considered to be significant.

In the studies of inhibition, $10^{-3}M$ malonate was approximately 38 percent effective in inhibiting succinate oxidation to the 5 percent level of confidence; $10^{-3}M$ sulfide inhibited ascorbate oxidation approximately 22 percent to the 1 percent level of confidence; fumarate oxidation was depressed approximately 50 percent by $10^{-3}M$ sulfite to the 1 percent level of confidence, and $10^{-3}M$ cyanide caused approximately 65 percent inhibition of cytochrome *c* to the 2 percent level of confidence.

These results suggest that *Ulothrix zonata* (i) is similar to the *Avena coleoptile* (*5*) and carrot root (*6*) in being sensitive to malonate; (ii) differs from *Scenedesmus* (*7*), *Chlorella* (*8*) and the fungus *Myrothecium* (*9*) in having a cyanide-sensitive mechanism; and (iii) is able to utilize several intermediates of tricarboxylic acid substrates as an energy source.

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16 September 1957

Basic Chromosome Number of Four in the Subfamily Panicoideae of the Gramineae

The chromosomal condition of the grass family has long been known for its extreme polymorphism. Polyploidy is particularly common, and almost all described types of polyploidy can be found in this family. Aneuploidy is also occasionally met with in the family but seems to be more or less restricted to those members that have subsexual methods of reproduction. Structural hybridity is of common occurrence (*1*), and special methods for genome building are found in *Saccharum* and other genera.

A great many basic chromosome numbers have been recorded for the family. In a recent publication, Darlington and Wylie (*2*) list all numbers from 4 to 15, as well as several larger ones, as basic for certain elements of the family. There is, however, a considerable difference in the frequency of occurrence of these numbers.

Four is of special interest since it is the smallest number so far encountered, the most recent addition, and also the least frequent of any of the basic numbers. Prior to this report it was known only in two closely related tribes (Aveneae and Stipeae) in the subfamily Festucoideae and in only four diploid species.

In the present study *Iseilema laxum* Hack. from Assam, India, was found to have four pairs of chromosomes. *Iseilema* is a member of the tribe Andropogoneae of the subfamily Panicoideae and is phylogenetically very far removed from the other genera with $n = 4$. A rather thorough cytological study was made of this accession of *I. laxum*, and it was found to be regular throughout the meiotic divisions.

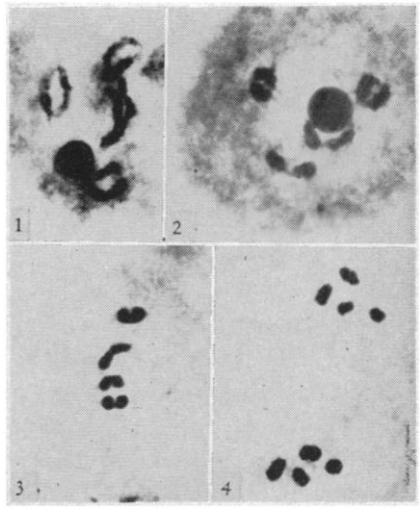
In spite of the small number of chromosomes, pachytene preparations were far from good, and it was not possible to distinguish the chromosomes one from another. However, the chromosomes were much contracted by diplotene and thus were quite satisfactory for study (Fig. 1); diakinesis was also distinct (Fig. 2), and at metaphase I the chromosomes had maximum contraction and stained much darker and sharper than in previous stages (Fig. 3).

Chiasma frequency was determined at each stage and was found to be 2.1 per bivalent at diplotene, 1.8 at diakinesis, and 1.56 at metaphase I. The gradual and constant decrease in chiasma frequency is as expected, and is interpreted to be the result of terminalization. Occasionally, at metaphase I, all four bivalents were closed with a chiasma in each arm, but the most common condition was three closed and one open bivalent. In some instances two open bivalents were seen, and in 6 of 110 cells three of the four bivalents were open. Also two cells were seen where one bivalent had become completely terminalized in both arms and the chromosomes were no longer attached.

The length of the chromosomes of 25 cells was measured at metaphase I. These chromosomes were all of approximately the same length, and were found to average 5.1 μ as closed bivalents and 7.7 μ as open bivalents. These chromosomes are rather large for the Andropogoneae (*3*) but are smaller than those of *Elyonurus* and *Sorghum* (*4*).

Anaphase and telophase were normal (Fig. 4), except that one pair of chromosomes invariably had a precocious separation. This is undoubtedly the bivalent that terminalized early at metaphase I.

This report of $n = 4$ in *Iseilema* not only represents the first such report in the subfamily Panicoideae but also the first instance of a tropical grass with such a number. All previous reports [*Milium scabrum* Rich. (*5*), *Airopsis tenella* Cass. (*6*) *Holcus gayanus* Boiss. (*7*), *Periballia laevis* Asch. and Graebn. (*6*)] have been of species of the temperate Mediterranean region.



Figs. 1–4. Meiosis in *Iseilema laxum* (× 1200). Fig. 1. Diplotene showing nine chiasmata. Fig. 2. Diakinesis with two closed and two open bivalents. Fig. 3. Metaphase I with three closed and one open bivalent. Fig. 4. Anaphase I showing normal distribution of the chromosomes.

It now appears that $n = 4$ may possibly be widespread in the Gramineae, or at least not restricted to a small phylogenetic segment of the family. One feature common to all $n = 4$ species is that they are all rather specialized (advanced) grasses. Not only are they specialized members of their respective tribes but the tribes themselves are rather advanced.

From this there is, at least, a slight suggestion that 4 is a number derived from some larger basic number in the family. However, it must be admitted that too few tribes have been studied cytologically to state categorically that no primitive members of the family have a basic number of 4.

Nevertheless, this appears as the best hypothesis at this time. The tenets of this hypothesis present no objections to the conclusions of Litardiere (*6*) that 4 is derived from 5, or to the conclusion of Flovik (*8*) that 5 is likely to be the basic number for the entire family.

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Stainless-Steel Closures for Replacement of Cotton Plugs in Culture Tubes

The science of bacteriology became established on a practical basis following the discovery in 1853 by Schroeder and von Dusch (*1*) that filtering air through cotton removed airborne microorganisms. Pasteur (*2*) demonstrated later that a capillary space sufficiently long and curved accomplished the same purpose. During the intervening century not only have technical improvements sometimes failed to keep abreast of the demands in the ever expanding field of microbiology, but distinct disadvantages in the use of cotton have been observed. Inhibitory substances from cotton have been reported for pneumococci (*3*), tubercle bacilli (*4*), a diphtheroid and *Hemophilus pertussis* (*5*), *Brucella abortus* (*6*), and *Histoplasma capsulatum* (*7*). The closures described in this report eliminate the undesirable features of cotton plugs, screw caps, and caps of glass, plastic, or aluminum, while offering many desirable features.

The closures (*8*) are fabricated of type 305 stainless steel and so have the noncorrosive properties typical of that metal. This type of stainless steel does not present the problem of contamination of the culture medium by stray metallic ions. As shown in Fig. 1, the closures consist of a cylindrical body closed at one end. At the juncture of the end wall and cylindrical body are located three equally spaced indentations whose functions are to insure an air passageway between the end of the tube and the closure and to center the closure over the end of the culture tube. In the cylindrical body there are three equally spaced resilient fingers which are integrally connected to the body of the closure near its open end; their free ends are directed inward and toward the closed end of the closure. These fingers allow for normal variation in the outside diameter of culture tubes of a given size yet grip the tubes sufficiently to prevent the closures from coming off if the tubes are tilted or shaken and to enable the tubes to be picked up by their closures.

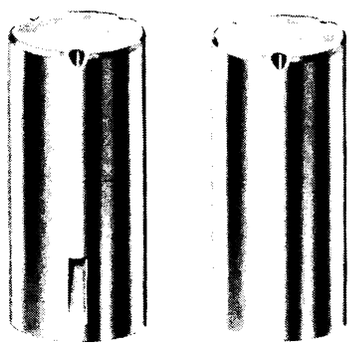

Fig. 1. Stainless steel closures for 16-mm bacteriological culture tubes. Type 16 (left) has fingers for engaging tubes; Type 16A (right) is made without fingers.

While tubes designated as 16 mm O.D. (outside diameter) vary from 16.54 to 15.46 mm (0.651 to 0.608 in.) outside diameter, the type 16 closures will accommodate tubes from 16.54 to 15.3 mm O.D. If it is desirable to check a supply of tubes which has accumulated over many years and from many sources, this may be done with a "go–no-go" gauge with openings of 0.652 and 0.602 in. The length of the closures, 1.5 in., permits easy manipulation with the fingers and creates between the walls of the closure and tube a capillary space which is sufficiently narrow, long, and curved to prevent airborne microorganisms from gaining entrance to the culture tubes. Hundreds of tubes of sterile media maintained their sterility when stored under varying conditions of temperature for periods varying from 5 to 25 weeks.

In contrast to cotton, there is no fire hazard, glassware is not fouled with oily substances during sterilization, the physical and chemical states of the culture medium are not altered by the presence of fibers, and the annoying feature of the irritation of the nostrils of workers with fine cotton dust is eliminated. The closures may be cleaned readily. Nothing projects into the tube; thus the greater portion of the culture tube is usable. Covering the top and a considerable area of the uppermost exterior surface of the culture tube with an impervious metal prevents the collection of dust and microorganisms around the rim of the culture tube and the contamination of cultures by growth of fungi through cotton plugs. The flaming of the open ends of culture tubes before an inoculating needle is inserted—a procedure which has become a ritual with bacteriologists—is now unnecessary.

The loss of culture media by evaporation from tubes with the closures is nearly one-half of that from tubes with cotton plugs. The closures allow an adequate exchange of gasses under aerobic and anaerobic conditions. This was determined by the pigment production of *Serratia marcescens* and *Chromobacterium violaceum*, the growth of *Salmonella typhosa* and *Salmonella schottmuelleri* on and in Russell's medium, the rate of diffusion of oxygen into thioglycollate medium, and the growth of *Clostridium tetani* and *Clostridium botulinum*.

In addition to the many technical advantages, there are several economical features of the stainless steel closures. By being used repeatedly, they save materials and labor. When a closure has been used 25 times it has realized savings equivalent to its original cost. The closures permit empty Vacutainer tubes (*9*) to be used as culture tubes. Culture tubes with closures need only be balanced in the centrifuge trunions before centrifuging; there are no cotton plugs to make secure. Since there are fewer storage problems, the closures are more convenient than cotton plugs on expeditions or field trips. The closures can be color coded readily by placing on their tops a dot of colored ink as used with the Marktex Tech-Pens (*10*). The colored markings withstand repeated sterilizations and washings but may be removed readily with acetone.

The majority of the tests which have been briefly summarized above have been performed with closures (*11*) for test tubes of 16 mm O.D. which are commonly employed in bacteriological work. Closures of similar type have been developed and tested for tubes of 13, 18, 20, 25, and 38 mm O.D.

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3 September 1957