Table 2. Properties of mouse hepatitis virus (MHV) complement-fixing antigen.

Item	Antigen-titration immune serum 140	Infectivity		
		10^{-1}	10^{-4}	10^{-6}
MHV lot 186				
Crude 10%, centrifuged at 10,000 rev/min (20 min)	1:8	9/9*	6/7	0/6
Sediment of centrifugation at 40,000 rev/min (1 hr)	1:4	12/12		
Sediment dried	1:2	6/6		
Sediment dried + benzene 3X	–			
Supernatant of centrifugation at 40,000 rev/min (2 hr)	1:16	1/14		
Supernatant of centrifugation at 40,000 rev/min, heated 61°C (20 min)	1:4			
Supernatant of centrifugation at 40,000 rev/min + ⅓ vol. ether	1:4			
Supernatant dried	1:4			
Supernatant dried + benzene 3X	1:2			
Normal liver				
Crude 10%, centrifuged at 10,000 rev/min (20 min)	–			
Supernatant of centrifugation at 40,000 rev/min (1 hr)	–			

* Mice showing hepatitis lesions/mice inoculated.

A box titration of mouse hepatitis virus with serum from immunized and from normal mice is outlined in Table 1. Extracts from normal mouse liver did not fix complement in the lowest dilutions of serum and antigen. Table 2 shows the results of treating aliquots from a single pool of infected mouse livers by the various procedures outlined. Similar results were obtained when two additional pools were subjected to many of the same procedures. Complement-fixing antigen was extracted from infected liver tissue that was centrifuged at 10,000 rev/min for 20 minutes. The supernatant fluid from such preparations maintained approximately the same level of complement-fixing ability when it was subjected to a further centrifugation at 140,000 g (40,000 rev/min) for 2 hours, even though the infectivity of the preparation was greatly reduced by this procedure. A decline in complement-fixing antigen in the supernatant of the latter procedure was detected following heating, ether extraction, desiccation, and desiccation and benzene extraction; however, these procedures did not cause complete extinction of antigenicity. Control antigens processed from "normal" baby mouse livers were negative. Virus content in the antigens declined from 10^{-4} in the original liver preparations (10,000 rev/min for 20 minutes) to less than 10^{-1} in the supernatant fluid of the ultracentrifuged antigen.

The complement-fixing antigen extracted from liver tissue infected with mouse hepatitis virus appears to be specific and relatively tolerant of desiccation, heating, ether, and benzene. The relatively virus-free supernatant fluid, following ultracentrifugation, appears to be a better antigen than the resuspended virus-containing sediment (Table 2). Failure to sediment the complement-fixing antigen along with the virus would support the assumption that this antigen is a soluble one (*9*).

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Respiration in Ulothrix zonata

The investigations of Genevois (*1*) and of Eny (*2*) suggest that the oxidative mechanisms in thallus plants do not differ significantly from those in higher plants. In pursuing this parallelism, we have investigated the ability of *Ulothrix zonata* to oxidize certain organic substrates.

Stock cultures of the alga were maintained at room temperature under natural daylight in closed culture dishes containing 50 ml of beef extract (0.05 percent, wt./vol.) to which had been added 50,000 units of penicillin. Prior to experimentation, the filaments were harvested, washed thoroughly in distilled water, and exposed to 10,000 units of penicillin per milliliter of fresh culture fluid for 6 to 8 hours.

The rate at which the plant oxidizes succinate, malate, fumarate, citrate, and ascorbate was determined by using 1.0 ml of intact cells in accordance with methods previously described (*3*). Homogenates were used according to the methods of Umbreit, Burris, and Stauffer (*4*) for the determination of the rates of oxidation of cytochrome *c*, oxalacetate, and α-ketoglutarate. The homogenates were prepared by destroying the cellular structure of the plants in a hand-powered glass homogenizer of approximately 6.0-ml capacity at a temperature of 3.0°C. Sodium sulfide ($10^{-3}M$), sodium sulfite ($10^{-3}M$), sodium malonate ($10^{-3}M$), and sodium cyanide ($10^{-3}M$) were used, respectively, in the reacting systems of fumarate, ascorbate, succinate, and cytochrome *c* to determine their effect upon the rate of oxidation of the preceding substrates.

Table 1 gives the endogenous Q_{O_2} values and the Q_{O_2} values with added substrates (corrections for the residual respiration have been made). As shown in the table, the rate of oxidation of cytochrome *c* was considerably more rapid than the rate of oxidation of the other substrates, while citrate appeared to be oxidized more slowly than any of the others. All of the Q_{O_2} values are below

Table 1. Endogenous and above endogenous respiratory rates in *Ulothrix zonata*. Values given for Q_{O_2} [where Q_{O_2} is measured in microliters of oxygen per milligram (dry wt) per hour] are means representing averages obtained from six to eight vessels of three separate experiments after correction for endogenous respiration.

Condition	Molar soln.	Q_{O_2}*	σ*
Endogenous		1.99	0.49
Succinate	0.050	6.76†	0.68
Fumarate	0.030	6.62‡	0.62
Malate	0.030	4.22‡	0.62
Oxalacetate	0.003	3.90†	0.94
Ketoglutarate	0.003	3.92†	0.61
Citrate	0.030	3.72†	0.53
Ascorbate	0.050	11.48†	0.58
Cytochrome *c*	0.0002	33.32†	2.48

* Standard deviation of the mean.
† Significant to the 1 percent level of confidence.
‡ Significant to the 2 percent level of confidence.

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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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.

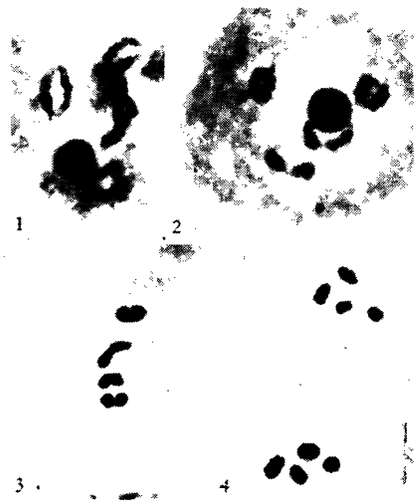
In spite of the small number of chromosomes, pachytene preparations were far from good, and it was not possible to distinguish the chromosomes one from another. However, the chromosomes were much contracted by diplotene and thus were quite satisfactory for study (Fig. 1); diakinesis was also distinct (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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